would be interesting to monitor patients on completion of their therapeutic program at identical time points and using the same sampling modality.

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Key words: AML, inv(16), CBFB-MYH11, quantitative RT-PCR, ASCIT, fludarabine.

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References


Myeloproliferative Disorders

Hypereosinophilic syndrome and cyclic oscillations in blood cell counts. A clonal disorder of hematopoiesis originating in a pluripotent stem cell

We studied a patient with hypereosinophilic syndrome (HES) who had myeloproliferative features, was unresponsive to imatinib mesylate, and showed cyclic oscillations in blood cell counts. No rearrangement in PDGFRα, PDGFRB and ETV6 genes was detected. Clonal analysis of hematopoiesis consistently showed skewed X-chromosome inactivation patterns in both granulocytes and T-lymphocytes, indicating a clonal myeloproliferative disorder originating in a pluripotent stem cell.

The term hypereosinophilic syndrome (HES) is used to define conditions characterized by elevated eosinophil counts (persistently greater than 1.5×10^9/L), variable damage to end organs such as the heart, lungs, skin, joints and nervous system, and no ascertainable cause for the eosinophilia.1 Abnormal clones of T cells producing interleukin-5 were found in some patients, suggesting that HES can be a clonal T-cell disorder.2 Recent reports,3,4 however, indicate that in most instances HES is a myeloproliferative disease caused by constitutive activation of tyrosine kinases. These observations explain the remarkable efficacy of imatinib mesylate, at least in a portion of HES patients. Myeloproliferative disorders typically result from clonal expansion of mutated hematopoietic stem cells and clonal analysis of hematopoiesis using X-chromosome inactivation patterns has shown that the same clinical phenotype may arise from clonal proliferation of different hematopoietic progenitors.5 A 41-year-old woman presented in 1995 with episodes of pruritus, pulmonary symptoms including wheezing, dyspnea, and cough, fatigue and facial edema. These manifestations worsened in the following years and the patient ultimately required prednisone and inhaled β-agonists to control her symptoms. In October 2002, a complete blood count showed: hemoglobin 13.0 g/dL, white cell count 17.3×10^9/L (eosinophils 30%), and platelets 442×10^9/L. A bone marrow biopsy showed hypercellularity (>70%) with granulocytic hyperplasia and marked eosinophilia. Cytogenetic analysis was normal and polymerase chain reaction analysis was negative for the BCR-ABL rearrangement. Serum eosinophil cationic protein was markedly elevated (246 µg/L, normal range 0–15 µg/L). Chest X-ray and an echocardiogram were normal; stool examination for ova and parasites was negative, and rheumatologic investigations were unremarkable. A diagnosis of idiopathic hypereosinophilic syndrome was made. In February 2003, due to worsening clinical symptoms, treatment with hydroxyurea, 1 g per day, was started. Skin and lung symptoms persisted, so a trial of imatinib, 100 mg daily, was initiated in April 2003. Despite increasing the dose to 400 mg daily, there was no significant improvement in the patient’s hypereosinophilia. Hydroxyurea, 2 g per day, was started again, but this woman benefited most from prednisone: her current treatment (January 2004) consists of prednisone, 12.5 mg/day, and hydroxyurea, 500 mg/day. As shown in Figure 1A, during 2003 marked periodic oscillations were observed in both WBC and platelet count, irrespective of the treatment given (hydroxyurea or imatinib mesylate). In particular, the platelet count fluctuated between 136 and 817×10^9/L. When we examined eosinophil and lymphocyte trend, it was apparent that these cell types also fluc-
Hybridization (FISH) was performed as mRNA expression showed elevated values of rearrangements. Quantitative evaluation of the percentage of fusion gene,1 the range from 0 to 5).

Hydroxyurea

Values ≥ 3.0 indicate ≥ 75% expression of one allele and the expansion of an abnormal clone.

Clonal analysis of hematopoiesis was performed by studying X-chromosome inactivation patterns in purified populations of circulating granulocytes and T lymphocytes by means of an assay for the human androgen receptor (HUMARA) gene. This showed skewed X-chromosome inactivation patterns in both granulocytes and T lymphocytes (Table 1), and fluctuations in these patterns were observed.

The hypereosinophilic syndrome of our patient has many features of a chronic myeloproliferative disorder. In fact, this patient showed skewed X-chromosome inactivation patterns in granulocytes,2 over-expression of granulocyte PRV-1 mRNA, and cyclic oscillations in blood cell counts.3 Cyclic leukocytosis has been previously described, particularly in patients with chronic myeloid leukemia, while cyclic thrombocytosis has been reported in patients with polycythemia vera receiving hydroxyurea.4 However, a case of eosinophilic leukemia with spontaneous oscillations of WBC count, platelet count, hemoglobin level and bone marrow cellularity has been recently reported.5

The patient reported here consistently showed skewed X-chromosome inactivation patterns in both granulocytes and T lymphocytes (Table 1). Although she did not have any of the molecular lesions described so far as causing eosinophilic leukemia, in particular the FIP1L1–PDGFRA fusion gene,6 the above data indicates that our patient has a clonal myeloproliferative disorder originating in a pluripotent stem cell capable of differentiating into granulocytes and T-lymphocytes. From a clinical point of view, it may be worth investigating whether HES with cyclic oscillations in blood cell counts represents a distinct nosologic entity. Based on the present case, we suggest using low doses of hydroxyurea in these patients, since higher doses are associated with greater fluctuations in blood cell counts.

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Key words: hypereosinophilic syndrome, hematopoiesis, X-chromosome inactivation.

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Table 1. Evaluation of X-chromosome inactivation patterns in purified populations of circulating granulocytes and T lymphocytes by means of a HUMARA assay.

<table>
<thead>
<tr>
<th>Date</th>
<th>Granulocytes</th>
<th>T lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>November 2002</td>
<td>3.9</td>
<td>3.3</td>
</tr>
<tr>
<td>May 2003</td>
<td>2.6</td>
<td>2.9</td>
</tr>
<tr>
<td>September 2003</td>
<td>3.0</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Values ≥ 3.0 indicate ≥ 75% expression of one allele and the expansion of an abnormal clone.
8. Vlodopick H, Rupp EM, Edwards CL, Goswitz FA, Beauchamp JJ. Spon-
taneous cyclic leukocytosis and thrombocytosis in chronic granulo-
9. Tefferi A, Elliott MA, Kao PC, Yoon S, El-Hemaidi I, Pearson TC. Hydrox-

Table 1. Area under receiver operating characteristic (ROC) curves for VIDAS D-dimer and the three soluble fibrin monomer (SFM) assays for different categories of DVT (all DVT; proximal DVT; DVT ≥ 4 cm) and for untreated and treated patient groups. Potentially significant differences in coagulation and fibrinolysis. Anticoagulation will decrease thrombin generation while it has less effect on D-dimer production. As a consequence, less fibrin monomer will be formed. In this prospective study, we included consecutive in and outpatients with a clinically suspected first episode of lower limb DVT and no signs of pulmonary embolism (PE), after they had given their signed informed consent. Complete lower limb venous ultra-
sonography (US) was used to exclude or confirm DVT. A full examination of the proximal and distal (infra-popliteal) veins was performed as previously described. A DVT was considered confirmed when both vein non-compressibility and direct visu-
alization of the thrombus were present.

FM: fibrin monomer. SF: soluble fibrin. DVT: deep vein thrombosis. [ ] 95% confidence interval. Each SFM test was compared with VIDAS D-dimer by considering the area under the curve which represents a global test accuracy. The test with the greater area is better. A test whose 95% confidence interval area includes the 0.5 value is not informative. Potential differences between the areas under the curve were assessed by the χ² test. The difference was significant at the 5% level (p value < 0.05). At the time of diagnosis, 91 patients were under prophylactic anticoagulation with low-molecular-weight-heparin (n=81) or unfractionated-heparin (n=10) and 26 patients were under therapeutic anticoagulation with oral anticoagulants (n=7), low-molecular-weight-heparin (n=11) or unfractionated heparin (n=8).