Del(20q) in patients with chronic lymphocytic leukemia: A therapy-related abnormality involving lymphoid or myeloid cells

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Abstract

Del(20q), a common cytogenetic abnormality in myeloid neoplasms, is rare in chronic lymphocytic leukemia. We report 64 patients with chronic lymphocytic leukemia and del(20q), as the sole abnormality in 40, a stemline abnormality in 21, and a secondary abnormality in 3 cases. FISH analysis revealed an additional high-risk abnormality, del(11q) or del(17p), in 27/64 (42%) cases. In most cases, the leukemic cells showed atypical cytologic features, unmutated *IGHV* genes and ZAP70 positivity. The del(20q) was detected only after chemotherapy in all 27 cases with initial karyotypes available. With a median follow-up of 90 months, 30 patients (47%) died, most as a direct consequence of chronic lymphocytic leukemia. Eight patients developed a therapy-related myeloid neoplasm, seven with a complex karyotype. Combined morphologic and FISH analysis for del(20q) performed in 12 cases without morphologic evidence of a myeloid neoplasm localized the del(20q) to the chronic lymphocytic leukemia cells in 5 (42%) cases, and to myeloid/erythroid cells in 7 (58)% cases. The del(20q) was detected in myeloid cells in all 4 cases of myelodysplastic syndrome. In aggregate, these data indicate that chronic lymphocytic leukemia with del(20q) acquired after therapy is heterogeneous. In cases with morphologic evidence of dysplasia, the del(20q) likely resides in the myeloid lineage. However, in cases without morphologic evidence of dysplasia, the del(20q) may represent clonal evolution and disease progression. Combining morphologic analysis with FISH for del(20q) or performing FISH on immunomagnetically-selected subpopulations to localize the cell population with this abnormality may help guide patient management.

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Keywords

chronic lymphocytic leukemia; del(20q); disease progression; unmutated *IGHV* genes; combined morphologic and FISH analysis

Introduction

Interstitial deletion of the long arm of chromosome 20, del(20q), is a common recurrent cytogenetic abnormality in myeloid malignancies, including myeloproliferative neoplasms, myelodysplastic syndromes, and acute myeloid leukemias, reported in approximately 10%, 4%, and 2% of cases, respectively (1–3). In myeloproliferative neoplasms, the presence of del(20q) appears to have no adverse effect on patient survival (4, 5). Similarly, del(20q) as the sole cytogenetic abnormality in patients with myelodysplastic syndromes is associated with good survival and a low risk of leukemic transformation (6, 7). In contrast, del(20q) has been associated with a poor response to treatment and reduced survival in acute myeloid leukemia (4).

In patients with chronic lymphocytic leukemia, the common recurrent cytogenetic abnormalities identified by fluorescence *in situ* hybridization (FISH) analysis in about 80% of patients include del(11)(q22.3), del(13)(q14.3), +12, and del(17)(p13.1) (8). Each of these cytogenetic subtypes is associated with distinct clinical, prognostic, and pathologic features (8). Deletion 20q is unusual in lymphoproliferative disorders including chronic lymphocytic leukemia. The clinical features of chronic lymphocytic leukemia with del(20q) have been described in detail in only a single case report (9). Deletion 20q in chronic lymphocytic leukemia without clinical information is reported in seven other publications as single cases (10–16).

We report the clinicopathologic, morphologic, immunophenotypic, and molecular genetic features of 64 cases of chronic lymphocytic leukemia with del(20q), the largest series to date. We performed combined morphologic and FISH analysis for del(20q) in a subset of cases. Our results indicate that chronic lymphocytic leukemia with del(20q) is heterogeneous. In a small subset of patients, we identified the del(20q) in myeloid or erythroid cells, where it may represent an age- or therapy-related myeloid neoplasm. In the majority of the patients, we identified the del(20q) in chronic lymphocytic leukemia cells, where it is likely a manifestation of disease progression. These two groups require different therapeutic approaches.

Materials and Methods

Case selection

We searched the files of our Clinical Cytogenetics Laboratory for cases of chronic lymphocytic leukemia with del(20q) between 1/1//1991 and 5/31/2014. The cases were reviewed, and the diagnoses of chronic lymphocytic leukemia and myeloid neoplasms were characterized using the morphologic and immunophenotypic criteria as specified in the World Health Organization classification (17, 18). The clinical data were obtained by review of medical records.

Morphologic examination

We reviewed H&E-stained bone marrow core biopsy and clot specimens, as well as Wright-Giemsa-stained aspirate smears and touch imprints. The bone marrow cellularity and pattern of lymphocytic infiltration were assessed in the core biopsy specimens; the pattern was classified as nodular, interstitial, diffuse, or a combination of these patterns. We performed 500-cell differential counts on aspirate smears or touch imprints. We paid particular attention to the cytologic features of the lymphocytes with respect to atypical morphologic features, including indented or clefted nuclei, plasmacytoid features, and the presence of prolymphocytes. The percentages of plasmacytoid lymphocytes, defined as cells with eccentrically placed nuclei, moderately abundant cytoplasm, and/or cartwheel-like chromatin, and lymphocytes with indented nuclei were recorded. Dysplasia in myeloid cells, erythrocytes, and megakaryocytes was assessed based on the criteria as specified in the World Health Organization classification (18).

Immunophenotypic analysis

Flow cytometry immunophenotyping was performed on bone marrow aspirates using a FACScan instrument (Becton Dickinson, San Jose, CA) as described previously (19). The lymphocyte population was gated using right-angle side scatter and CD45 expression. The panel of monoclonal antibodies included reagents specific for CD3, CD5, CD10, CD11c, CD19, CD20, CD22, CD23, CD38, CD79b, FMC-7, surface immunoglobulin light chains, and IgM/IgD.

We calculated chronic lymphocytic leukemia scores according to the system proposed by Matutes and colleagues (20) and subsequently modified by Moreau and colleagues (21). Scores were based on five variables: expression of dim surface immunoglobulin (1 point), CD5 (1 point) and CD23 (1 point); dim or absent CD22/CD79b (1 point); and absent FMC-7 (1 point). Cases with a score of 4 or 5 were considered to have a typical immunophenotype; cases with a score of less than 4 were considered to have an atypical immunophenotype.

Immunohistochemical stains were performed on formalin-fixed, paraffin-embedded bone marrow core biopsies or clot sections. Staining for ZAP70 (dilution 1:500, Upstate Cell Signaling Systems, Lake Placid, NY) was performed as described previously (22). Cases with nuclear staining in 20% of the neoplastic cells were considered positive. Nuclear staining of admixed non-neoplastic T-cells served as an internal control. We also performed immunostain for cyclin D1 (SP4, Lab Vision/NeoMarkers, Fremont, CA) in a subset of cases.

Conventional cytogenetic and fluorescence i*n situ* hybridization analyses

Conventional cytogenetic analysis was performed on metaphase cells from bone marrow aspirates or peripheral blood cultured for 24 hours without mitogens and for 72 hours with lipopolysaccharide, using standard techniques. Giemsa-banded metaphases were analyzed, and results were reported using the International System for Human Cytogenetic Nomenclature.

FISH analysis for the common chronic lymphocytic leukemia-associated cytogenetic abnormalities was performed in 46 cases on interphase nuclei from cultures of bone marrow or peripheral blood using a panel of probes designed to detect deletions of 11q22.3 (*ATM*), 13q14.3, 13q34, and 17p13 (*TP53*), and trisomy 12, according to the manufacturer's instructions (Vysis/Abbott, Des Plaines, IL). FISH to detect del(20)(q12) was performed using the LSI D20S108 probe (Vysis/Abbott).

Combined morphologic and fluorescence *in situ* hybridization analysis

Combined morphologic and FISH analysis was performed as described previously (23). Briefly, Wright-Giemsa-stained bone marrow aspirate smears were reviewed to select target cell populations, and images were captured using bright-field microscopy. The smears were then destained using 1% acid alcohol (1% HCl in 70% ethanol), treated with protease II (Vysis/Abbott), and hybridized with a fluorescently-labeled LSI D20S108 probe (Vysis/Abbott). The target cell populations were then matched under fluorescent microscope, and photographed.

Somatic mutation status of the *IGHV* genes

Sequence analysis of the immunoglobulin heavy chain variable region (*IGHV*) gene was performed in 33 cases using total RNA extracted from bone marrow aspirates or peripheral blood as described previously (24). Patients' sequences were aligned to the germline sequences in the V-BASE 2 database (25). The *IGHV* mutation status was designated as "unmutated" if there were fewer than 2% mutations (98% homology to germline sequences) or as "mutated" if there were 2% or more mutations (<98% homology) compared with the germline sequences (26).

Results

Clinical findings

We identified 64 cases of chronic lymphocytic leukemia with del(20q), 53 men and 11 women, representing <1% of all chronic lymphocytic leukemia cases. At initial diagnosis of chronic lymphocytic leukemia, the median age was 57 years (range, 31–77). The patients presented with absolute lymphocytosis (n=35), anemia (n=1), or thrombocytopenia (n=1) detected on routine physical examination, lymphadenopathy (n=21), or absolute lymphocytosis found during evaluation for B-symptoms (n=3) or chest pain (n=2); clinical presentation information was unavailable for one patient.

The clinical and laboratory data when patients presented to our institution are summarized in Table 1. Forty-two patients had received treatment for chronic lymphocytic leukemia and 22 were treatment-naive. Twelve patients (19%) reported B-symptoms, 56 (88%) had generalized lymphadenopathy, and 25 (39%) had splenomegaly. Forty-eight patients (75%) had absolute lymphocytosis (range, $5.0–324.3\times10^9$/L; median, 25.4×10^9/L; reference range, $1.0–4.8\times10^9$/L), 43 (67%) were anemic (hemoglobin range, 4.1–13.9 g/dL; median, 11.8 g/dL; reference range, 14.0–18.0 g/dL for men and 12.0–16.0 g/dL for women), and 36 (56%) were thrombocytopenic (range, $4–139\times10^9$/L; median, 82×10^9/L; reference range, $140–440\times10^9$/L). Serum lactate dehydrogenase was elevated in 30 patients (47%) (range,

621–3357 U/L; median, 752 U/L; reference range, 313–618 U/L), as was serum β_2-microglobulin (4 mg/L) in 26 patients (40%) (range, 4.1–10.3 mg/L; median, 5.9 mg/L; reference range, 0.7–1.8 mg/L). The patients were in Rai stages 0 (n=2; 3%), I (n=21; 33%), II (n=9; 14%), III (n=4; 6%) and IV (n=28; 44%).

Sixty-two patients received treatment for chronic lymphocytic leukemia at our institution; 58 received fludarabine, cyclophosphamide and rituximab (FCR)-based therapy, 2 received fludarabine and cyclophosphamide, 1 received bendamustine and rituximab, and 1 received rituximab. Fourteen patients subsequently underwent bone marrow transplantation. Two patients remain untreated.

Of the nine patients (14%) who subsequently developed diffuse large B-cell lymphoma (Richter's transformation), seven died, five after therapy with cyclophosphamide, vincristine, doxorubicin and dexamethasone (hyper-CVAD), and two after therapy with cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) and bone marrow transplantation. Two patients remain alive, one with persistent chronic lymphocytic leukemia following treatment with ABT-199, a BCL2 inhibitor, and one in clinical remission following treatment with hyper-CVAD and bone marrow transplantation.

Ten patients (16%) received a diagnosis of a myeloid neoplasm. Three patients had a myeloid neoplasm at presentation to our institution: one had a 10-year history of *JAK2* V617F-positive polycythemia vera and received hydroxyurea before developing chronic lymphocytic leukemia; one had a 2-year history of chronic lymphocytic leukemia treated with FCR, and presented with both chronic lymphocytic leukemia and myelodysplastic syndromes; and one treatment-naïve patient developed myelodysplastic syndrome two months after developing chronic lymphocytic leukemia. After presenting to our institution, seven additional patients developed a myeloid neoplasm following therapy for chronic lymphocytic leukemia; one patient developed *JAK2* V617F-positive primary myelofibrosis 139 months after treatment and six developed myelodysplastic syndromes after treatment (median, 67 months; range, 28–167). Thus, the myeloid neoplasms in eight patients, seven with myelodysplastic syndrome and one with myeloproliferative neoplasm, are considered therapy-related.

With a median follow-up of 90 months from the initial diagnosis of chronic lymphocytic leukemia (range, 12–252), 30 patients (47%) died of chronic lymphocytic leukemia (n=21, including 7 with development of diffuse large B-cell lymphoma), graft vs. host disease (n=5), secondary acute myeloid leukemia (n=2), or lung cancer with persistent chronic lymphocytic leukemia (n=2). Thirty-four patients (53%) remain alive, 19 with persistent chronic lymphocytic leukemia and 15 in clinical remission.

Morphologic and immunophenotypic findings

At presentation to our institution, all patients showed bone marrow involvement by chronic lymphocytic leukemia. The morphologic features of a representative case are illustrated in Figure 1A. The core biopsies showed a median cellularity of 60% (range, 20–95%). Lymphoid cells infiltrated the bone marrow in an interstitial (n=24), diffuse (n=10), nodular (n=8), or mixed (n=22) pattern, and involved 5–100% of the total cellular elements (median,

80%). The aspirate smears showed predominantly small lymphoid cells, but 43 cases (67%) showed atypical cytologic features. Thirty-one cases contained a subset (15%) of lymphoid cells with indented and irregular nuclear contours, and 28 showed a subset (15%) of plasmacytoid lymphocytes. Fifteen patients had increased (5%) prolymphocytes (range, 5–15%; median, 7%) (Figure 1B). Three patients also showed a myeloid neoplasm in addition to chronic lymphocytic leukemia, two with myelodysplastic syndromes (Figure 2) and one with polycythemia vera.

Despite the presence of atypical cytologic features in 43 cases, the leukemic cells demonstrated typical immunophenotypes in 62 cases (97%), with a chronic lymphocytic leukemia score of 4 or 5 (17, 20, 21). Flow cytometry immunophenotyping demonstrated that the neoplastic cells in all 64 cases were positive for CD5, CD19, and dim surface monotypic immunoglobulin light chain (40 kappa, 23 lambda, 1 lacking expression of surface light chains). In addition, the neoplastic cells were positive for CD23 in 62/64 (97%), dim to moderate CD20 in 60/64 (94%), CD11c in 37/54 (69%), CD38 in 41/60 (68%), CD79b (dim) in 22/55 (40%), CD22 (dim) in 20/54 (37%), and FMC-7 (dim) in 4/64 (6%) cases. In all cases assessed, the cells were negative for CD3 (n=64) and CD10 (n=45). Two cases showed atypical immunophenotypes, with a chronic lymphocytic leukemia score of 3; one showed moderate expression of CD20 and FMC-7, and the other lacked CD23 and expressed FMC-7. Immunostain demonstrated that 30/36 cases (83%) expressed ZAP70, with >90% of the leukemic cells positive. Immunostain for cyclin D1 performed in 17 cases, including the two cases with atypical immunophenotypes, was negative.

Cytogenetic and molecular diagnostic findings

Overall, at the time the del(20q) was identified, 46 cases showed simple abnormal karyotypes (1–2 abnormalities) and 18 showed complex abnormal karyotypes (3 abnormalities). The del(20q) was the sole abnormality in 40 cases. In two cases (one simple and one complex) it was the sole abnormality in the stemline, i.e., the most basic clone of the tumor cell population, and in eight cases (one simple and seven complex) it was one of two or more stemline abnormalities. In twelve cases (three simple and nine complex) the del(20q) was the sole abnormality in one clone in cases that showed clonal divergence, i.e., two or more cytogenetically-unrelated clones. It was a secondary abnormality in only three cases (two simple and one complex).

The del(20q) was detected only after chemotherapy for chronic lymphocytic leukemia in all 27 cases with karyotypes at initial diagnosis available for comparison, with a median interval from the time of initial diagnosis to the detection of del(20q) of 65 months (range, 2–212). The results of conventional cytogenetic analysis performed at the time of initial chronic lymphocytic leukemia diagnosis were unavailable for 37 cases.

FISH analysis to assess the common recurrent chronic lymphocytic leukemia abnormalities performed in 46 cases at presentation to our institution demonstrated at least one abnormality in 37 (80%) (Table 2). Deletions of 11q22.3, 13q14.3, 17p13.1, and trisomy 12 were detected in 16 (35%), 20 (43%), 11 (24%), and 15 (33%) cases, respectively. Twenty-five cases (54%) had one FISH abnormality, seventeen cases (37%) had two abnormalities, three cases (7%) had three abnormalities, and one case (2%) had four abnormalities. Two

cases with del(13)(q14.3) also showed del(13)(q34), suggesting monosomy 13. Interphase FISH analysis for del(20)(q12) performed as part of the initial clinical evaluation at our institution was positive in all 27 cases tested.

Of the eight patients who developed a therapy-related myeloid neoplasm, the del(20q) was identified in three before the diagnosis of therapy-related myelodysplastic syndrome (12, 17, and 27 months), in one before the diagnosis of primary fibrosis (160 months), in three at the time of diagnosis of therapy-related myelodysplastic syndrome, and in one after the diagnosis of myelodysplastic syndrome (15 months). In order to determine the lineage of the cells that harbored the del(20q), we performed combined morphologic and FISH analysis in 16 cases with aspirate smears available for study, 13 previously-untested cases and three case previously assessed by interphase FISH. We identified the del(20)(q12) in myeloid and/or erythroid cells in 11 cases (69%): all four cases tested with therapy-related myelodysplastic syndrome (Figure 3A and 3B), one case with slight dysplasia that did not meet the morphologic criteria for myelodysplastic syndrome, and six cases without myeloid dysplasia. We identified the del(20)(q12) in lymphoid cells in five cases (31%), none with morphologic evidence of a myeloid neoplasm (Figures 3C and 3D). Thus, in 11 cases without evidence of myelodysplasia, combined morphologic and FISH analysis localized the del(20q) to the chronic lymphocytic leukemia cells in about half of cases, and to the myeloid/erythroid cells in the other half. Upon review, we could identify no karyotypic abnormalities that would have allowed us to distinguish between these cases.

Sequence analysis to evaluate the somatic mutation status of the *IGHV* genes performed in 33 cases demonstrated that 28 cases were unmutated and 5 were mutated.

Discussion

Because del(20q) is a common cytogenetic abnormality in myeloid neoplasms of older adults (1–3) and unusual in lymphoproliferative disorders, it would be reasonable to assume that the presence of a clone with del(20q) in a chronic lymphocytic leukemia patient represents an evolving myeloid neoplasm, either age-related or secondary to chemotherapy. However, we observed that in a subset of chronic lymphocytic leukemia patients with del(20q), the abnormality was present in a fraction of interphase nuclei by FISH, i.e., non-dividing cells, that far exceeded the fraction of myeloid cells in the bone marrow aspirates, and was more consistent with the extent of involvement by chronic lymphocytic leukemia. In addition, most of these cases lacked morphologic features of myelodysplasia either at the time the del(20q) was detected or at follow-up. Based on these observations, we hypothesized that, in at least a subset of cases, the del(20q) resided in the chronic lymphocytic leukemia, rather than in the myeloid cells. We performed combined morphologic and FISH analysis to determine the lineage of the cells that contained the cytogenetic abnormality. Although our sample size is small, in approximately half of the patients we identified the del(20q) in the chronic lymphocytic leukemia cells; in the other half we identified it in the myeloid and/or erythroid cells. Thus, chronic lymphocytic leukemia with del(20q) is a heterogeneous group.

There are few reports in the literature of chronic lymphocytic leukemia with del(20q). We found a single well-documented case report. The patient, an 80-year-old woman, presented with generalized lymphadenopathy, anemia, and thrombocytopenia. Small lymphoid cells, some with plasmacytoid features, infiltrated the bone marrow. Cytogenetic analysis revealed del(20)(q11.2) as the sole cytogenetic abnormality. The patient received supportive care, and died 12 months later from bilateral bronchopneumonia and ischemic heart disease (9). The cytogenetic features of a handful of other cases have been reported in the literature (10–16).

A number of features suggest that del(20q) in chronic lymphocytic leukemia is associated with disease progression and poor prognosis. In general, the neoplastic cells showed one or more markers of poor prognosis, including atypical cytologic features, unmutated *IGHV* gene, ZAP70 expression, and one of the high-risk recurrent cytogenetic abnormalities (8, 27–32). With a median follow-up of less than 8 years, almost all patients required therapy for chronic lymphocytic leukemia and almost half died, most as a consequence of chronic lymphocytic leukemia. Most of these cases lacked morphologic features of myelodysplasia either at the time del(20q) was detected or at follow-up. Only a small subset of the patients developed a myeloid neoplasm.

The presence of del(20q) as either the sole or a stemline karyotypic abnormality in more than 95% of our cases raises the possibility that it is a critical genetic event. It also suggests the presence of a gene (or genes) on the long arm of chromosome 20 that functions as a tumor suppressor. In myeloid neoplasms, potential candidate genes that map to this region include *Dido* (33), *L3MBTL1* (34), and *MYBL2* (35). There are few genetic studies of del(20q) in lymphoproliferative lesions. However, loss of heterozygosity (LOH) in the region of 20q11.2 to 20q13.3 has been described in chronic lymphocytic leukemia patients, raising the possibility of a tumor suppressor gene in this region (36, 37). In a study of 19 patients with large cell transformation, Lee et al. described LOH in a region that corresponds to 20q12 within large cells in over half of the cases studied (36). Recently, Clifford, et al. has identified *SAMHD1*, located at chromosome 20q11.23, as a tumor suppressor in chronic lymphocytic leukemia. The protein encoded by *SAMHD1* normally degrades intracellular deoxynucleoside triphosphates, and is involved in the response to DNA damage and in cell proliferation and survival. They found that *SAMHD1* is recurrently mutated in 11% of recurrent/refractory chronic lymphocytic leukemia cases. Further, these mutations were associated with copy-neutral LOH or loss of the other allele (38).

In conclusion, our results show that chronic lymphocytic leukemia cases with del(20q) that acquired after therapy is heterogeneous. In cases with morphologic evidence of myelodysplasia, the del(20q) likely resides in the myeloid cells. However, in cases that lack morphologic evidence of myelodysplastic syndrome, del(20q) may represent clonal evolution and chronic lymphocytic leukemia progression. In the absence of morphologic evidence of a myelodysplastic syndrome, either combining morphologic analysis with FISH for del(20q), or performing FISH on immunomagnetically-selected subpopulations in order to localize the cell population in which this abnormality resides may help guide patient management.

Supplementary Material

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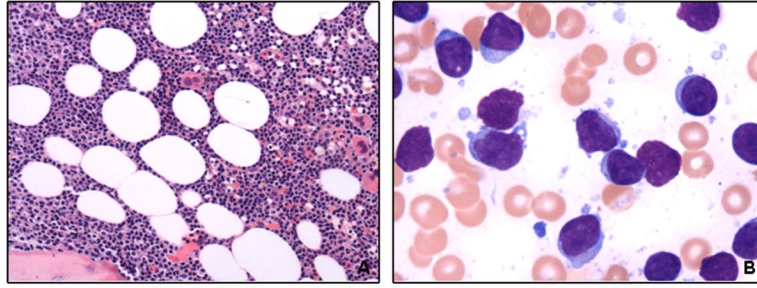
Figure 1. Morphologic findings of chronic lymphocytic leukemia with del(20q)
(A) Bone marrow biopsy from a representative case shows chronic lymphocytic leukemia cells extensively infiltrating the marrow (H&E, 200x). (B) The aspirate smear shows atypical chronic lymphocytic leukemia cells with irregular nuclear contours and plasmacytoid differentiation (Wright-Giemsa, 1 000x).
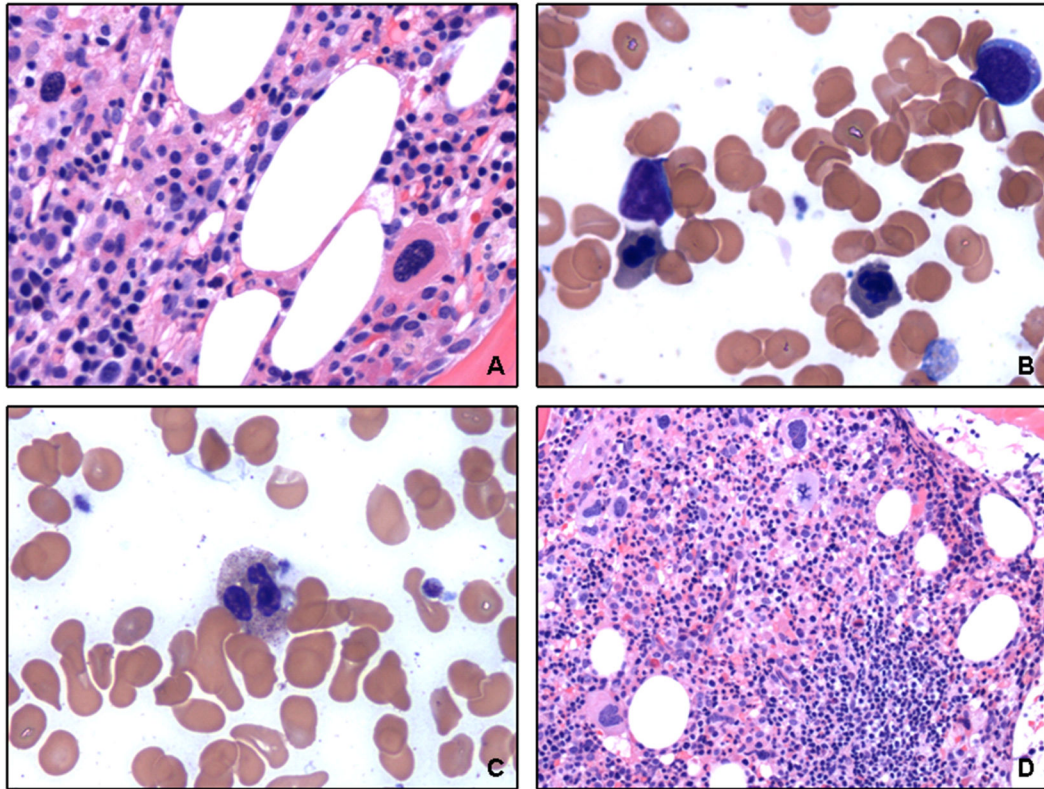
Figure 2. Morphologic findings of a case of chronic lymphocytic leukemia with myelodysplastic syndrome
Bone marrow biopsy and aspirate smear demonstrate dysplastic features in (A) megakaryocytes (H&E, 400x), (B) erythrocytes (Wright-Giemsa, 1 000x), and (C) granulocytes (Wright-Giemsa, 1 000x), as well as (D) residual chronic lymphocytic leukemia (H&E, 200x).
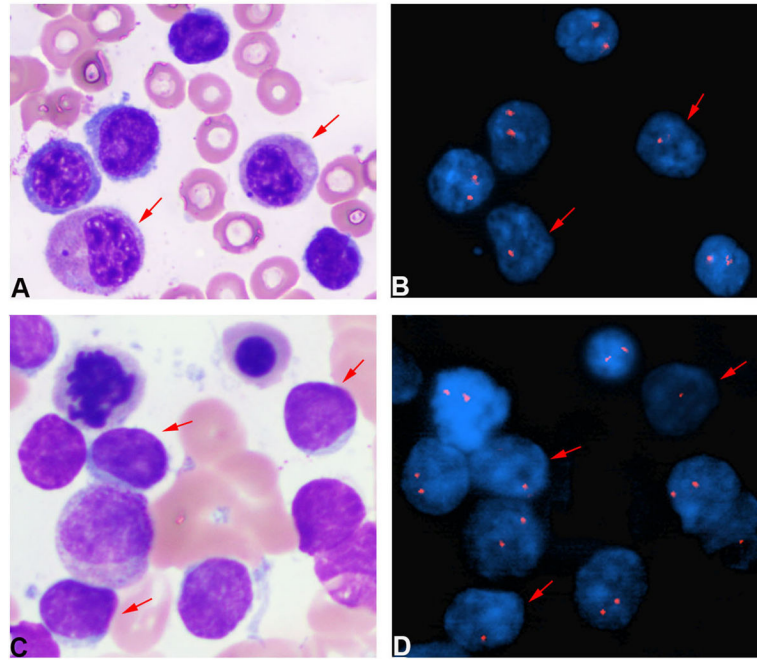
Figure 3. Combined morphologic and FISH analysis
(A and B) The del(20q) is identified in myeloid and erythroid cells in a case of myelodysplastic syndrome (100x). (C and D) The del(20q) is identified in lymphoid cells in a case without morphologic evidence of a myeloid neoplasm (100x).

Table 1

Clinical findings[*]

	Number	Median	Range
Gender			
Male	53		
Female	11		
B-symptoms	12		
Lymphadenopathy	56		
Splenomegaly	25		
Lymphocytosis (x10^9/L)	48	25.4	5.0–324.3
Anemia (Hgb, g/dL)	43	11.8	4.1–13.9
Thrombocytopenia (x10^9/L)	36	82	4–139
LDH (U/L)	30	752	621–3357
β2M (mg/L)	26	5.9	4.1–10.3
Rai stage			
0	2		
I	21		
II	9		
III	4		
IV	28		

[*] At presentation to our institution

Abbreviations: Hgb, hemoglobin; LDH, lactate dehydrogenase; β2M, β-2 microglobulin

Table 2

Cytogenetic and molecular genetic features

	Number
del(20q)*	
sole abnormality	40
sole abnormality with clonal divergence	12
stemline abnormality	9
secondary abnormality	3
FISH	
del(13)(q14.3)	20/46
del(11)(q22.3)	16/46
trisomy 12	15/46
del(13)(q34)	2/46
del(17)(p13)	11/46
Unmutated *IGHV* genes	28/33

*On conventional karyotypic analysis