Simultaneous diagnosis of CLL and CML in a single patient with evidence for two different cell clones

Bonnet C.[1], Menten-Dedoyart C.[2], Lambert F.[3], Gothot A.[4], Baron F.[1,2], Caers J.[1,2], Herens C.[3], and Beguin Y.[1,2]
(1): Clinical Hematology; (2): GIGA-I³ ; (3): Human Genetics; and (4): Laboratory-Hematology,
University and University Hospital of Liège, Liège, BELGIUM.

1. CASE REPORT:

Co-occurrence of two chronic-stage myeloid and lymphoid haematological malignancies is a rare phenomenon reported in ≤1% of patients. Here we report the case of a 74-years-old man presenting concomitantly with both Philadelphia positive chronic myeloid leukemia (Phi+ CML) and B-cell chronic lymphocytic leukemia (B-CLL). We aimed to know if two independent clones were present or if a common origin could be demonstrated for these two diseases. CLL cells and granulocytes were purified using a combination of gradient density centrifugation and/or fluorescence-activated cell sorting (FACS) and further analyzed using FACS, fluorescent *in situ* hybridization (FISH) and molecular assays. The presence of the *BCR-ABL1* fusion gene (FG) and the 13q14 deletion (del13q) were evaluated by FISH allowing to study the clonal involvement of both myeloïd and lymphoïd lineages. With this approach we were able to show that two independent clones were simultaneously coexisting in this patient.

2. AUTOMATED FULL BLOOD COUNT :

Haemoglobin: 12.3 gr%

Platelets: 455 x 10^9/L
White blood cell count: 85 x 10^9/L
Neutrophils: 43.8 x 10^9 /L
Lymphocytes: 32.7 x 10^9 /L
Activated Lymphocytes: 1.28 x 10^9 /L
Basophils: 0.85 x 10^9 /L
Myelocytes: 0.85 x 10^9 /L
Metamyelocytes: 4.68 x 10^9 /L
Myeloblasts: 0.85 x 10^9 /L

→ **Myeloproliferative neoplasm ?**

" presence of some GUMPRECHT shadows"

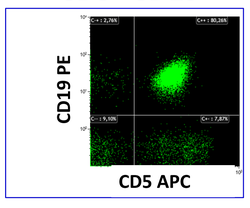
LDH: 1,031 IU/L

→ **Chronic Lymphocytic Leukemia?**

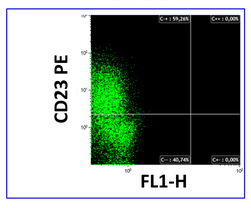
3. FLOW CYTOMETRY ANALYSIS:

Monoclonal B cell population ($CD19^+$, $CD20^{low}$, $CD5^+$, $CD23^{partial}$, S.Ig. K^+, $CD22^-$, $FMC7^-$) ⇔ Matutes score: 5 points

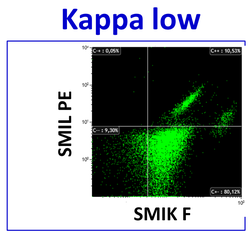
$CD5^+$ $CD19^+$



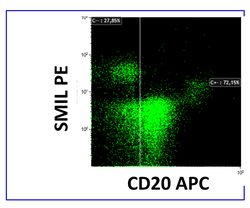
$CD23^+$



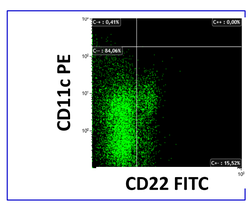
Kappa low



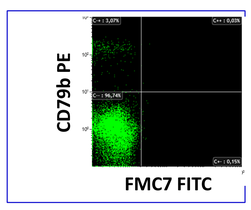
$CD20^{low}$



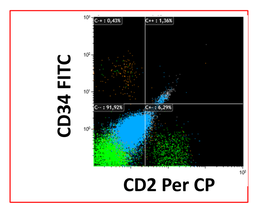
$CD22^{low}$



$FMC7^-$ $CD79b^-$



No CD34+/CD117+ cell population



CONCUSION (I):
Simultaneous diagnosis of a chronic phase CML & CLL.

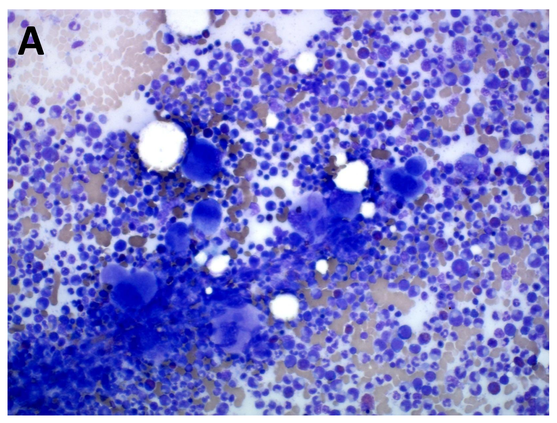
→ **Did the two diseases originated from the same clone?**

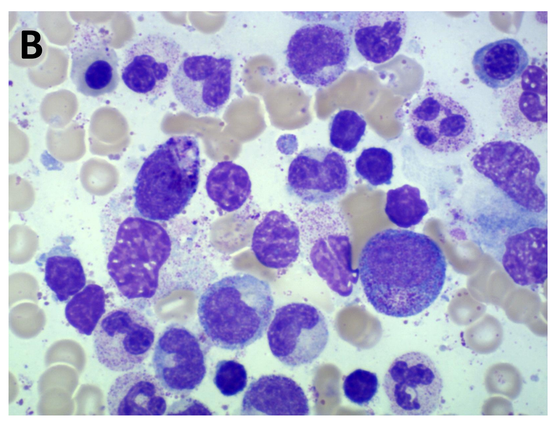
6. FISH RESULTS ON PURIFIED CELL FRACTIONS:

On the blood sample, 41% of the nuclei were characterized by a *BCR-ABL1* rearrangement and 46% by a deletion of the 13q14 locus (D13S319 probe).
CD5+/CD19+ cells were sorted by FACS (> 95% purity) whereas the granulocytes were isolated by combining density gradient centrifugation and FACS (>90% purity). FISH were then subsequently performed on the cell sorted fractions and clearly show that CML and CLL are two distinct diseases in this patient. No *BCR-ABL1* rearrangement was found within the B-lymphocytes fraction (200 nuclei observed) while 98% of the cells showed a deletion of the 13q14 locus. Conversely, within the granulocytes fraction, 95% of the nuclei were characterized by a *BCR-ABL1* rearrangement while only four nuclei were found deleted for 13q14.

	Granulocyte fraction				B-lymphocyte fraction			
FISH probe	BCR-ABL ES		D13S319		BCR-ABL ES		D13S319	
FISH Result	Normal nuclei (%)	Positive nuclei (%)	Normal nuclei (%)	Positive nuclei (%)	Normal nuclei (%)	Positive nuclei (%)	Normal nuclei (%)	Positive nuclei (%)
	5	95	96	4	100	-	2	98

4. BONE MARROW SMEAR :





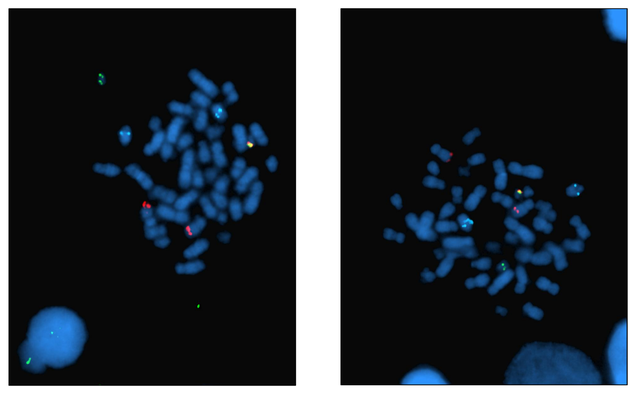
May-Grunwald-Giemsa stain, magnification x 10 (A) & x100 (B)

5. CYTOGENETICS AND MOLECULAR STUDIES:

Karyotype, FISH and molecular studies were performed on blood and bone marrow samples from this patient.

Sample		*Blood*	*Bone Marrow*
Date of sample :		20/06/2012	04/07/2012
Type of culture :		MTX synchronisation	MTX synchronisation or DSP30-IL2 stimulation
Cytogenetic results :		45,X,-Y,t(9 ;22)(q34 ;q11) [20]	45,X,-Y,t(9 ;22)(q34 ;q11) [20]*
FISH results :	BCR-ABL ES probe	Positive in 41% of the nuclei	/
	D13S319 (13q14) + LAMP (13q34)	Deleted in 46% of the nuclei	/
	D12Z3 (c12 centromere)	Normal	/
	ATM (11q22.3)	Deleted in 21% of the nuclei	/
	TP53 (17p13)	Normal	/

*: Only six metaphases were observed and analyzed after a DSP30-IL2 stimulated culture; all of them showed the t(9;22)(q34;q11) translocation.
MTX: methotrexate; DSP30: CpG- oligonucleotide promoting the proliferation of B-lymphocytes; IL2: interleukin-2.
DNA and total RNA and were extracted from bone marrow. RT-PCR showed the presence of a P210 (e13a2) *BCR-ABL1* chimeric transcript whereas genomic PCR demonstrated a clonal heavy chain immunoglobulin gene rearrangement.



All the mitoses with the Philadelphia chromosome show a *BCR-ABL1* rearrangement (1G,2R,1Y signal pattern) and 2 normal chromosomes 13 (no deletion of the 13q14 locus, [in blue]).
Red : ABL probe (9q34) - Green : BCR probe (22q11) - Blue : D13S319 probe (13q14)

7. CONCLUSION (II):

We conclude that the patient presented with two different diseases originating from two different clones.

8. FOLLOW-UP AND PERSPECTIVES:

The patient, who was first treated with hydroxyurea (for 10 weeks) and later with imatinib (for 3 months thus far), experienced a rapid hematologic remission of his CML component, but with persistence of lymphocytes with Gumprecht shadows. A similar case report has been recently reported (*D'Arena et al., JCO, Vol 30, 2012*) but in this case the CML occurred 7 years after CLL diagnosis. Further follow-up of our case is required to see if the targeted therapy of CML with imatinib will work well and, like it was previously reported for one patient (*J. Yoon et al., Leukemia Research, 2011*), if such therapy will also acts on the CLL cells through decreasing phosphorylation of STAT3 and mcl-1 level with induction of CLL cells apoptosis.

UNIVERSITÉ de Liège — GIGA — CHU de Liège