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A case of SRSF2 mutation in chronic lymphocytic leukemia

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ABSTRACT

Chronic lymphocytic leukemia (CLL) is characterized by extremely variable clinical course indicating substantial differences in the biology of the disease. Molecular characterization provides new insights useful for treatment decision making. We report on a patient diagnosed with CLL, whose disease was characterized by episodes of rapid progression and disease stabilization, and in which a *SRSF2* gene mutation was identified in the absence of other commonly known mutations of CLL. To the best of our knowledge this is the first case of *SRSF2* gene mutation ever reported in CLL.

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Chronic lymphocytic leukemia (CLL) is the most frequent hematologic neoplasm in Western countries. The clinical course of chronic lymphocytic leukemia ranges from a very indolent disorder with a normal lifespan, to a rapidly progressive disease leading to death. The variable clinical course of CLL is driven, at least in part, by the disease molecular heterogeneity. This makes it difficult to select treatment choice and estimate survival [1]. Most common chromosome abnormalities of CLL are deletions (6q, 11q, 13q, or 17p) and less frequently chromosomal gains (trisomy 12). Molecular markers with prognostic impact include inactivating mutations of *TP53*, *NOTCH1*, *SF3B1* and baculoviral IAP repeat containing 3 (*BIRC3*) genes. Molecular genotyping of CLL enhances our understanding of the clinical heterogeneity of the disease and provides new insights useful for treatment decision making [2,3].

Splicing machinery dysfunctions have been associated to CLL, in particular *SF3B1* gene mutations (splicing factor 3B subunit 1) which are present in about 15% of patients [4]. To our knowledge, no *SRSF2* gene mutation has ever been detected in CLL. In order to broaden our knowledge on the frequency of *SRSF2* splicing mutations in CLL we studied systematically peripheral blood samples with a DNA high-resolution melting analysis test [5] in a cohort of 115 patients diagnosed with CLL (Table 1) and found only 1 mutated case.

We report on a patient diagnosed with chronic lymphocytic leukemia (CLL) whose disease was characterized by marked clinical heterogeneity with episodes of rapid progression and disease stabilization and in which a *SRSF2* gene mutation was identified in the absence of other commonly known mutations of CLL.

A female patient aged 55 was observed in December 1995 at Sant' Eugenio hospital in Rome with lymphocytosis. The patient past medical records included: malaria 7 years before, bilateral oophorectomy and hysterectomy due to uterine fibroma, and hypothyroidism under medication with levothyroxine sodium. Laboratory tests disclosed $19{,}7 \times 10^9$ L leukocytes ($15{,}5 \times 10^9$ L lymphocytes), hemoglobin 130 g/L, 218×10^9 L platelets and normal levels of β2-microglobulin and lactate dehydrogenase. The microscopic analysis of blood smear was consistent with a lymphoproliferative process with multiple mature-like small lymphocytes with scant cytoplasm, regular nucleus (dense chromatin without nucleoli) and presence of Gümprecht cells. Flow cytometry analysis showed positivity for: CD5, CD23, CD 19 (LLCr score 5) CD38 and ZAP-70, with restriction to lambda light chain. The IGHV

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Table 1
Clinical characteristics.

Parameter	Category	Study population (n = 110)
Gender	Male (%)	57 (51.8%)
Age (years old), median (range)		64 (39–83)
RAI stage	0	37 (33.6%)
	I–II	73 (66.4%)
WBC ($\times 10^9$/L), median (range)		19,52 (7,64–270,38)
Lymphocytes ($\times 10^9$/L), median (range)		13,70 (4,20–258,30)
Beta-2-microglobulin	Abnormal high level (%)	28 (25,5%)
Karyotype by fluorescence in situ hybridization (FISH)	Normal	26 (23.6%)
	High risk	38 (34.5%)
	Deletion 13p	46 (41.8%)
Lymphadenopathy/splenomegaly at diagnosis	Presence	37 (33,6%)
Bulky mass	Presence	4 (3,6%)
Lymphocytes doubling time	< 12 months	36 (32,7%)
Necessity of treatment	Yes	81 (73.6%)
First time treatment[a]	FC	53 (48,18%)
	RFC	5 (4,54%)
	RB	10 (9,09%)
	Leukeran + R	3(2,72%)
	Leukeran	8(7,27%)
Days to start treatment, median (range)		977 (7–3935)
Infection grade IV[b]		19 (17,3%)
Response to treatment	Complete remision	50 (61,73%)
	Partial remision	24 (29,63%)
	Failure/progression	1 (1,23%)
	No valuable	6 (7,41%)
Relapse in patients who achieved complete remission		18 (36%)
Days to relapse[c]		1258 (386–2725)

[a] FC – fludarabine + cyclophosfamide. RFC – rituximab + fludarabine + cyclophosfamide. RB – rituximab + bendamustine. R – rituximab.
[b] Median value of the days passed since complete remission after first line therapy until relapse occurred.
[c] All patients presenting infection grade IV received treatment (p = 0.01).

status of the patient was unmutated. Karyotype studies showed del13q. Sequencing analysis of the following genes resulted negative for molecular alterations: *NOTCH1* (Notch homolog 1, translocation-associated [Drosophila]) exon 34, *NRAS* (neuroblastoma RAS viral [v-ras] oncogene homolog) exons 1 and 2, *KRAS* (Kirsten rat sarcoma viral oncogene homolog) exons 1 and 2 and *TP53* (tumor protein 53) exons 3, 4, 5, 6, 7 and 8 (exons 9–11 were excluded due to low mutation frequency). High resolution melting analysis screening for common mutations of *IDH1/2* (isocitrate dehydrogenase 1 and 2 [NADP+]) and *SF3B1* (exons 12–15) genes resulted as well negative. Clinically, the patient presented only one small cervical lymphadenopathy and no systemic symptoms. CLL was diagnosed as Rai stage I (Rai modified) and Binet stage A according to the International Working group CLL criteria.

The patient did not receive treatment until February 1997 (14 months later) when she rapidly progressed presenting splenomegaly and an increased number of lymphadenopathies (Rai II and Binet B), requiring treatment. A course of intravenous fludarabine 25 mg/Kg/day for 5 days every 28 days for a total of 5 cycles was prescribed. In June 1997, treatment outcome assessment showed only partial response (50% reduction of lymphadenopathy).

In December 1998, disease progression was observed with splenomegaly, increased number of lymphadenopathies and rapid increase of blood lymphocytes (lymphocyte doubling time < 12 months). A course of oral chlorambucil 10 mg/day with prednisone 25 mg/day for 10 days every month was given for a total of 9 cycles. Maintenance therapy was started with subcutaneous interferon α-2b using alternating doses of 1.5 MU and 3 MU 3 times / week and the patient remained in partial remission for the following 7 years.

A third disease progression occurred in March 2006 and a course of fludarabine 25 mg/Kg/day was given for a total of 6 cycles, followed by intravenous rituximab 375 mg/Kg every 28 days for a total of 4 cycles. Complete remission was achieved at the end of treatment. An additional cycle of rituximab was administered in June 2010, At present, the patient remains in complete remission with residual neutropenia and thrombocytopenia requiring treatment with low dose of prednisone.

SRSF2 mutation was confirmed by Sanger sequencing of different blood DNA samples from the patient during her last two follow up visits to the hospital in April and June 2014 (Fig. 1). Unfortunately, DNA from disease onset was not available, making it difficult to correlate the clinical behavior of the disease with the presence of *SRSF2* mutation. The anomaly could have been acquired as a late event following different treatment lines or, alternatively, it might have been present at diagnosis in the bulk leukemic population or at sub-clonal level. At diagnosis the patient presented 13q- as a sole genetic abnormality (which has been associated to good prognosis) and overexpression of ZAP-70 which is known to be associated to poor prognosis.

In recent years, there has been growing interest regarding the role of spliceosome machinery in neoplastic diseases following evidence of the involvement of splicing factor mutations in diverse cancers [6]. *SRSF2* (Serine-Arginine Splicing Factor 2) is a member of the serine/arginine (S/A) rich family pre-mRNA splicing factors. *SRSF2* mutations are primarily detected in hematologic diseases, at a reported frequency of 5.5% in refractory anemia with ringed sideroblasts and refractory cytopenia with multilineage dysplasia with ringed sideroblasts, 11.6% in myelodysplastic syndrome (MDS) without ringed sideroblasts, 6.5% in acute myeloblastic leukemia secondary to MDS, 0.7% in de novo acute myeloid leukemia, 1.9% in myeloproliferative neoplasms, 18.8% in secondary acute myeloid leukemia evolving from myeloproliferative neoplasm and 28.4% in chronic myelomonocytic leukemia (CMML) [6,7]. In MDS and CMML, *SRSF2* point mutations mostly occur in exon 1 of the *SRSF2* gene, at amino acid position P95 (95% and 93% of cases respectively). In the remaining minority of cases insertions/deletions and frameshifts can be found around amino acid P95 and also point mutations in other positions such as P96, P84, P85 and P86 [7,8] have been reported. In MDS and CMML, *SRSF2* mutated cases show higher transformation rate, shorter time to progression to acute myeloid leukemia and shorter overall survival compared to unmutated cases [7,9,10]. *SRSF2* mutations are thought to interfere with cellular homeostasis via defective splicing of certain genes such as *RUNX1* (runt-related transcription factor 1 gene) with a loss-of-function effect seen in MDS and CMML [8]. Recent publications have liked *SRSF2* mutations with older male patients with MDS whose clinical course remains stable with no co-occurrence of other mutations [11].

The frequency of other splicing mutations in CLL besides *SF3B1* such as *SRSF2* has been studied recently and it is known that mutations in this gene are uncommon [12–14]. In our study, we found one case of *SRSF2* mutation in a patient with CLL with a very variable clinical course and many relapses. Little can be assumed in this case due to the lack of similar reports in literature, however it is possible that the mutation could have contributed to the worsening of her disease.

Another assumption that arises from this case is that the presence of myelodysplasia secondary to treatment could explain the presence of *SRSF2* mutation in this patient. This brings us to think on many relapsed CLL cases with residual cytopenias where no other molecular abnormalities or disease markers can explain poor clinical outcomes.