

## CASE REPORT

# Association of myelodysplastic syndrome with CD5+, CD23+ monoclonal B-cell lymphocytosis

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## INTRODUCTION

Myelodysplastic syndromes (MDS) are clonal disorders characterized by bone marrow (BM) failure and an increased risk of transformation into acute myeloid leukemia (AML). Typically, MDS patients are elderly and are already anemic, leucopenic and/or thrombocytopenic upon presentation. Despite their myeloid origin, many MDS cases show abnormalities in B-cells, usually related to a decrease in the B-cell production and/or an increase in apoptosis (1,2). Such alterations may be due to an increased production demand for essential hematopoietic cells, such as red cells and neutrophils. Alternatively, a blockade on B-cell maturation might occur (2).

A low level of monoclonal B-cells ( $<5 \times 10^9/L$ ) in otherwise healthy individuals characterizes a condition called monoclonal B-cell lymphocytosis (MBL). The overall reported frequency of MBL is between 0.5% and 12% of adults, depending on the age of the population and the sensitivity of the flow cytometry approach used to detect the B-cell clones (3,4). MBL is more frequently observed among the relatives of patients with familial chronic lymphocytic leukemia (CLL) and in individuals exposed to toxic environments. Although coexistence of CLL and AML or CML has been sporadically reported, the actual frequency of MBL in association with other hematological neoplasias remains to be established.

## CASE DESCRIPTION

Here, we report a 61-year-old female patient diagnosed with both MDS and MBL. She complained of a three-month history of fatigue associated with sustained anemia and thrombocytopenia detected on a routine blood test. Splenomegaly or lymphadenomegaly were not observed. Her hemoglobin level was 3.8 g/dL, her absolute white blood cell (WBC) count was  $7.6 \times 10^9/L$  (neutrophils 68%; eosinophils 1%; lymphocytes 23%; monocytes 9%) and her platelet count was  $34 \times 10^9/L$ . Hyposegmented neutrophils were observed on blood film examination. The BM aspirate

was hypercellular and showed increased blast cells (9%), marked dysplastic abnormalities in the erythroid, granulocytic and megakaryocytic lineages and no ring sideroblasts. The BM biopsy showed mild reticulin fibrosis without the presence of lymphoid aggregates or infiltrates. Cytogenetic analysis was inconclusive. A diagnosis of refractory anemia with excess blasts-1 was established according to the WHO classification and the patient received supportive therapy consisting of red blood cell transfusions.

Five months later, the patient's myeloblasts had increased to 15% (BM) and an abnormal karyotype (46,XX,del(9)(q22) [20]) was detected. Immunophenotyping of BM cells was performed using a large panel of monoclonal antibodies in a four-color combination to analyze precursor cells, granulocytic, monocytic and erythroid BM compartments (5) (Table 1). Multiparameter flow cytometry confirmed the increased number of myeloblasts (Figure 1) with an aberrant (CD7<sup>+</sup>, CD56<sup>+</sup>), immature (CD34<sup>+</sup>, CD117<sup>+</sup>, HLA-DR<sup>+</sup>) myeloid (CD13<sup>+</sup>, CD33<sup>+</sup>) phenotype. No CD34<sup>+</sup> B-cell precursors were detected and phenotypic abnormalities were identified in the maturing neutrophils (e.g., aberrant CD13/CD11b expression) and in the monocytic (e.g., CD2<sup>+</sup>, CD56<sup>+</sup>) and erythrocytic (e.g., CD71<sup>lo</sup>) compartments (Figure 1). In addition, 8% of the marrow cells were mature T-lymphocytes and 9% were mature B-lymphocytes that presented aberrant CD25 and CD22<sup>dim</sup> expression. A detailed study was performed due to this B-cell phenotype, which demonstrated that 88% of B-cells were monoclonal, surface kappa light chain restricted and a CD5<sup>+</sup>, CD19<sup>+</sup>, CD23<sup>+</sup> and CD25<sup>+</sup> phenotype (Figure 2).

Further analysis of the peripheral blood (PB) demonstrated the presence of monoclonal B-lymphocytes with a CD19<sup>+</sup>, CD20<sup>dim</sup>, CD22<sup>dim</sup>, CD5<sup>+</sup>, CD23<sup>+</sup>, CD79b<sup>dim</sup>, CD25<sup>+</sup>, CD43<sup>+</sup> and CD38<sup>+</sup> phenotype, lacking in reactivity to FMC-7, sIgM, CD10, CD103, and CD11c. The absolute PB B-lymphocyte count was  $3.0 \times 10^9/L$  and a diagnosis of CD5+CD23+ MBL was made.

The patient was treated for MDS with daunorubicin and cytarabine, with no response and died six months later with AML and the persistence of the PB monoclonal B-cell population ( $2 \times 10^9/L$ ).

The reported case followed the guidelines of the local ethics committees and the Helsinki Declaration. This patient was included in a large study on MDS (6) and informed consent was obtained after the study was approved by the Institutional Review Board of UNIFESP (Brazil).

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No potential conflict of interest was reported.

**Table 1** - Monoclonal antibody reagents used for the immunophenotypic characterization of myelodysplastic syndrome (modified from Matarraz S. et al., *Leukemia* 2008;22:1175-83) (5).

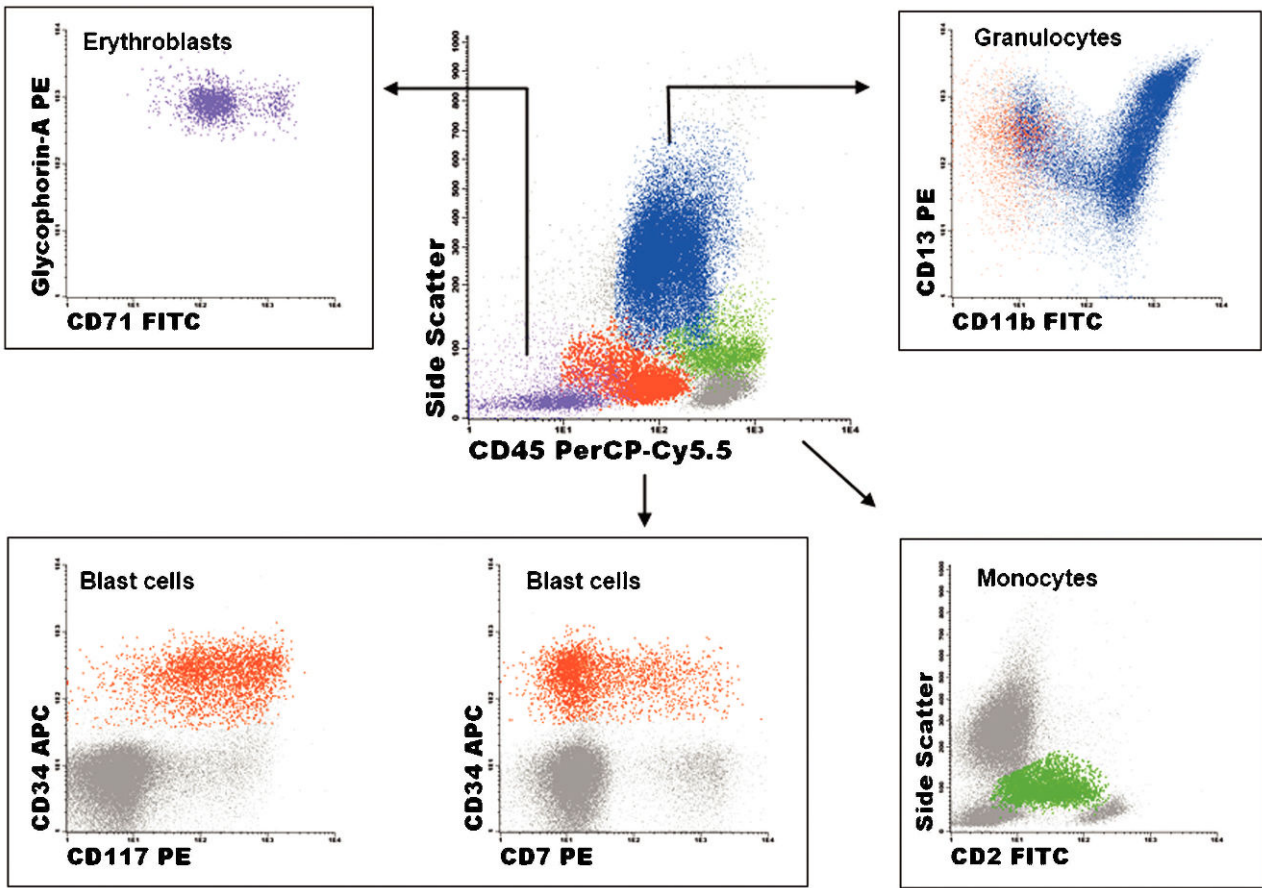
|     | FITC   | PE            | PerCP-Cy5.5 | APC         |
|-----|--------|---------------|-------------|-------------|
| 1.  | HLA-DR | CD117         | CD45        | CD34        |
| 2.  | HLA-DR | CD123         | CD45        | CD34        |
| 3.  | CD11b  | CD13          | CD45        | CD34        |
| 4.  | CD36   | CD64          | CD45        | CD34 + CD14 |
| 5.  | CD15   | CD16          | CD45        | CD34        |
| 6.  | CD2    | CD56          | CD45        | CD34        |
| 7.  | CD65   | NG2           | CD45        | CD34        |
| 8.  | CD71   | Glycophorin-A | CD45        | CD34        |
| 9.  | CD61   | CD33          | CD45        | CD34        |
| 10. | CD22   | CD25          | CD45        | CD34        |
| 11. | nTdT   | cMPO          | CD45        | CD34        |
| 12. | cCD3   | CD7           | CD45        | CD34        |
| 13. | CD19   | CD79a         | CD45        | CD34        |

## DISCUSSION

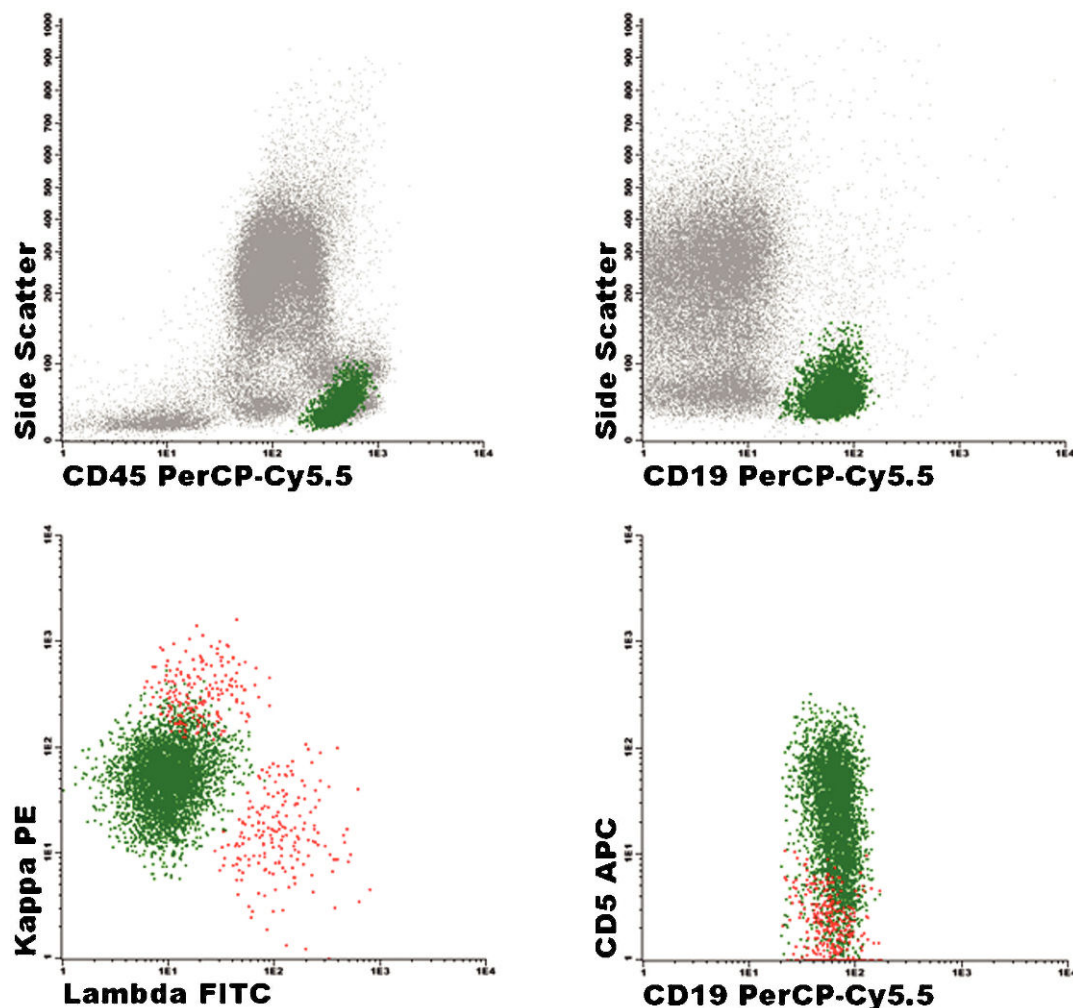
Association of MDS and B-chronic lymphoproliferative disorders (B-CLPD) is an uncommon finding that has been sporadically described (Table 2). We found that 31 cases had been reported since 1974. The median age at presentation was 72 years (range: 49-95) and the male-to-female ratio was

1.2:1. Most cases corresponded to patients with MDS and CLL (19/31) and no significant association with specific subtypes of MDS was observed. Florensa et al. showed a frequency of 1% of B-CLPD in a series of 1198 MDS patients (CLL 0.5%, lymphoplasmacytic lymphoma 0.4% and multiple myeloma 0.1%) (7). At present, the general consensus is that these associations may occur randomly (8-10). In line with this idea, we found only one case report supporting the existence of an ontogenic association between both disorders: trisomy 8 was detected in 55% of CD13<sup>+</sup> neutrophils and in 13% of CD19<sup>+</sup>/CD5<sup>+</sup> B lymphocytes in a case with MDS and systemic vasculitis, suggesting a common stem cell precursor had generated the two neoplastic cell populations (11).

Low numbers of circulating monoclonal B-cells in otherwise healthy individuals has been investigated in the last ten years. A MBL diagnosis is confirmed by the presence of  $<5 \times 10^9/L$  monoclonal B-cells associated with a normal physical examination and negative history for lymphoproliferative disease, as observed in our case (12). The progression rate to CLL has been described as approximately 1-2% of MBL cases per year (12). MBL has been observed in a significant number of healthy individuals and in, particular, in elderly people. Despite its frequency, the association of MBL in patients with other hematological neoplastic diseases (e.g., MDS) remains to be established.



**Figure 1** - Immunophenotypic analysis of bone marrow cell compartments: the blast cells (orange) are CD34<sup>+</sup> and CD117<sup>+</sup> with partial expression of CD7; the erythroblasts (purple) present glycophorin-A and low expression of CD71; the maturing granulocytes (blue) demonstrate an anomalous maturation pattern of CD13/CD11b expression, with increased levels of CD13 and CD11b during intermediate maturation stages; the monocytes (green) express aberrant CD2.



**Figure 2** - Immunophenotypic analysis of bone marrow B-cells: monoclonal B-lymphocytes (green) are CD19<sup>+</sup>, CD5<sup>+</sup>, CD45<sup>++</sup> and presents dim  $\kappa$  light chain restriction. Residual polyclonal B-lymphocytes are illustrated in red. The same immunophenotypic B-cells were detected in the peripheral blood.

To the best of our knowledge, this is the first report describing the association of MDS and CD5<sup>+</sup> with CD23<sup>+</sup>MBL. Interestingly, despite the presence of a monoclonal B-cell population, no CD34<sup>+</sup>B-cell precursors were identified in the patient's BM, as usually occurs in MDS cases.

Caballero et al. reported a patient with AML associated with CLL in which the progression of CLL disease was observed after treatment for AML and remission was achieved (13). In contrast, the lymphoid clone remained stable in our case, without change after therapy during the follow-up.

MDS develops in a multistep way. An increasing number of accumulated genetic abnormalities lead to ineffective hematopoiesis. In addition, it has been noted that immune dysfunction in MDS could also contribute to the development of cytopenia in some groups of patients. Accumulating evidence has demonstrated the association of MDS and autoimmune manifestations, T-cell mediated myelosuppression and cytokine-induced cytopenia (14,15). Immunosuppressive therapy in selected MDS patients results in high rates of hematological recovery with improved survival, especially in young patients and in the presence of HLA-DR15 (16). In addition, autoimmune complications are well recognized in CLL, occurring in

10% to 25% of patients at some time during the course of the disease. Autoimmunity in CLL predominantly targets blood constituents, most commonly red cells. The association of MBL and immune dysfunction is uncertain, although Mittal et al. (17) reported a high prevalence (20%) of CLL phenotype lymphocytes in 31 patients with autoimmune disorders (AIHA, idiopathic thrombocytopenic purpura and Evans' Syndrome), suggesting the importance of these clones in the pathogenesis of autoimmune blood disorders.

For the reasons stated above, the coexistence of a monoclonal B-cell disorder with MDS deserves special interest. This is particularly true because multiparameter flow cytometry immunophenotyping is the method of choice to detect MBL. Although numerous reports had described the immunophenotypic abnormalities in MDS, it has only recently begun to be applied during the routine work-up for the diagnosis and prognosis of potential MDS cases (18). Moreover, our case illustrates the importance of well-designed flow cytometry panels, capable of analyzing all hematopoietic cell populations because more than one neoplastic disorder may be present in the same case. We suggest the addition of one screening tube in the MDS investigational panel, containing CD19, CD5, anti- $\kappa$  and  $\lambda$

**Table 2 - Previous reports of MDS and B-chronic lymphoproliferative disorders.**

| References  | N (31) | Age<br>y/Gender           | N. of cases<br>Subtype of CLPD | Light<br>Chain      | MDS<br>subtype*                 | Cytogenetic   |
|---|--------|---------------------------|--------------------------------|---------------------|---------------------------------|---|
| 1. Papayannis AG et al. Br J Haematol. 1974;28:125-129. | 1      | 71/M                      | CLL                            | NR                  | RARS                            | 47, XY, +mar [25]/46, XY (10)   |
| 2. Escribano LE et al. Sangre. 1977;22:639-645          | 1      | 66/M                      | CLL                            | NR                  | RARS                            | 46, XY  |
| 3. Manoharan A et al. Br J Haematol. 1981; 48:111-116.  | 1      | 74/F                      | Lymphocytic Lymphoma           | λ                   | CMML                            | NR  |
| 4. Greenberg BR et al. Br J Haematol. 1983;53:125-133.  | 1      | 79/F                      | CLL+MM                         | λ (CLL)             | RARS                            | 46,XX   |
| 5. Camba L et al. J Clin Pathol. 1985;38:297-300.       | 1      | 80/F                      | PLL                            | λ                   | RA                              | NR  |
| 6. Copplestone JA et al. Br J Haematol. 1986;63:149-159 | 5      | 81.4 (67-95)/<br>1 M, 4 F | 2 B-NHL;<br>3 CLL              | NR; NR κ<br>(3 CLL) | RAEB; 2 RA;<br>2 RARS           | Normal in all cases   |
| 7. Bracey AW et al. Am J Hematol. 1989;30:174-180.      | 1      | 72/M                      | CLL                            | NR                  | RAEB                            | 46, XY, t(1;7), -7, +8  |
| 8. Bastion Y et al. Leukemia. 1991;5:1006-1009.         | 1      | 49/M                      | CLL                            | κ                   | RAEB                            | 46, XY, -4, +der(4),<br>-12, +der (12)                                  |
| 9. Shulze R et al. Clin Investig. 1992;70:1082-1084     | 1      | 60/M                      | LPL                            | κ                   | CMML                            | NR  |
| 10. Uematsu M et al. Int J Hematol. 1995;62:45-51.      | 1      | 89/F                      | B-NHL                          | κ                   | RARS                            | NR  |
| 11. Florensa L et al. Leuk Lymphoma. 1996;23:609-612.   | 11     | 79.3 (59-86)/<br>8 M, 3 F | 6 CLL, 5 LPL                   | NR                  | 3 RA, 2 RARS,<br>2 RAEB, 4 CMML | NR  |
| 12. Sylvester LS et al. Leuk Res. 1997;21:619-621.      | 1      | 85/F                      | CLL                            | κ                   | RCMD                            | Myeloid clone: 46, XX, del(13)/<br>Lymphoid clone: 47, XX, +12<br>46,XX |
| 13. Mitterbauer G et al. Ann Hematol. 1997;74:193-197.  | 1      | 69/F                      | CLL                            | κ                   | RAEB                            |   |
| 14. Lai R et al. Am J Clin Pathol. 1999;111:373-378.    | 1      | 67/M                      | CLL                            | NR                  | RARS                            | NR  |
| 15. Mossafa H et al. Leuk Lymphoma. 2001;41:337-341.    | 1      | 66/M                      | CLL                            | κ                   | RCMD                            | 46, XY, add(1),del(11)<br>(10)/47, XY, +3 (4)                           |
| 16. Cauwelier B et al. Leuk Lymphoma. 2001;41:337-341   | 1      | 76/F                      | B-NHL                          | λ                   | RAEB                            | Myeloid clone: +13 (FISH)   |
| 17. Aviv H et al. Leuk Lymphoma. 2004;45:1279-1283.     | 1      | 63/M                      | CLL                            | κ                   | RCMD                            | 46, XY, +12, del (14)(q21)<br>(1)/47, XY,+8 (1)/46, XY [23]             |

\*All cases were reclassified according to the WHO criteria; N - number of cases; NR—not reported; CLPD—Chronic lymphoproliferative disorder; NHL—Non-Hodgkin Lymphoma; LPL—Lymphoplasmacytic Lymphoma; PLL—Prolymphocytic Leukemia; MDS—Myelodysplastic Syndrome; RARS—refractory anemia with ring sideroblasts; CMML—chronic myelomonocytic leukemia; RA—refractory anemia; RCMD- refractory cytopenia with multilineage dysplasia; RAEB—refractory anemia with excess blasts.

APC, allophycocyanin; FITC, fluorescein isothiocyanate; PE, phycoerythrin; PerCP, peridinin chlorophyll protein.

light chains, which could be expanded when necessary. Widespread use of flow cytometry in the routine evaluation of MDS patients will potentially contribute to defining the actual frequency of the association between MBL and MDS and it may provide new insights into the association of these disorders.

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## AUTHOR CONTRIBUTIONS

Sandes AF and Chauffaille ML attended the patient and provided clinical data. Silva MR performed the bone marrow anatomopathological study, and Chauffaille ML was responsible for the cytogenetic experiments and analysis. Sandes AF and Siufi GC were responsible for data acquisition and flow cytometric immunophenotyping analysis. Sandes AF, Orfao A and Yamamoto M planned the study, interpreted the data and drafted the manuscript. All co-authors approved the final version of the manuscript.

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