Interestingly, we did reveal an association between increased VEGF levels and thrombotic complications.

An increase in either intratumoral angiogenesis or serum levels of VEGF has been demonstrated in a variety of hematologic malignancies.[5] The possible prognostic relevance of these measurements in chronic myeloproliferative disorders (CMD) has been reported by Musolino *et al.* who found significantly higher VEGF levels in CMD patients with vascular complications.[6] It has also been reported that tumor-released VEGF activates endothelial cells to become prothrombotic causing platelet adhesion and activation.[3] Musolino *et al.* observed endothelium activation in CMD patients and thromboembolic complications.[7]

Therefore, it is speculated that increased VEGF might be responsible for the endothelial activation. Other authors have reported increased VEGF in CMD accompanied by thrombocytosis that could limit the interpretation of this finding since platelets are a major source of VEGF.[8] We found increased VEGF levels in patients with PV without thrombocytosis and this increase was not correlated with the platelet count.

Thus, in our study increased levels of VEGF might reflect a state of platelet activation and be responsible for occurrence of a thrombotic event. We, therefore, think that VEGF measurement might have a clinical use in categorizing high- and low-risk PV patients and be appreciated as an additional variable in clinical prognostic models. These data, if confirmed in larger studies, might also support the rationale for using angiogenesis inhibitors as antithrombotic agents.

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t(15;17) in acute promyelocytic leukemia is not associated with submicroscopic deletions on der(17)

We report a fluorescent *in situ* hybridization (FISH) study on 34 patients with acute promyelocytic leukemia. The study was designed to detect microdeletions in the derivative chromosome 17 which is the result of a reciprocal translocation t(15;17). No deletion was found.

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Acute promyelocytic leukemia (APL) is characterized by the reciprocal translocation t(15;17)(q22;q21), disrupting the PML and RAR-α genes, which are localized on chromosomes 15q22 and 17q21, respectively. The t(15;17) generates two chimeric genes: PML/RARα arises on der(15), whereas the reciprocal RARα/PML fusion is located on the der(17).[1-2] Microdeletions on the derivative chromosome carrying the reciprocal fusion gene have been recently reported in some leukemia translocations such as t(9;22) in chronic myeloid leukemia (CML) and inv(16), as well as in t(8;21) and 11q23 abnormalities in acute myeloid leukemia (AML).[3-7] In CML Ph$^+$ and AML with inv(16) these microdeletions were associated with a worse prognosis. Microdeletions in APL cases have been recently investigated, by FISH, in 30 APL patients by Kolomietz *et al.*,[8] who utilized the Vysis LSI PML/RARα translocation probe. This study did not reveal microdeletions. The Vysis probe, however, is not able to detect deletions in der(17), as it was specifically designed to detect the expressed fusion gene on der(15) chromosome. Therefore, their conclusion on the absence of deletions on der(17) has no experimental support. In contrast, we used appropriate FISH probes, specifically designed to detect deletions in der(17). Thirty-four APL patients were enrolled at diagnosis. All cases were tested by conventional cytogenetic and RT-PCR analysis to assess the presence of the t(15;17) and of the fusion gene PML/RARα, respectively. Co-hybridization FISH experiments were performed by using a mixture of two probes (BAC RP11-247C2 and PAC RP5-1112G21, from de Jong libraries), spanning the PML gene on chromosome 15 and RARα gene on chromosome 17, respectively (Figure 1a). Their precise positions were derived from the Ensembl database (*http://www.ensembl.org/*). On metaphases of APL patients these probes generated two clear fusion signals on der(15) and on der(17) chromosomes in addition to single signals on normal 15 and 17 chromosomes (Figure 1b). The absence of a fusion signal on der(17) would reveal microdeletions on this chromosome. Twenty metaphases were evaluated for each patient. The analysis did not reveal any microdeletion, since an evident fusion signal on der(17) was observed in all analyzed metaphases.

In our series there was 1(3%) case with cryptic PML/RARα fusion gene created by an insertional event, which is in agreement with the frequency of this abnormality in APL cases reported in literature.[9] In fact, this patient had apparently normal chromosomes 15 and 17 by conventional cytogenetic analysis while RT-PCR revealed expression of PML/RARα. Fluorescent *in situ* hybridization (FISH) experiments showed an abnormal hybridiza-

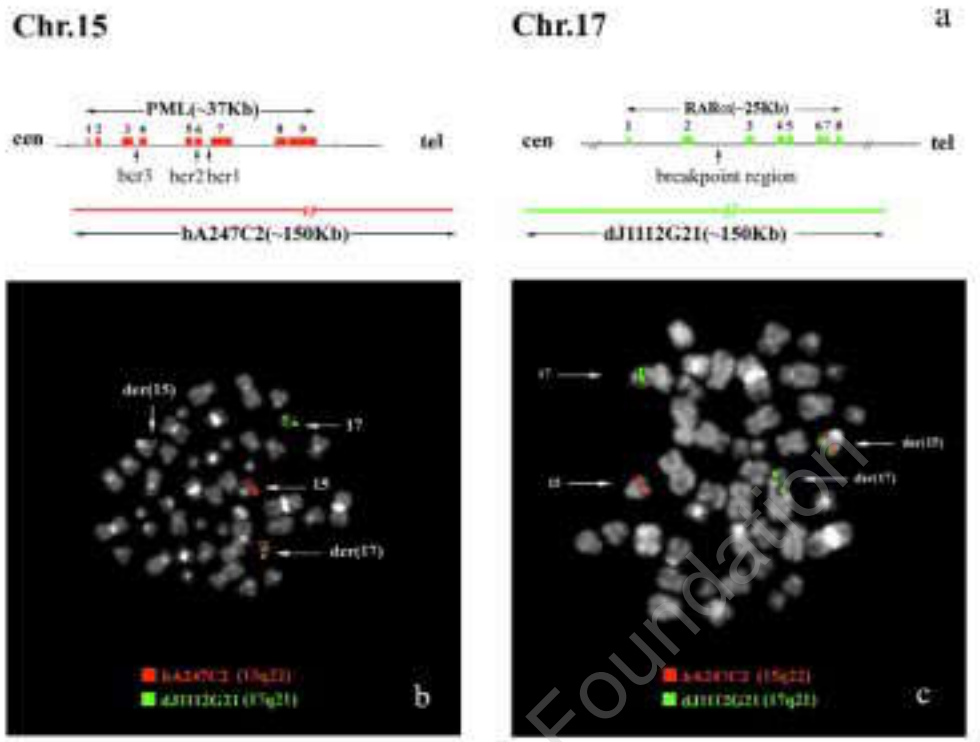

Figure 1. (a) The figure shows the position of probes RP11-247C2 and RP5-1112G21 in respect to PML and RARα genes. The size of both probes is approximately 150 Kb. (b) FISH pattern in a metaphase characterized by a t(15;17) rearrangement without detectable deletion. (c) FISH experiment performed using the same probes in the case showing a PML/RARα insertion. BAC RP11-247C2 showed signals of equal intensity on normal chromosome 15 and on der(15), while PAC RP5-1112G21 gave signals on both der(17) and der(15).

tion pattern: PAC RP5-1112G21 gave the classical splitting [signals on both der(17) and der(15); Figure 1c], while BAC RP11-247C2 showed signals of equal intensity on normal chromosome 15 and on der(15). No RP11-247C2 signal was detected on der(17). Co-hybridization of chromosome 15 WCP with RP5-1112G21 revealed a cryptic insertion of RARα gene in one chromosome 15.

The present study suggests that t(15;17) is not accompained by any deletion in der(17). Very small deletions (a few kb), escaping FISH detection cannot be excluded. Our conclusion could also be biased by the relatively small number of patients analyzed, and confirmation on larger studies are needed. Statistical analysis, however, allows exclusion of the deletion in more than 9% of patients with a 95% confidence level.[10]

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A new translocation t(11;13)(q13;q14) in a mature B-cell neoplasm

We present the case of a man affected by an unclassified mature B-cell neoplasm with a bone marrow culture stimulated with TPA showing a 46,XY, t(11;13)(q13;q14)[14]/46,XY [6] karyotype. Fluorescent *in situ* hybridization demonstrated that the BCL1 oncogene is translocated (not rearranged) to chromosome band 13q14 and that a copy of D13S319 locus is deleted. To our knowledge, this is the first reported case with this novel cytogenetic aberration.

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The lymphomas and leukemias of B-lymphoid cells are heterogeneous diseases associated with different cytogenetic aberrations.[1] Translocations that involve genes controlling progression through the cell cycle have been described: cyclin D1 on chromosome 11q13 is involved in the t(11;14)(q13;q32) in mantle cell lymphoma (MCL),[2] and recently a new translocation t(6;14)(p21.1;q32.3) has been reported involving cyclin D3 in mature B-cell malignancies.[3-5]

Translocation t(11;14)(q13;q32) has been detected in MCL, multiple myeloma (MM) and chronic prolymphocytic leukemia (PLL). Cytogenetic abnormalities of chromosome band 13q14 reported in mature B-cell disorders include deletions and in some rare cases translocations, deletion 13q being one of the most frequent cytogenetic aberrations in B-cell chronic lymphocytic leukemia (B-CLL).[6]

Here we present a case with unclassified mature B-cell neoplasm showing a not previously reported translocation, t(11;13)(q13;q14), as a sole anomaly.[7] The present case was studied by conventional cytogenetics and fluorescence *in situ* hybridization (FISH).

In November 1997, a 63-year old man was referred for study of a myelodysplasia. Physical examination revealed no abnormalities. Laboratory findings were: leukocyte count 3×10^9/L, Hb 13.1 g/dL, MCV 101 fL, and platelet count 104×10^9/L. A bone marrow aspirate showed a myelodysplastic syndrome (MDS), refractory cytopenia with trilineage dysplasia.

In October 2000 the patient had a white cell (WBC) count of 7.2×10^9/L with 46% lymphocytes and in November 2001 the WBC was 12.2×10^9/L with 69% of lymphocytes. Lymphocytes were small, with condensed chromation, single nucleolus, without cytoplasmic prolongations and some presented plasmacytic differentiation (Figure 1). Immunological studies by flow cytometry showed the following: $CD19^+$, $CD5^+$, $CD23^+$ weak (30%), $FMC7^+$ weak, $CD22^+$ weak, CD 79b$^+$, $CD10^-$, $CD25^+$, $CD11c^+$, $CD38^-$, $CD20^+$ bright, IgM^+, IgD^-, and monoclonal κ light chain with bright intensity (Matutes score 4/6).

In January 2002 the WBC count was 13.7×10^9/L with 74% of lymphocytes. A bone marrow aspirate showed 40% lymphocytes, 18% red cells, 38% white cells and 4% plasma cells. Immunologic study of lymphocytes from peripheral blood revealed: $CD5^+$ and $CD23^+$ weak (24%) and an immunophenotypic profile in the majority of plasma cells (82%) suggested myeloma cells ($CD38^+$, $CD19^+$, and $CD56^+$). The measurements of immunoglobulins in serum showed increased IgG and decreased levels of IgA and IgM: immunofixation revealed monoclonal heavy chain IgG and light chain κ. A 24-hour stimulated bone marrow culture with TPA showed a 46,XY, t(11;13)(q13;q14) [14]/46,XY [6] karyotype (Figure 2a). A diagnosis of an unclasified mature B-cell neoplasm was established. The patient did not receive any treatment.

FISH with locus-specific probes from LSI IgH/CCND1 or t(11;14)/BCL1/IgH (Vysis, Downers Grove, USA) 13q14 (D13S319) (Vysis) and 11q22.3-23.1 (ATM) (YAC clone 755b11) demonstrated that the BCL1 is translocated (not rearranged) into the derivative 13q (Figure 2b), ATM is translocated into 13q and the D13S319 locus is deleted (one copy).

To our knowlege this is the first reported case showing this cytogenetic aberration. It is noteworthy that in the present case two genes, both implicated in different B-cell malignancies, are involved, namely cyclin D1 and D13S319 locus; and that cyclin D1 is translocated and D13S319 locus is deleted. Deletion 13q is one of the most frequent aberrations observed in B-CLL, preferentially with typical morphology. It has been detected in MCL associated with t(11;14)(q13;q32), and is also found in MM related with a poor prognosis. It is interesting to remark that this patient could not be considered to have a B-CLL because the CD23 negativity and bright expression of CD20 and CD79b. In addition the diagnosis of MCL was discarded because BCL1/IgH rearrangement was not detected by FISH. Nevertheless, the finding of this novel cytogenetic aberration involving chromosomes 11 and 13 could explain the diagnosis of unclassified mature B-cell neoplasm according to the WHO classification.[1]

More reports of patients with t(11;13)(q13;q14) are needed to determine the exact prognostic value of this new non-random chromosome anomaly and to study the exact role of these genes in the pathogenesis of lymphoid disorders.

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