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Breast Cancer with the Presence of a Large Number of Atypical Cells in Peripheral Blood Mimicking Acute Myeloid Leukemia: A Case Report

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ESCCA 2023 Utrecht
Disclosure commercial conflict of interest

x	No, nothing to disclose
	Yes, as specified below:

Company Name	Specification

Case at Presentation

- A 75-year-old woman with a history of breast lobular adenocarcinoma treated with mastectomy and radiotherapy in 2021, reported to the emergency room complaining asthenia.

- **The laboratory tests showed:**

Leucocytosis (**23.4**x$10^3/\mu l$), anemia (Hb **72** g/l), slightly low platelet count (**138**x$10^3/\mu l$) and increased reticulocytes (**11%**)

Increased Calcium level: **13** mg/dl (normal range 8.6-10.3 mg/dl)

Increased LDH: **600** UI/l (normal range 140 - 280 UI/l)

Increased Indirect Bilirubin: **5** mg/dl (normal range 0.2-0.8 mg/dl)

Reduced Haptoglobin: **<0.1** g/l (normal range 0.5-2.2 g/L)

The Direct Antiglobulin Test (DAT) was **negative** and the renal function was normal

Tumor markers were negative

1st Question

1) These preliminary clinical features may be indicative of:

a) A reactive condition

b) Macroangiopathic hemolytic anemia

c) PNH

d) Other condition

c) PNH

Clinical indications for PNH testing and monitoring

- Intravascular hemolysis (with or without anemia) as evidenced by increased LDH, reduced or absent haptoglobin, hemoglobinuria and elevated plasma hemoglobin, especially if associated with iron deficiency, abdominal pain, esophageal spasms, thrombosis, neutropenia and/or thrombocytopenia.

- Bone marrow failure syndromes, including AA and MDS (especially RCUD).

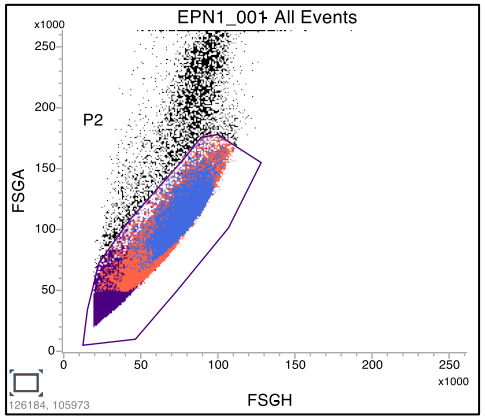
- Thrombosis with unusual features and/or occurring at unusual sites – e.g. hepatic veins/Budd-Chiari syndrome, other intra-abdominal veins (e.g. portal, splenic), cerebral sinuses or dermal veins – especially if accompanied by hemolysis with or without anemia and/or other unexplained cytopenias.

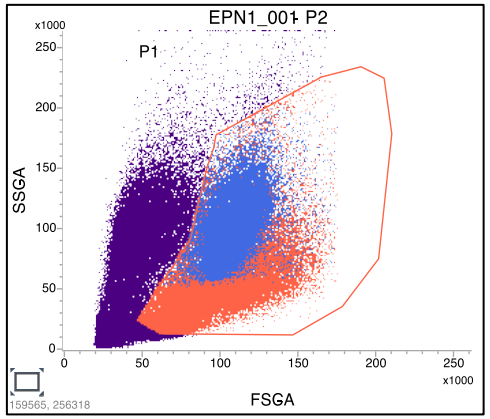
- Regular monitoring of PNH patients, including those receiving eculizumab.

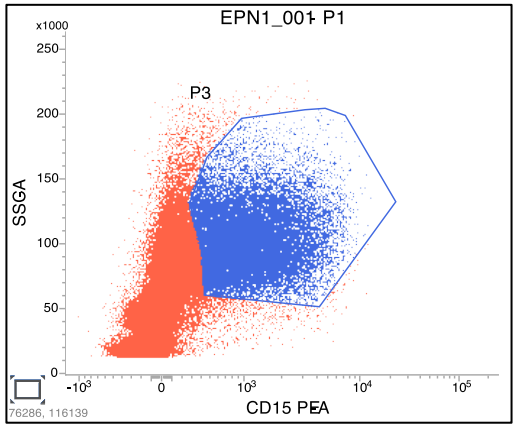
- Regular monitoring of AA-PNH and MDS-PNH patients

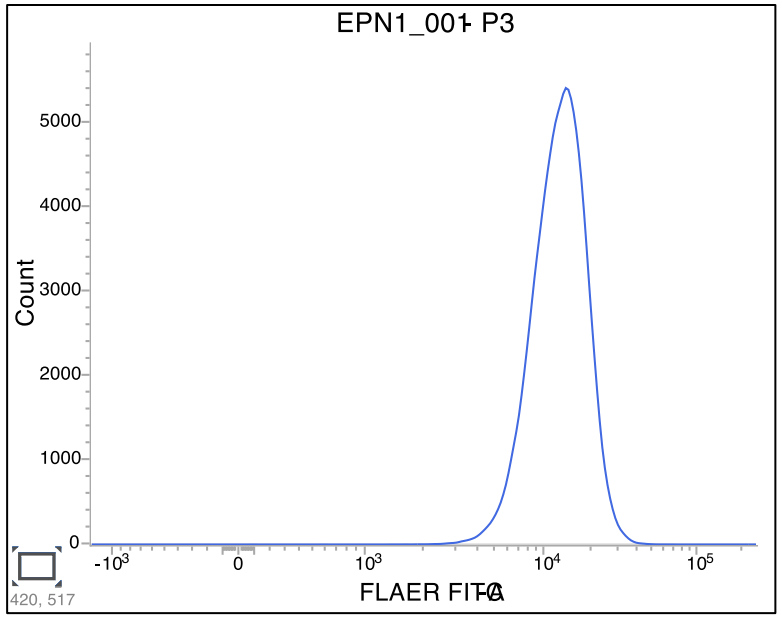
M. Lima Practical Laboratory Medicine 20 (2020) e00158

Screening of PNH (FLAER/CD15)









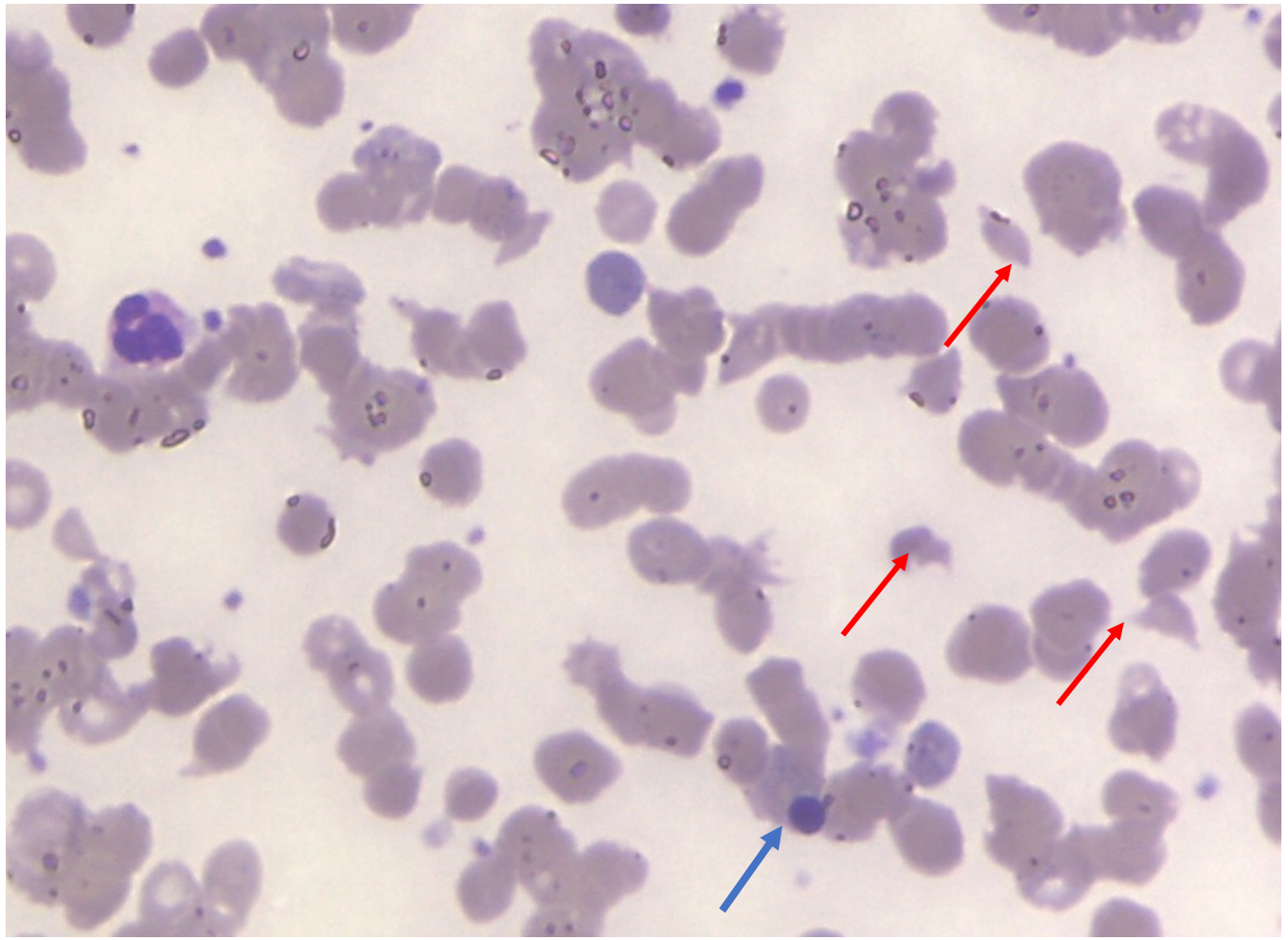
a) Macroangiopathic Hemolytic Anemia

- Cancer-related microangiopathic hemolytic anemia (CR-MAHA) is a paraneoplastic syndrome characterized by negative DAT, thrombocytopenia ($<150x10^3/ml$) and detctable schistocytes.
- Evidence of organ damage (renal failure or neurological symptoms) is frequent
- CR-MAHA is most commonly associated with gastric cancer, followed by breast, prostate and lung cancers. However hematological malignancies such as lymphoma make up 8% of cases.
- Drugs used in the setting of cancer are associated with CR-MAHA.
- Infections may also present elicit MAHA in cancer patients
- Disseminated Intravascular Coagulation in patients with unknown cancer may be the first clinical manifestation of MAHA

M. R. Thomas. Blood 2021; 137: 1310-1317

Peripheral Blood Smear

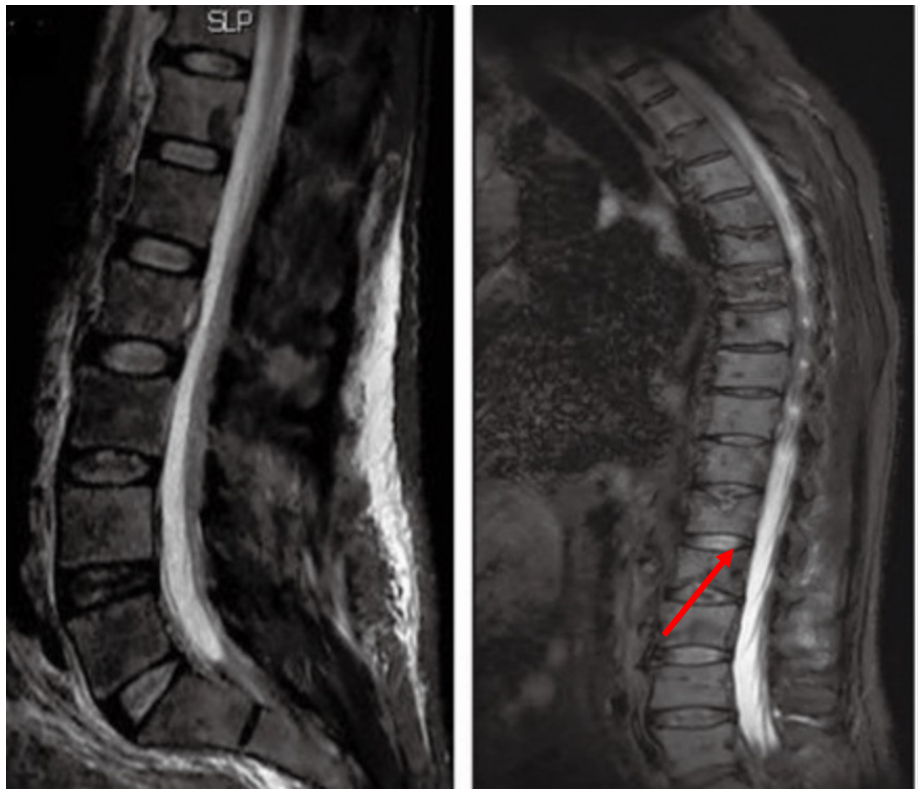
Marked red cell anisocytosis, a remarkable proportion of schistocytes (15% of erythrocytes - red arrows) and erythroblasts (3% of nucleated cells - blue arrow) were observed.



a) Macroangiopathic Hemolytic Anemia

Therefore, in the hypothesis of a Macroangiopathic Hemolytic Anemia related to cancer recurrence, total body CT scan and ^{18}F-FDG PET/CT were planned.

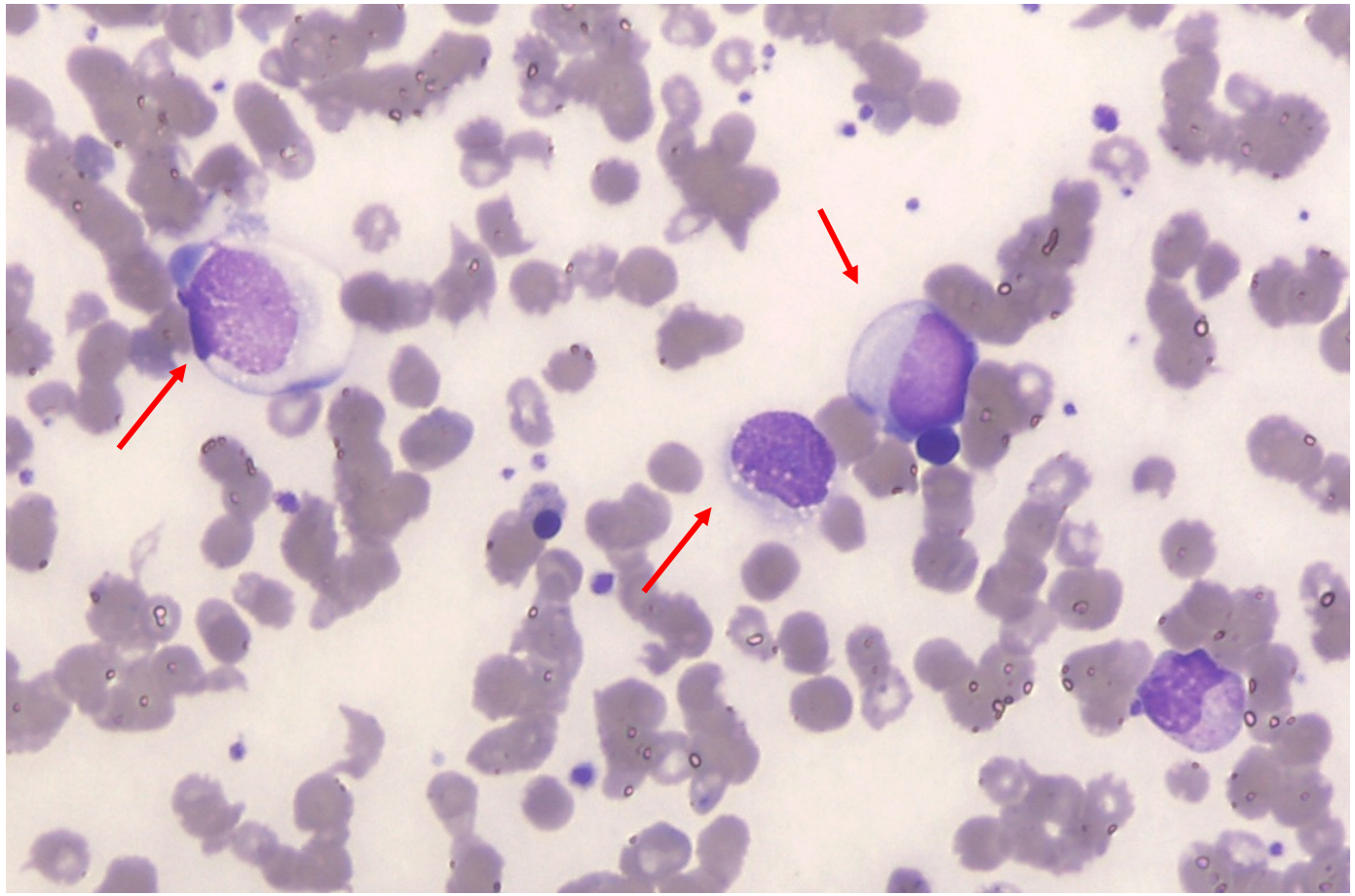
The former examination resulted negative, whereas the latter showed only a slight FDG uptake in the spine, ascribed to increased bone marrow activity, probably due to the hemolytic condition.



A New Peripheral Blood Smear was Ordered for Reanalysis

Immature granulocytes (promyelocytes, myelocytes and metamyelocytes) were observed.

A significant number of large mononuclear cells with eccentric nucleus, of uncertain lineage, (15% of nucleated cells - red arrows) was also noted.



2nd Question:

Based on morphological features, what could be the initial diagnosis?

a) A reactive condition

b) Macroangiopathic hemolytic anemia

c) Secondary Acute Myeloid Leukemia

d) Other condition

Bone Marrow Aspirate Immunophenotyping and Trephine Biopsy

To exclude the diagnosis of secondary acute leukemia due to the presence of circulating abnormal cells, bone marrow aspirate and trephine biopsy were performed.

The aspirate cell suspension was analyzed by multicolor flow cytometry.

SECONDARY MYELOID NEOPLASMS

The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours

- Myeloid neoplasms after cytotoxic therapy (MN-pCT) are included in this category.
- As in previous editions (Therapy-related AML according to WHO 2016), this entity includes AML, MDS, and MDS/MPN arising in patients exposed to cytotoxic (DNA-damaging) therapy for an unrelated condition.
- Chemotherapy is a risk factor for secondary myeloid neoplasms. Pre-existing malignant clones, secondary to the selection pressure of cytotoxic therapy, can proliferate in an altered marrow environment.
- The definition of MN-pCT requires a documented history of chemotherapy treatment or large-field radiation therapy for an unrelated neoplasm.
- The majority of AML-pCT and MDS-pCT are associated with TP53 mutations.

SECONDARY MYELOID NEOPLASMS

- Breast cancer, non-Hodgkin lymphomas, and Hodgkin lymphomas are the three primary malignancies most frequently associated with the development of AML-pCT (or t-AML).
- AML-pCT accounts for about 8% of all AML diagnoses with median age at diagnosis depending on the primary tumor, ranging between 40 to 66 years.
- In a retrospective analysis of 6 prospective multicenter trials of the German-Austrian AML Study Group (AMLSG), the median age at diagnosis was 57.8 years.
- AML-pCT is charcterized by a poor prognosis, with a lower overall survival, as compared to de novo AML.
- AML-pCT is usually associated to complex chromosomal abnormalities, multiple gene mutations and unusual immunophenotyping.

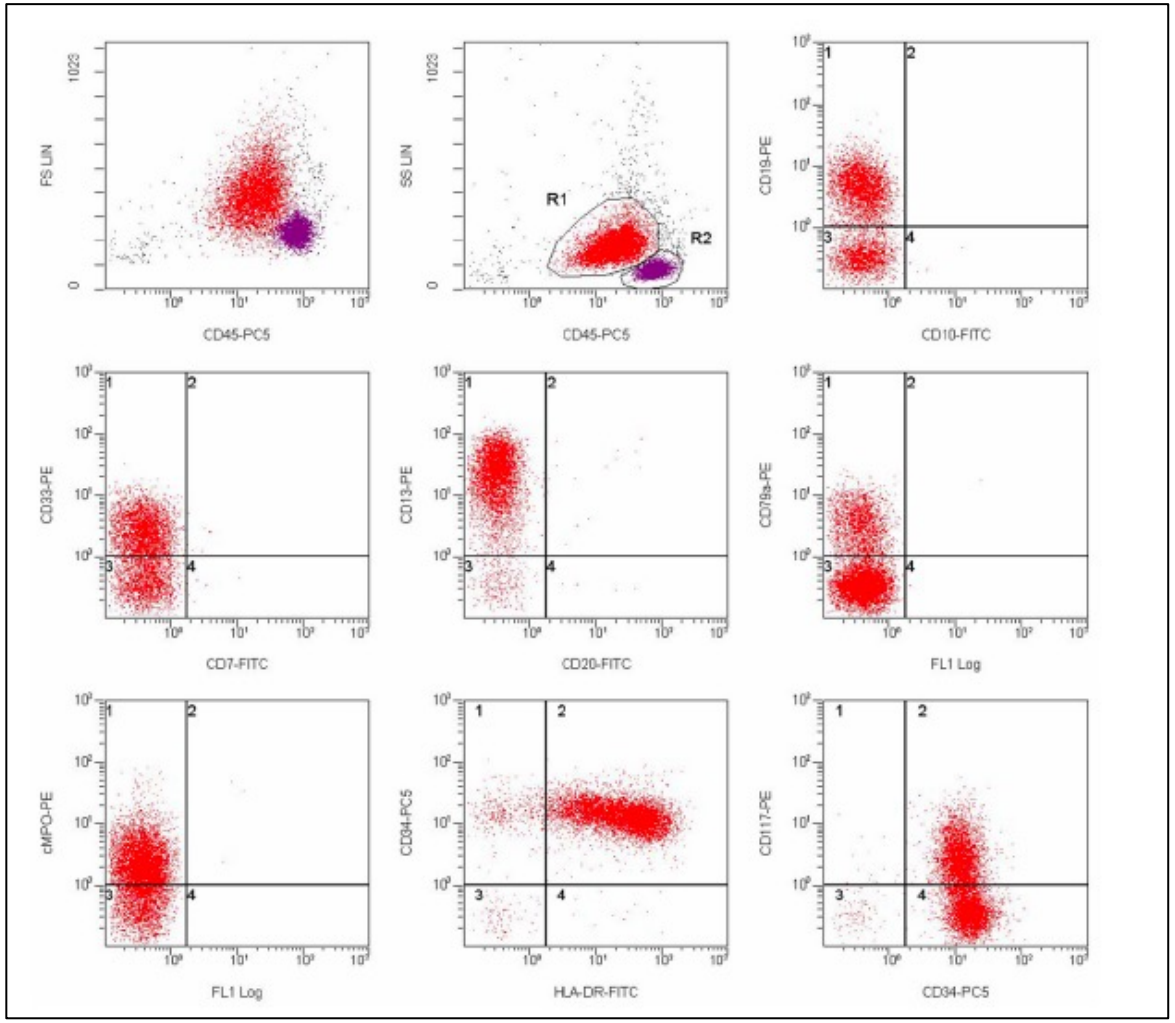
Case Report

Secondary mixed phenotype acute leukemia following chemotherapy for diffuse large B-cell lymphoma: a case report and review of the literature

Flow cytometric findings of a case of mixed phenotype acute leukemia.

Region 1 (R1) included 60.93% of all nucleated cells.

In R1 most cells co-expressed myeloid (cyMPO, CD13) and lymphoid antigens (CD19 and cyCD79a).



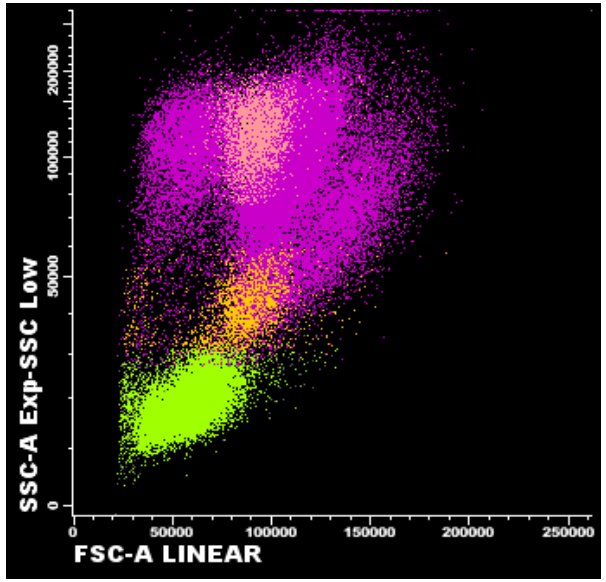
X. Gong. Int J Clin Exp Pathol 2018; 11(6): 3104-3110

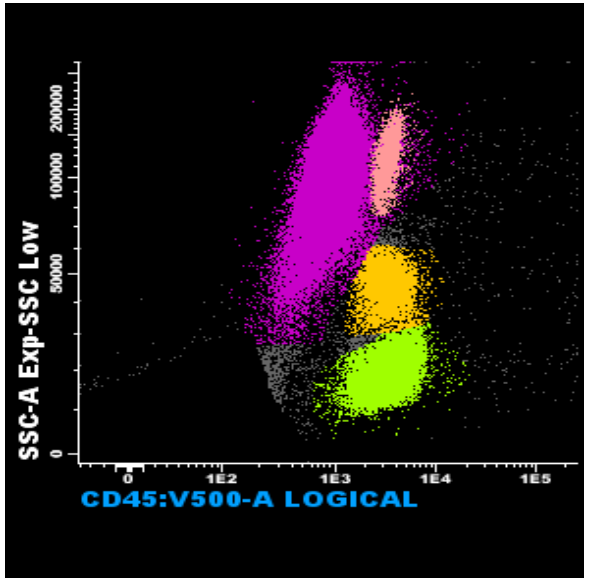
Incidence of Acute Myeloid Leukemia after Breast Cancer

- Alkylating agents and Topoisomerase II inhibitors, fundamental to the treatment of breast cancer, are the most likely contributors to this increased risk.
- Radiation therapy adds to the risk.
- Several studies have reported an increased incidence of acute myeloid leukemia after treatment of breast cancer with evidence of a dose-intensity relationship.
- It is estimated that one every twenty patients will develop a secondary non-breast cancer after 10 years, which corresponds to a 22% increase of relative risk, particularly for secondary AML and MDS.

Valentini CG. J Hematol Infect Dis. 2011;3(1):e2011069.

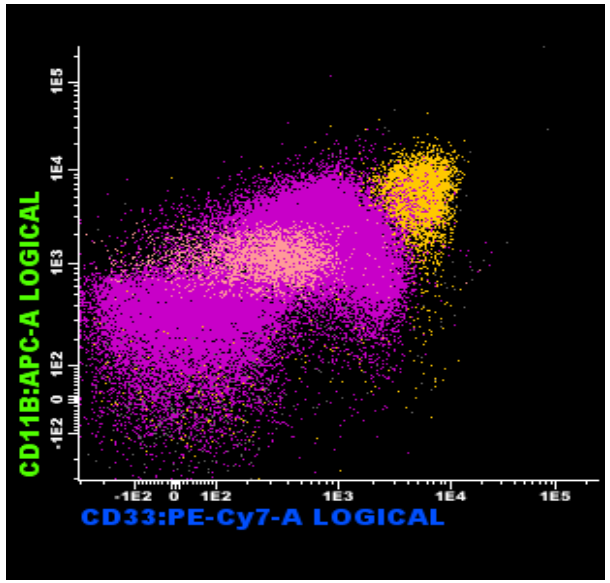
Case Report: The first myeloid flow cytometric panel on peripheral blood (1)

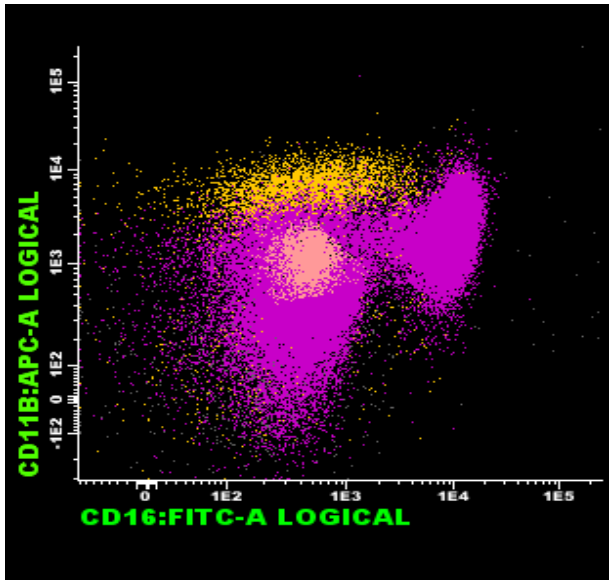


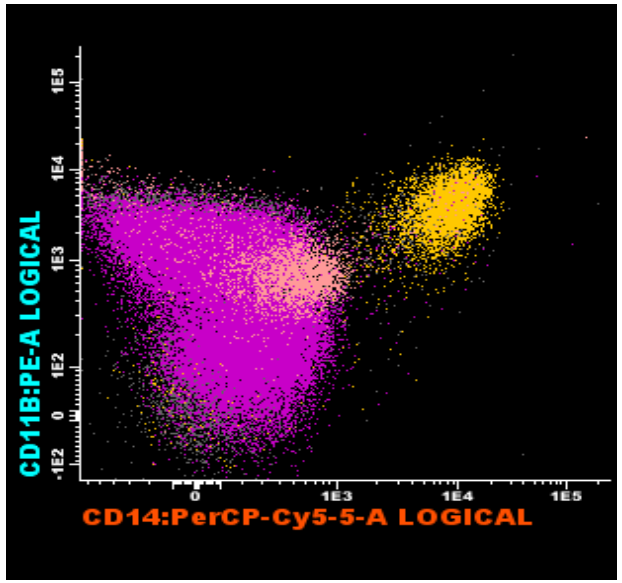


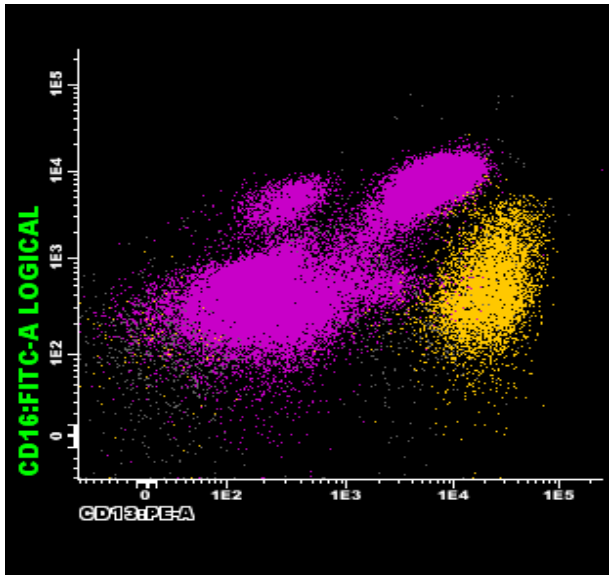
Population	Events	Total %	Partial %
EVENTS	100000	100.00	NA
Other EVENTS	1263	1.26	1.26
NUCLEATED CELLS	87654	87.66	87.66
LEUCOCYTES	87654	87.66	100.00
LYMPHOCYTES	17615	17.61	20.10
Other LYMPHOCYTES	17615	17.61	100.00
EOSINOPHILS	2686	2.69	3.06
NEUTROPHILS	62125	62.13	70.88
Other NEUTROPHILS	62125	62.13	100.00
MONOCYTES	5228	5.23	5.96
Other MONOCYTES	5228	5.23	100.00
DEBRIS OR DOUBLETS	11083	11.08	11.08

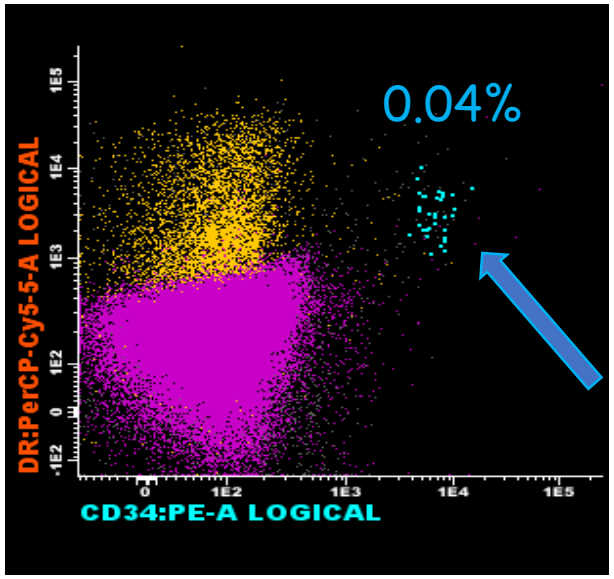
Case Report: The first myeloid flow cytometric panel on peripheral blood (2)



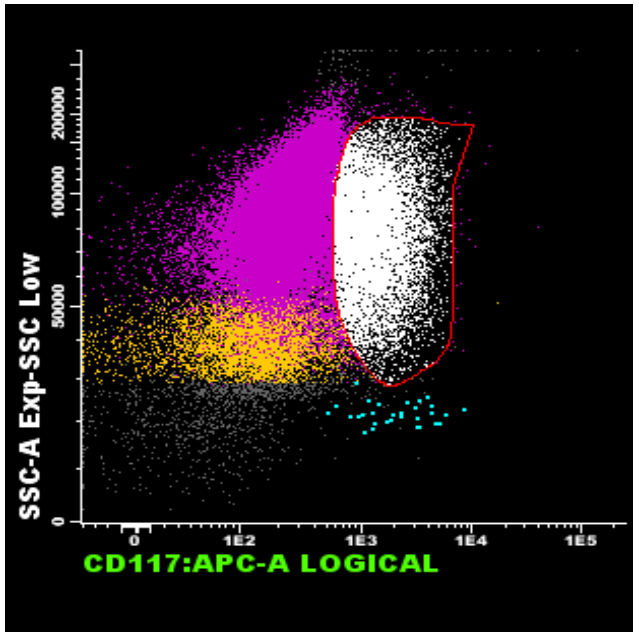


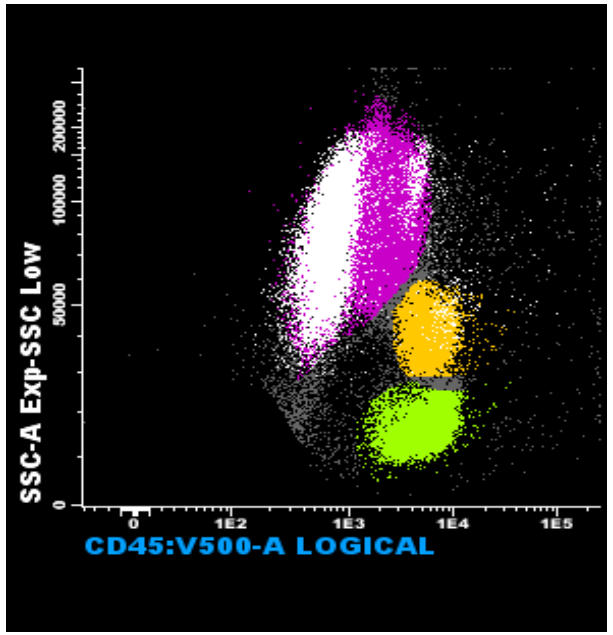


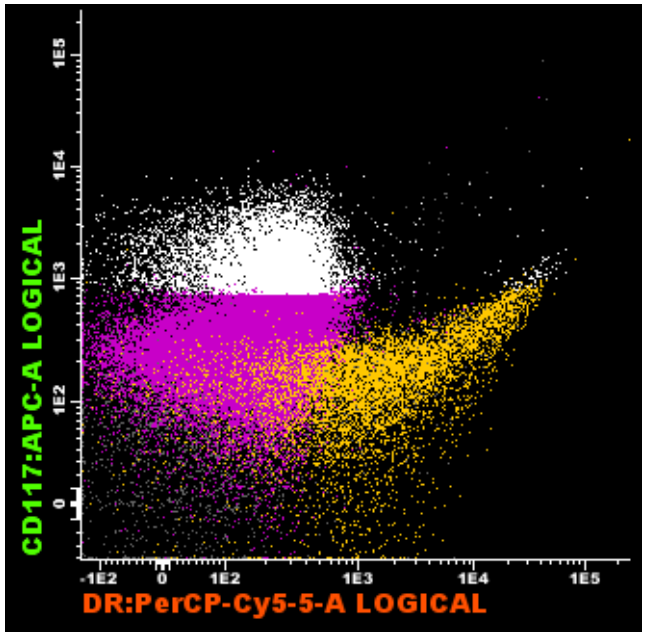


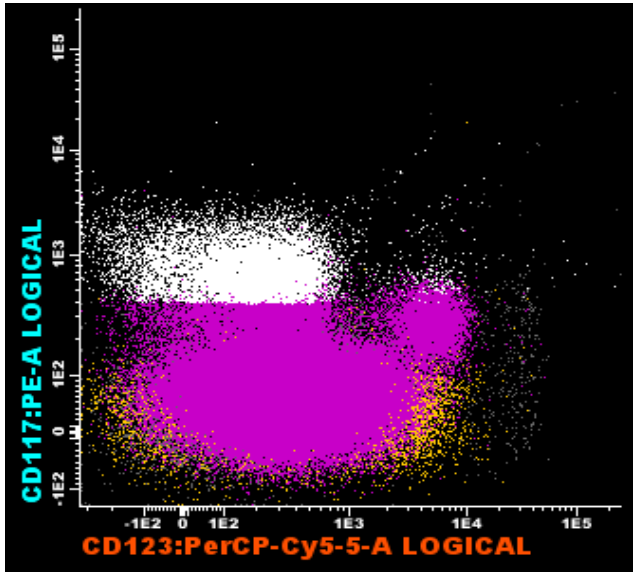


Case Report: The first myeloid flow cytometric panel on peripheral blood (3)









An atypical population **SSC++ CD117+** CD45 dim/negative was detectable (15%), showing negativity for all tested myeloid markers.

3rd Question:

Based on the first myeloid panel on peripheral blood, what is your orientative diagnosis?

a) Acute Myeloid Leukemia

b) Erythroid Acute leukemia

c) Plasma Cell Leukemia

d) Other

Breast Cancer and Myeloma

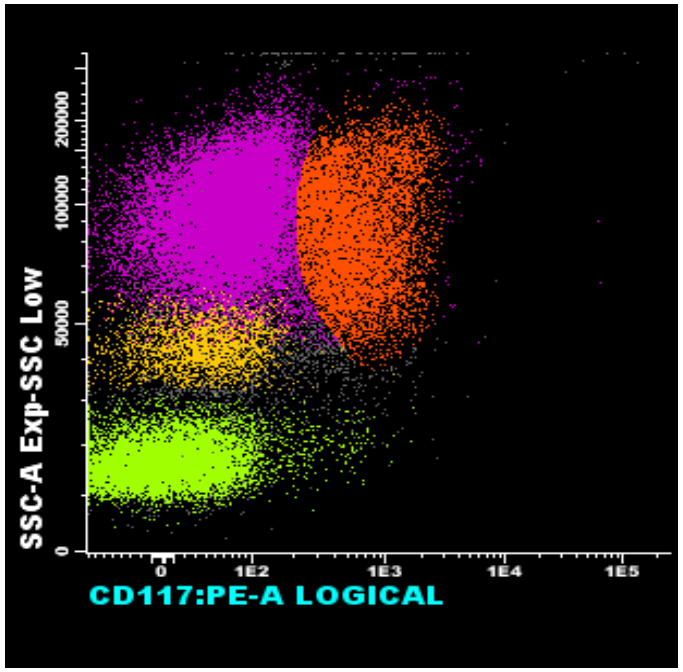
- Multiple Myeloma and metastatic breast disease may show common clinical features (hypercalcemia, anemia and bone lesions above all).

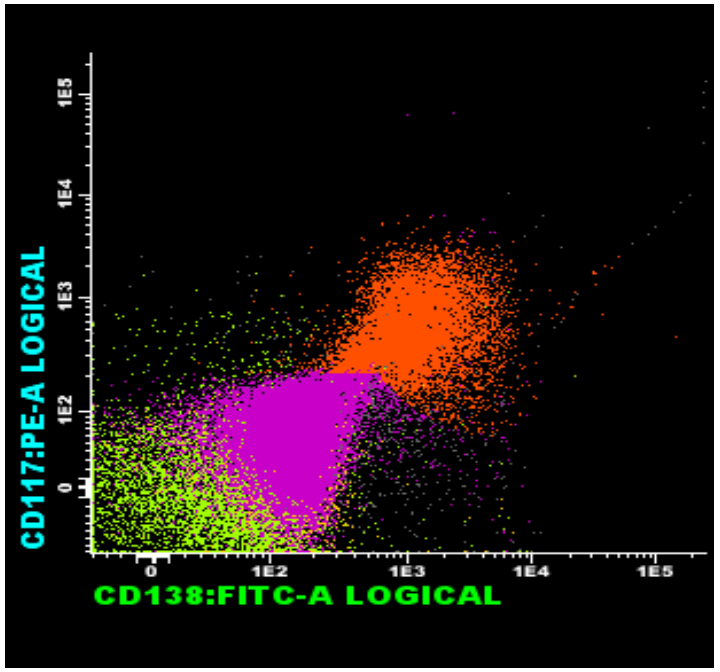
- However, in metastatic breast cancer the bone lesions are **both blastic and lytic**, while myeloma bone lesions are **purely osteolytic**, due to increased osteoclast activity and suppressed osteoblast activity.

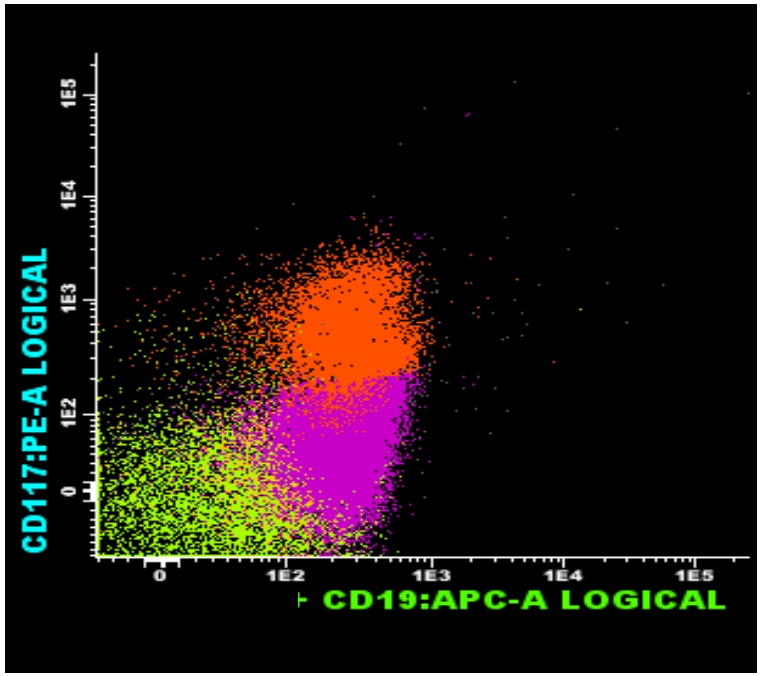
- In the literature a few cases of synchronous MM and breast cancer are reported. In most cases the diagnosis of breast cancer and MM were done separately. The diagnosis of breast cancer usually preceded the MM detection.

Sokołowski M. Current Problems in Cancer 2018; 42(2): 231–234.
Hough B. J Oncol 2010; 2010: 509530.

Case Report: The first peripheral blood panel was extended including MM markers

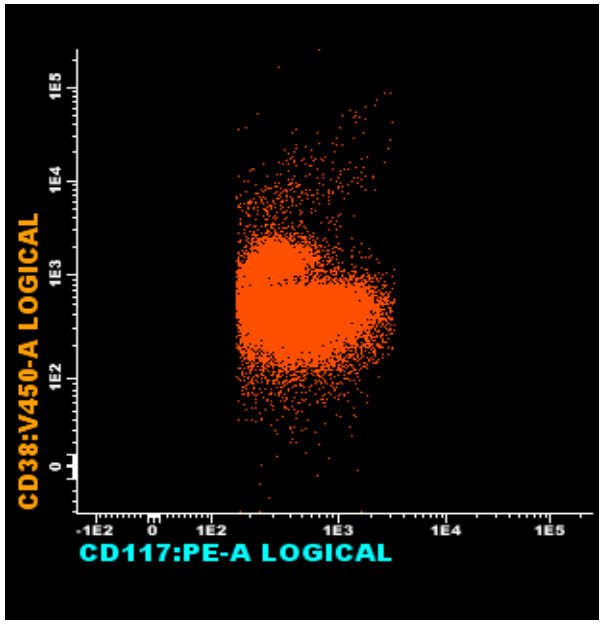


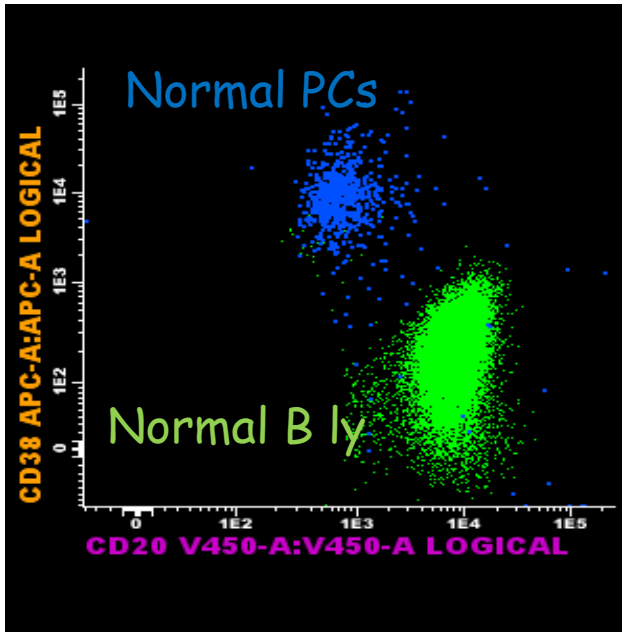


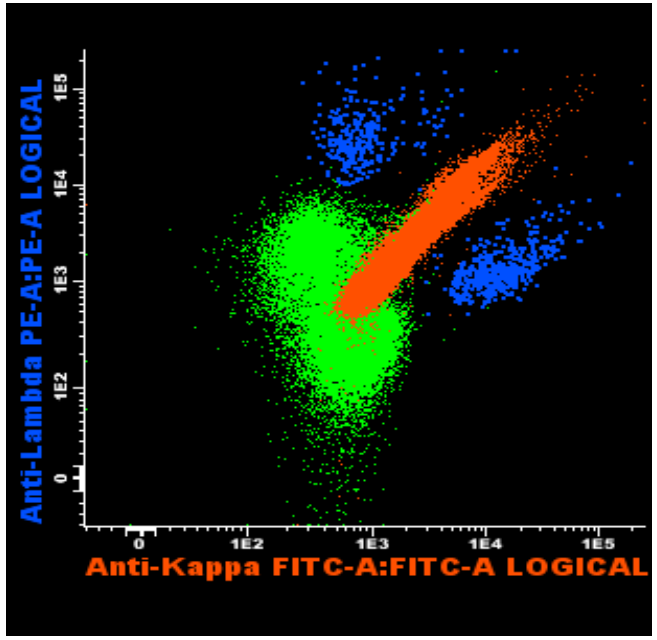


An atypical population CD117+ CD138+ CD19- was detected

Case Report: The first peripheral blood panel was extended including MM markers



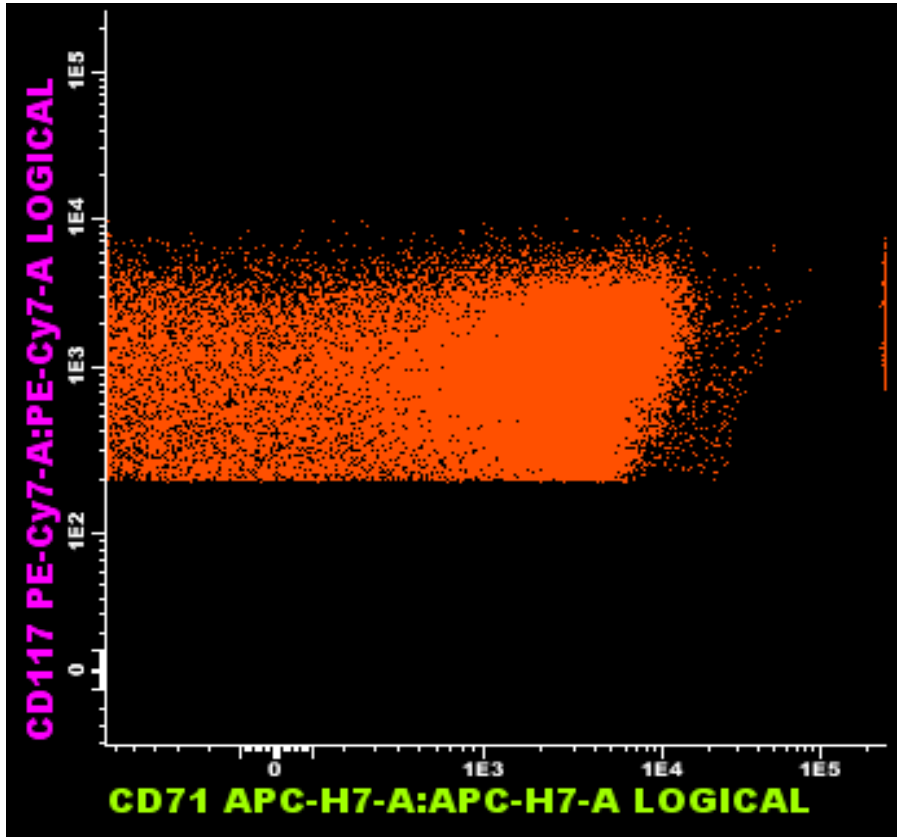




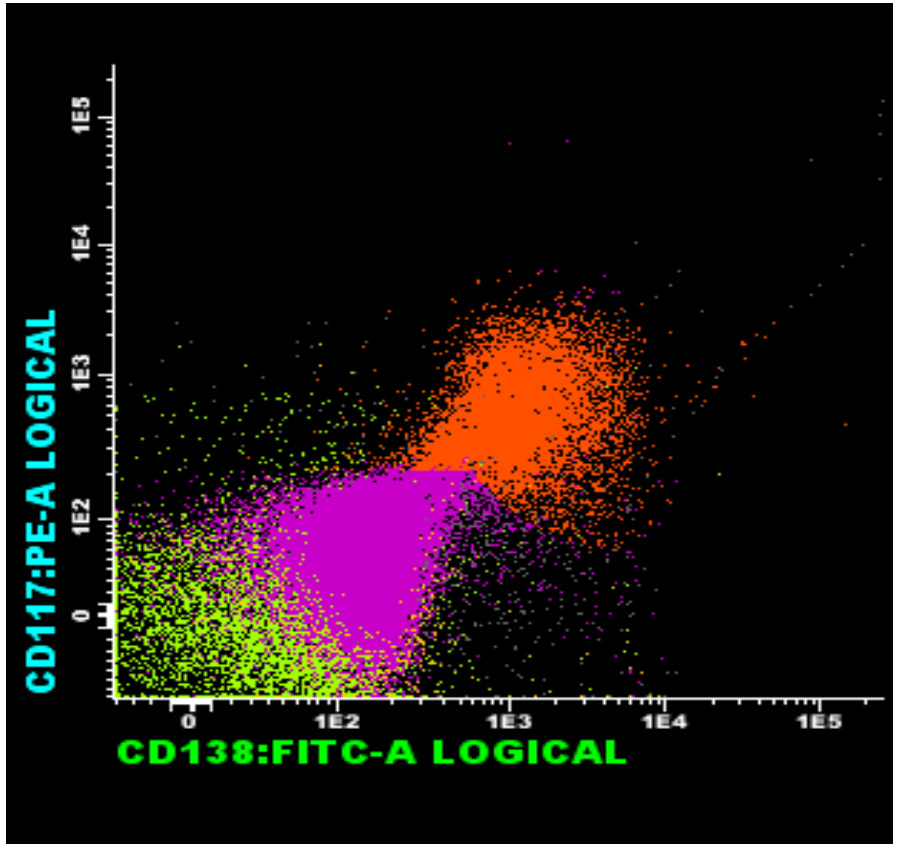
The atypical population was **CD117+ CD138+ CD19-**

but also CD38- cyKappa- cyLambda-

Erythroid markers



Plasma Cell markers



Atypical population:

CD117+ CD138+ CD19- CD38- CD71+ SSC++ CD45 dim/negative

4th Question

Based on the extended peripheral blood panel, which is your diagnosis?

a) Acute Myeloid Leukemia

b) Erythroid Acute leukemia

b) Plasma Cell Leukemia

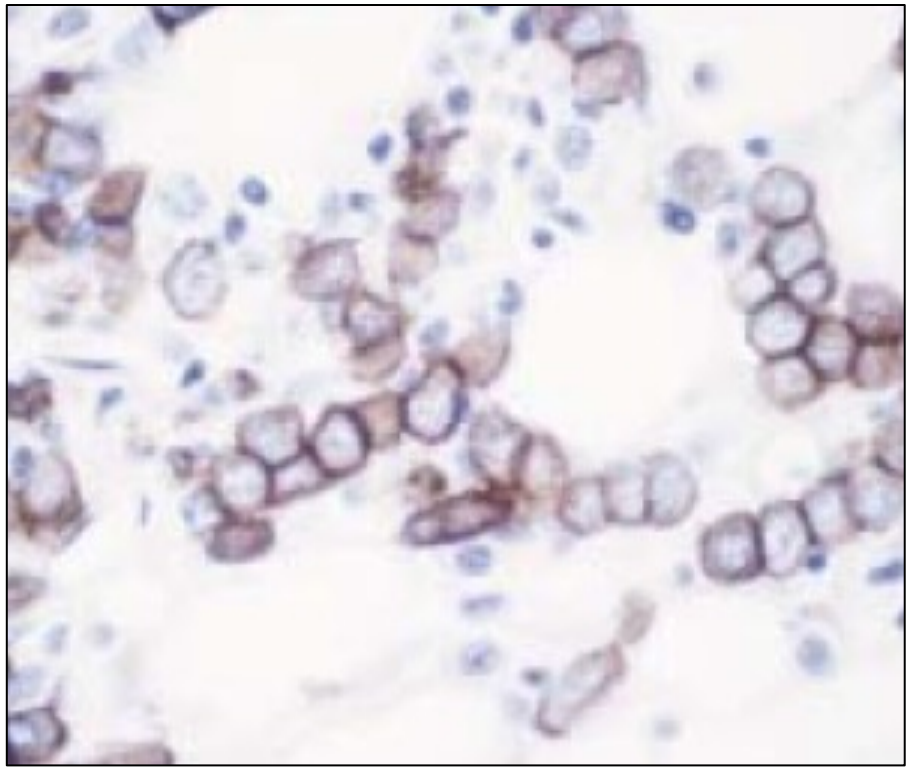
c) Other

CD138: Immunohistochemical Profile in Hematopoietic and Non-Hematopoietic Neoplasms

- CD138 is used to identify and quantitate normal and neoplastic plasma cells.
- Expression of CD138 is also typically observed on the surface of mature epithelial cells (squamous and transition types).
- In breast carcinomas, a weak CD138 expression is reported on malignant ductal cells, associated with extensive positive stromal staining.
- CD138 positivity has been also reported in a variety of soft tissue tumors.
- Because CD138 interacts with heparin binding growth factors, such as fibroblast growth factor-2, its accumulation in tumor stroma might contribute to angiogenesis, stromal proliferation, and tumor pathogenesis.
- CD138 expression is not observed in the stroma of normal breast tissue or benign stromal-epithelial neoplasms

O'Connell FP. Am J Clin Pathol 2004; 121: 254-263

CD138 : Immunohistochemical Profile in Non hematopoietic Neoplasms



Metastatic lobular carcinoma of breast in bone marrow biopsy specimen. Tumor cells exhibit plasmacytoid morphologic features and reveal strong membrane reactivity for CD138.

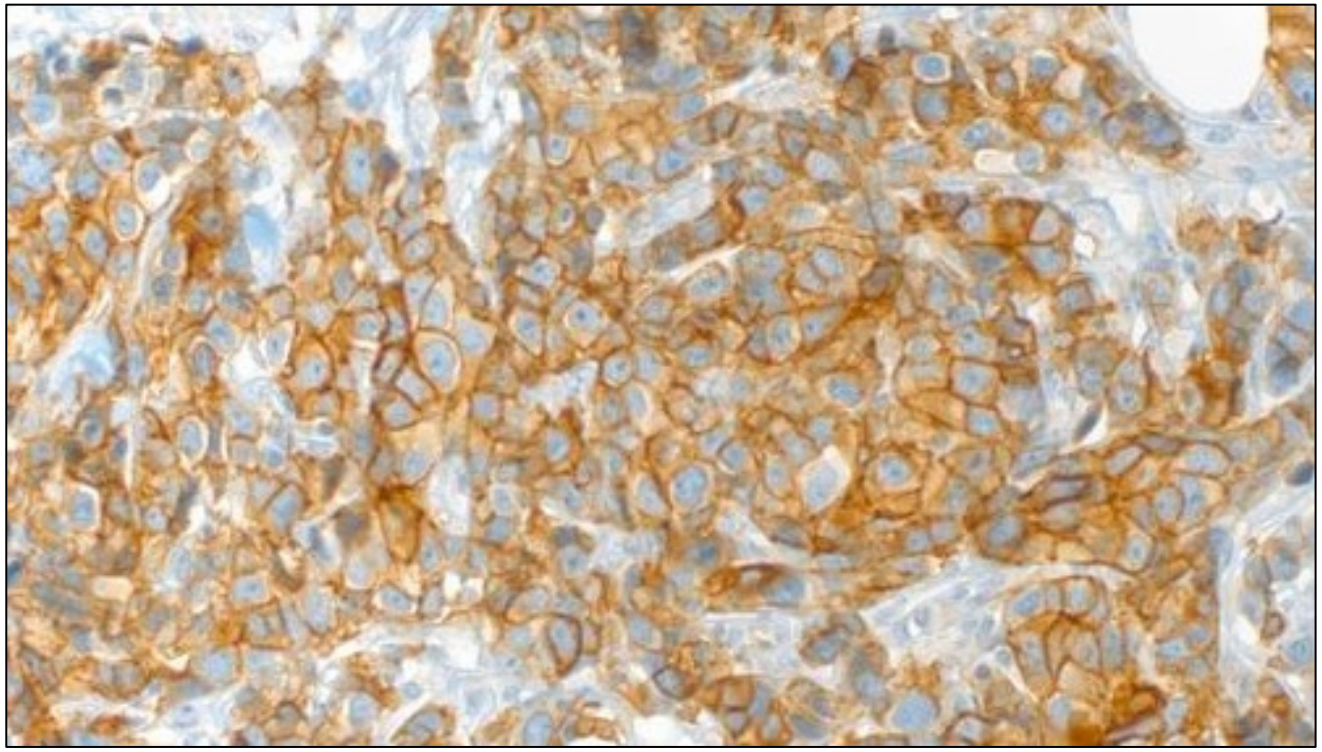
O'Connell FP. Am J Clin Pathol 2004; 121: 254-263

KIT (CD117): Expression in Normal and Neoplastic Tissues

- CD117 (KIT) is a type III tyrosine kinase receptor mediating cell signal transduction in several cell types.
- KIT-dependent cell types include mast cells, some hematopoietic stem cells, germ cells, melanocytes and erythroid precursors.
- Other KIT-positive normal cells include epithelial cells and subsets of cerebellar neurons.
- KIT is expressed in pulmonary and other small cell carcinomas, adenoid cystic carcinoma, renal chromophobe carcinoma, thymic, and some ovarian and few breast carcinomas.

Markku M. Applied Immunohistochemistry & Molecular Morphology 2005; 13(3): 205-220.

KIT (CD117): Expression in Neoplastic Tissues



Triple-negative breast cancer with positivity for CD117.

About 15% of all breast cancers were found to be positive for CD117.

CD117 was significantly higher expressed in distant metastatic and node-positive breast cancers.

Schulmeyer CE. Diagnostics 2023; 13: 372.

5th Question

What markers can be expressed on non-hematopoietic neoplasms?

a) CD56, CD117 or CD138, EpCAM

b) CD45, CD56, CD38, CD117

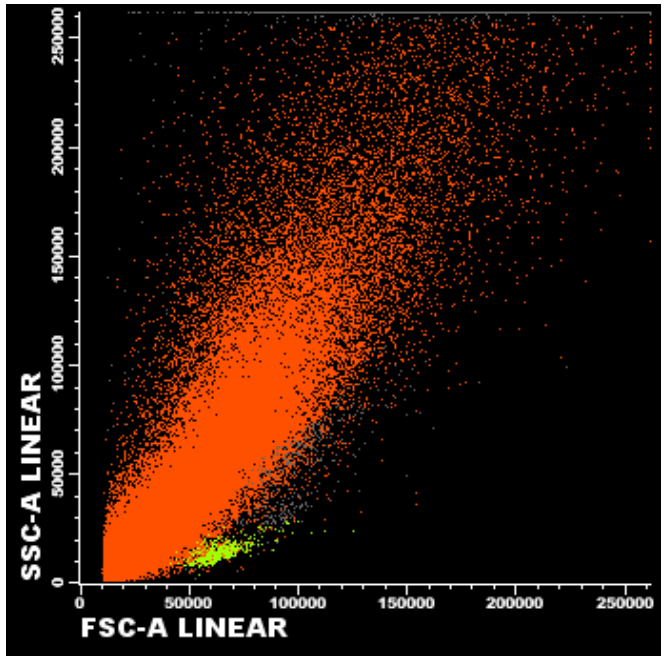
c) CD45, CD56, EpCAM

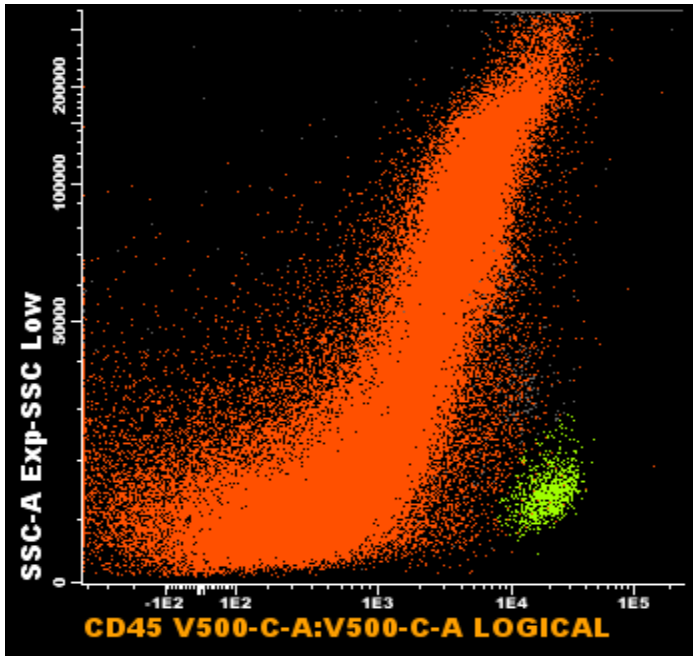
d) CD56, CD117, CD138, CD9

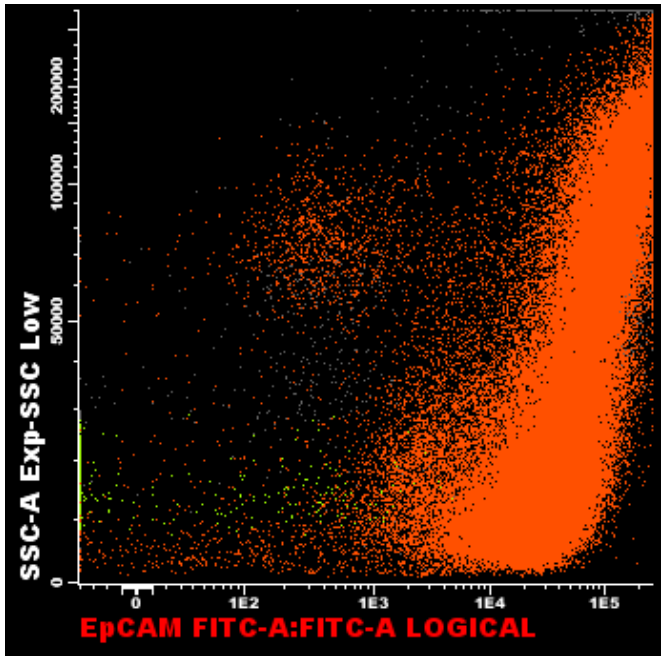
EpCAM (CD326): Expression in Non Hematopoietic Neoplasms

- Epithelial cell adhesion molecule (EpCAM) is a transmembrane cell surface glycoprotein that is highly expressed in epithelial cancers and at lower levels in normal epithelia.
- Due to its frequent overexpression in carcinomas, EpCAM has been used as a diagnostic marker.
- It is expressed in various epithelial cancers, such as breast cancer (ductal type more frequently than lobular type), upper digestive and respiratory tract, gastrointestinal cancer, cancers of the genital and urinary tract, neuroendocrine tumors, and some types of soft tissue sarcomas.
- Malignant melanoma and glioblastoma do not express EpCAM.
- EpCAM is not expressed on normal or neoplastic mesothelial cells.

Fine needle aspiration of a lymph node with epithelial metastases (EpCAM+)







An example of massive infiltration of carcinoma cells in a lymph node.

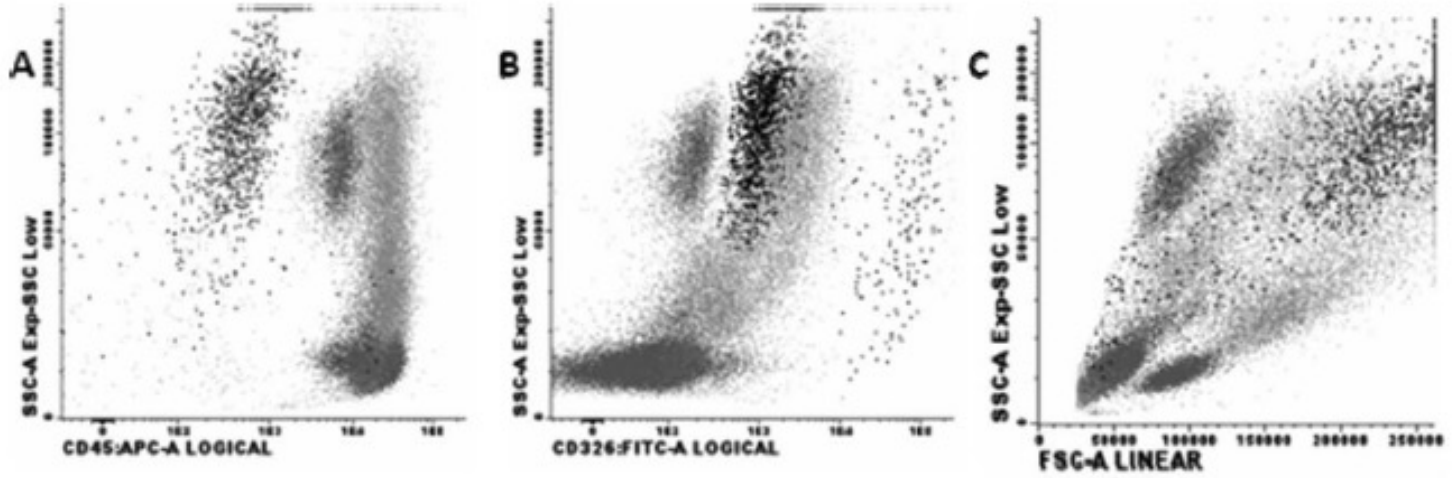
Neoplastic cells were CD45 negative, with strong positivity for CD326, high SSC and high FSC.

Cytometry Part B (Clinical Cytometry) 90B:289–294 (2016)

Original Article

Screening of Carcinoma Metastasis by Flow Cytometry: A Study of 238 Cases

Maria Acosta,* José Pereira, and Maria Arroz
Department of Clinical Pathology, Cytometry Laboratory, CHLO, Hospital S. Francisco Xavier, Estrada do Forte do Alto do Duque, Lisbon 1495-005, Portugal



Scanty infiltration (0.2%) of carcinoma cells in a pleural effusion with concomitant **mesothelial cells** (1.9%).

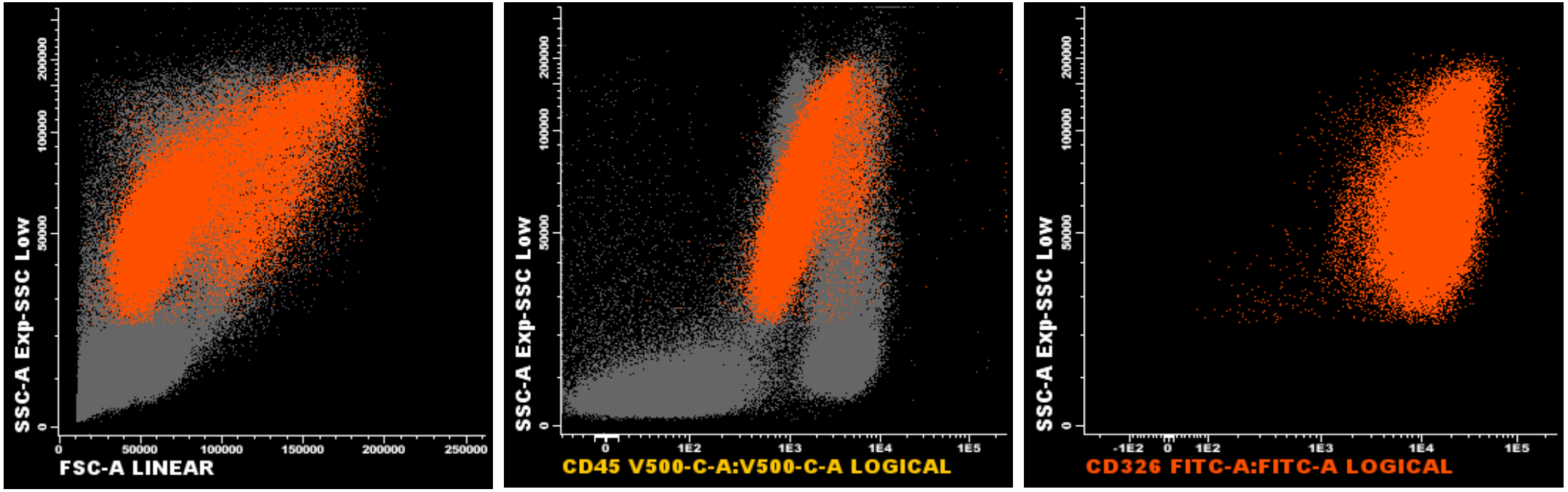
Table 1
Comparison of FCM Results With the Final Diagnosis

		FCM	
		Negative	Positive
Final Clinical Diagnosis	Without EN	146 (TN)	1 (FP)
	With EN	5 (FN)	86 (TP)

TP-True Positive, FP- False Positive, TN- True Negative, FN- False Negative, EN - Epithelial Neoplasia.

FCM has a good sensitivity (96.7%) and specificity (99.3%)

Case Report: CD326 Staining of Bone Marrow Cells



Atypical cells in the bone marrow were CD45 dim/negative, with strong positivity for CD326, high SSC and high FSC.

6th Question

Which is your final diagnosis?

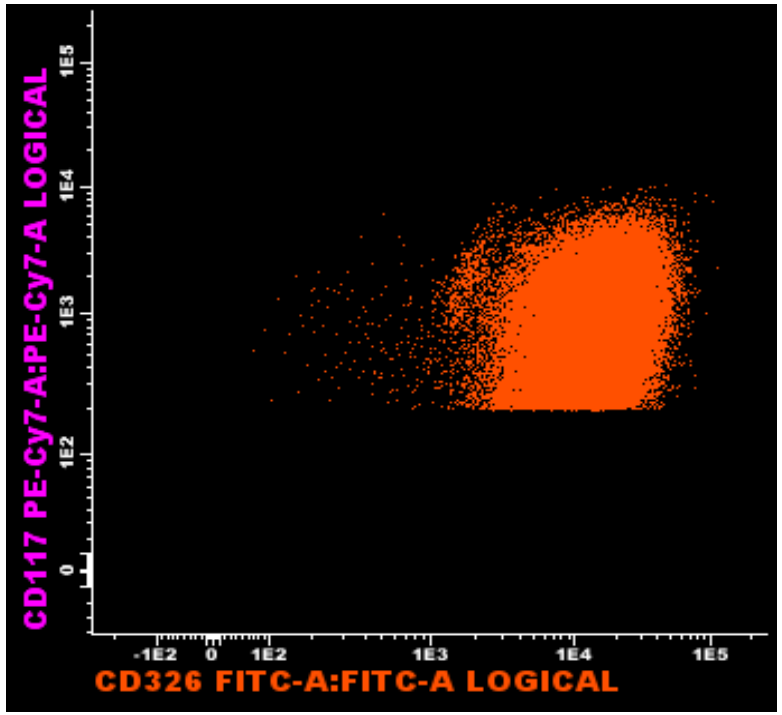
a) Acute Myeloid Leukemia

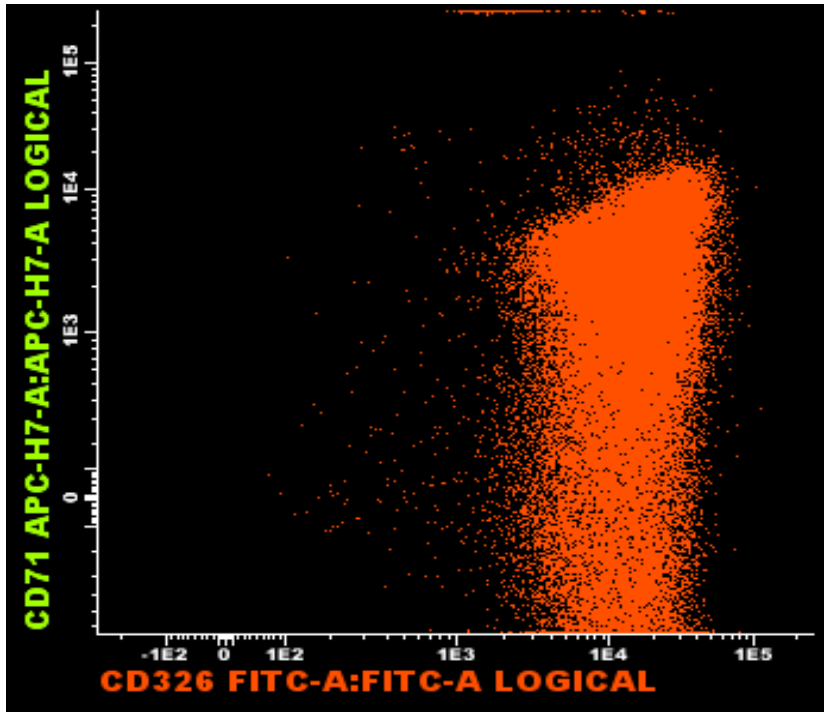
b) Erythroid Acute leukemia

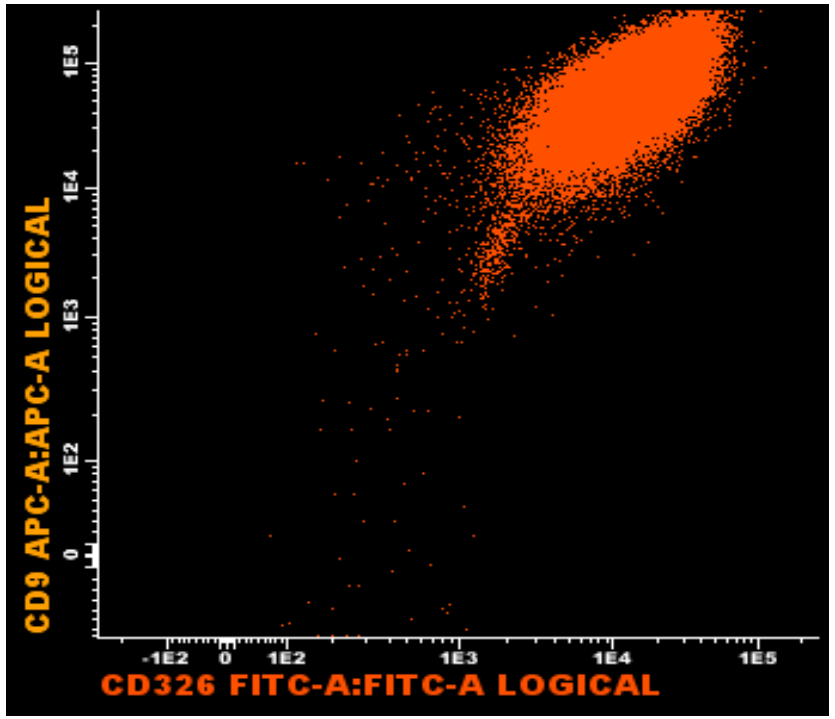
c) Plasma Cell Leukemia

d) Non-hematological neoplasm (BM infiltration of breast cancer cells)

Case Report: CD326 Expression Associated to Other Leukocyte Markers







The refined phenotype of the abnormal cell was:
CD71+ CD117+ CD138+ CD9++ and EpCAM+++

The utility of flow cytometry in the diagnostic workup of malignant effusions due to non-hematopoietic neoplasms

Phenotypic profiles in diverse non-hematological neoplasms

	CD45	EpCAM	CD10	CD56	CD71	CD81	CD9
Most carcinomas	–	+	–	–	–	–	–
Non-small cell lung carcinoma	–	+	–	–/+	–/+	–/+	–/+
Ovarian serous carcinoma	–	+	–	–/+	–	–	–/+
Breast carcinoma	–	+	–/+	–	–/+	+/–	+/–
Pancreatic carcinoma	–	+	–	–	–	–/+	–/+
Peritoneal serous carcinoma	–	+	–	–	–	–	–/+
Small cell lung cancer	–	+	–	–/+	–	–	–
Urothelial carcinoma	–	+	–	–	–	–	–/+
Primary lung sarcoma	–	–	–	+	–	–	+

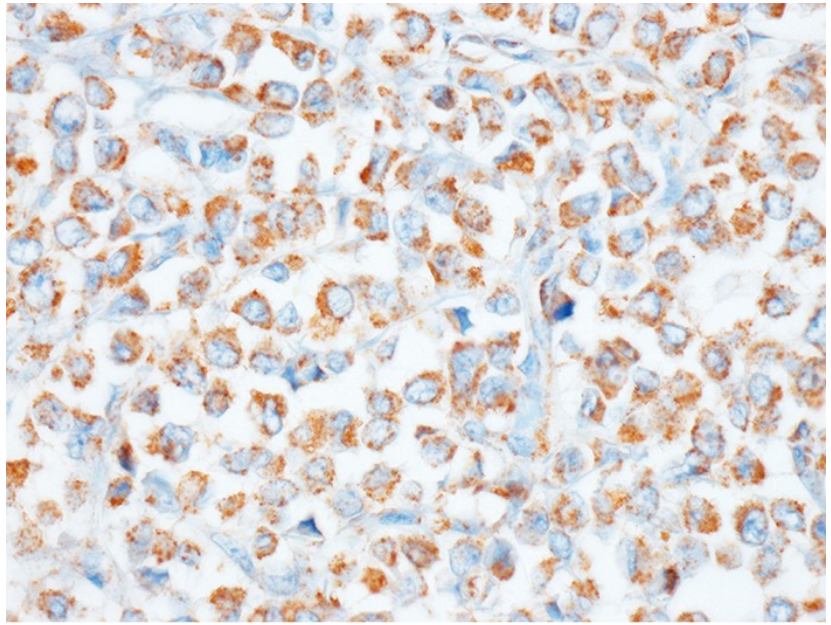
Wong-Arteta J. Cytometry 2020; 98: 504-515.

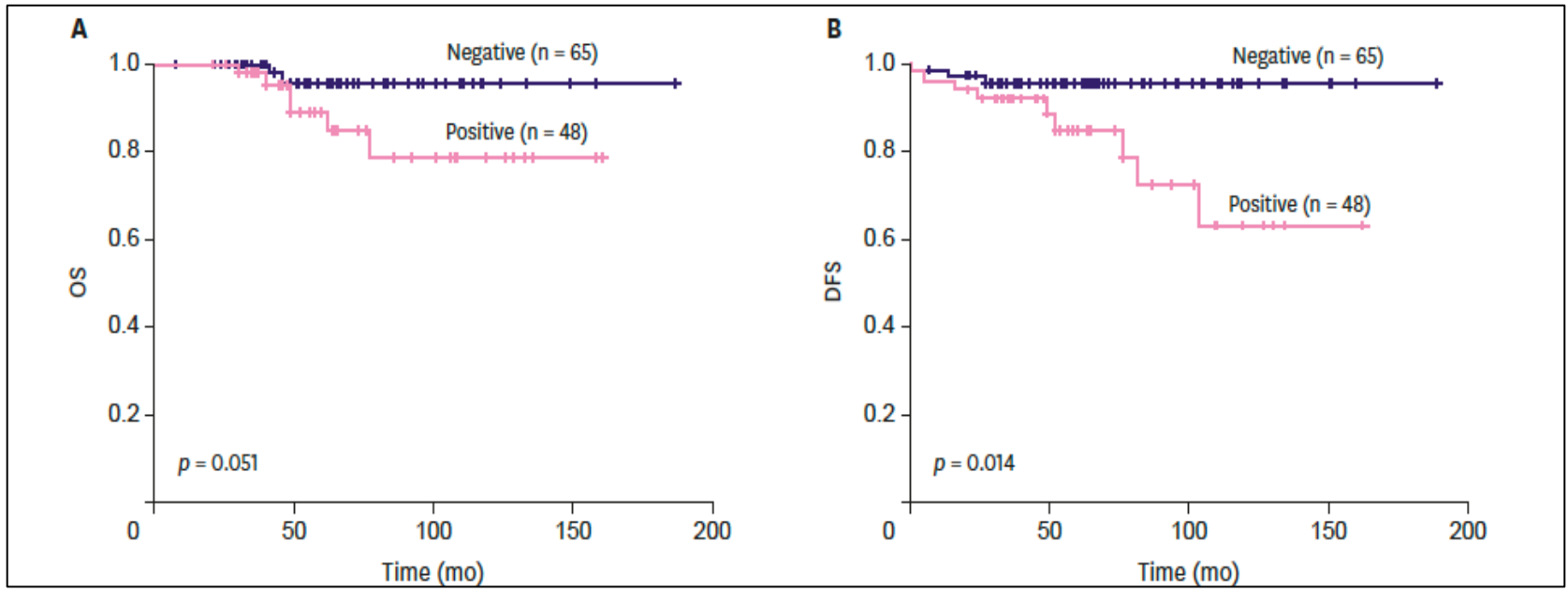
CD9 Expression in Tumor Cells

- CD9 is involved in a variety of biological processes such as cell adhesion, motility, and proliferation due to its interactions with integrins, growth factor receptors, transmembrane proteins, and signaling molecules.
- CD9 is widely expressed in various normal and cancer tissues.
- Different studies have reported conflicting results on the prognostic value of CD9 expression in various types of cancer.
- Non-neoplastic mammary epithelial cells were observed to be negative or weakly positive for CD9 expression, while the stromal inflammatory cells exhibited moderate-to-strong immunoreactivity for CD9.

Baek J. J Breast Cancer 2019 Mar; 22(1): 77-85

CD9 Expression in Tumor Cells is Associated with Poor Prognosis in Patients with Invasive Lobular Carcinoma (ILC)



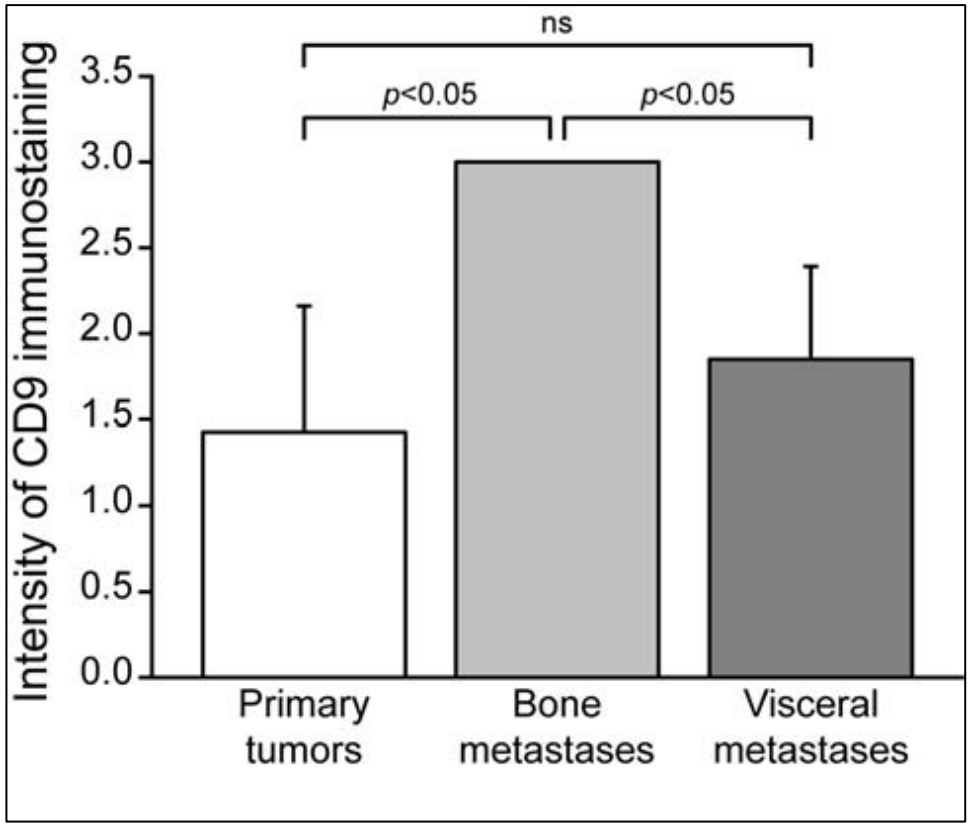


Diffuse and strong CD9 expression in tumor cells

CD9 expression in tumor cells could be a significant prognostic marker in patients with ILC.

Baek J. J Breast Cancer 2019 Mar; 22(1): 77-85

Overexpression of CD9 in Human Breast Cancer Cells Promotes the Development of Bone Metastases

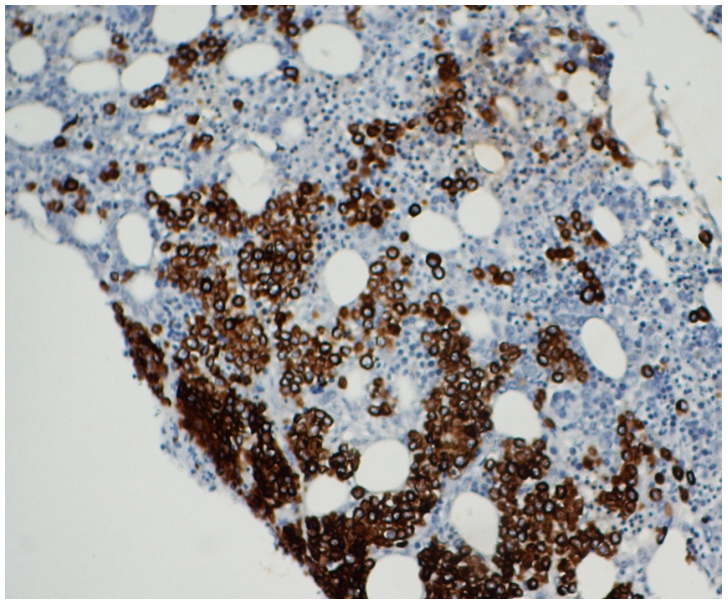


Intensity of CD9 immunostaining in primary tumors, bone metastases and visceral metastases.

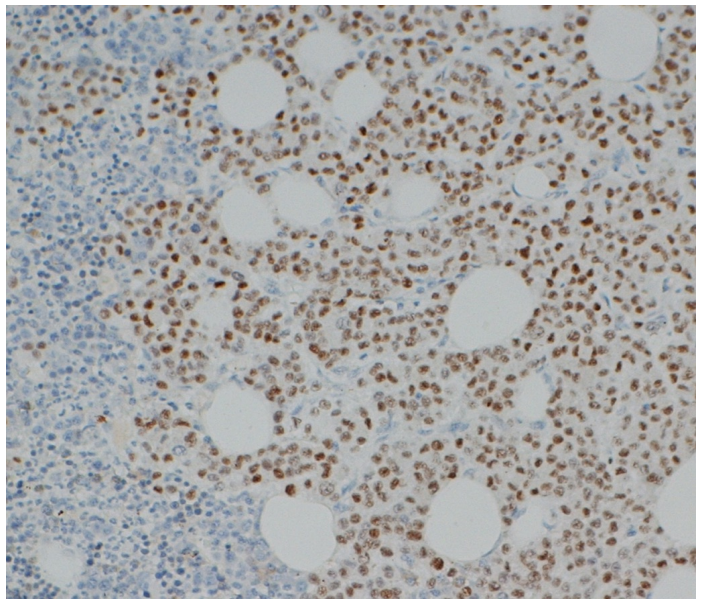
Higher levels of CD9 expression were detected in bone metastases than in visceral metastases or primary tumors.

Kischel P. Anticancer Research 2012; 32: 5211-5220

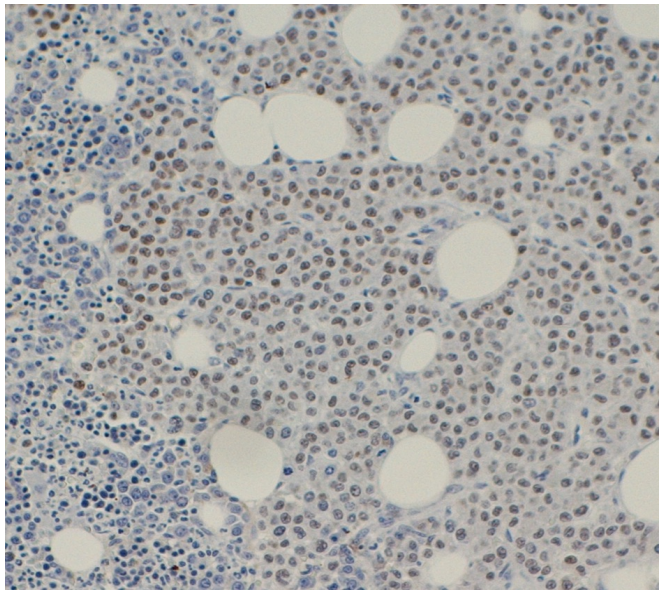
Case Report: Immunohistochemistry on bone marrow



Cytokeratin +



Estrogen Receptor +



GATA-binding protein 3 (GATA3)

Immunohistochemistry on bone marrow confirmed the massive infiltration of breast cancer cells, allowing to diagnose metastatic breast cancer with bone marrow involvement.

CONCLUSION: Flow Cytometry and Non-Hematopoietic Neoplasms

- Rare tumor cells can be sometimes detected in the peripheral blood of cancer patients, usually at very low concentration.

- Many studies have shown that the number of circulating tumor cells can be correlated with disease progression and poor prognosis in various carcinomas.

- Few papers report cases in which malignant tumor cells were detected in peripheral blood by conventional flow cytometry.

- In this case the flow cytometric analysis allowed to characterize the non-hematological nature of this abnormal population, circulating at unusually high level in the peripheral blood (15%).

CONCLUSION: Flow Cytometry and Non hematopoietic Neoplasms (non-HN)

- In non hematopoietic neoplasms Flow Cytometry may be used as a useful ancillary test for cytological examination, providing faster results when compared to immunohistochemistry.
- The combined use of both techniques improves the overall diagnostic accuracy.
- It is important to inspect carefully the events falling into the CD45-negative region and their behavior with the conjugated antibodies, to identify possible non-HN populations.
- Due to the possible coexistence of hematopoietic and non hematopoietic neoplasms, the visual cytomorphological analysis of bone marrow and peripheral blood smears is always recommended.
- Clear guidelines on this matter are however lacking.

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