at a rate, although these differences were not statistically significant (p>0.1)(Table 1). According to previous established limits (10% of the mean value in all cases for SD and 8% for CV), good results were obtained for both reproducibility and accuracy control tests.

Previous studies have demonstrated the reliability of the FACSCount cytometer for unstained samples stored for different periods.5,6 This study, using the same technical procedure, investigates the reliability of tests on samples stored after having been incubated with monoclonal antibodies. We show that samples, stored for 24 hours after preparation, remain stable and, therefore, that FACSCount flow cytometry is reliable, especially when the samples were stored at 4°C. These results refer only to this specific method and may not be necessarily be extended to other techniques.7

Table 1. Comparison of counts of samples analyzed on the same day as they were taken and after 24 hours’ storage.

<table>
<thead>
<tr>
<th>Group</th>
<th>Immediate</th>
<th>24 hours</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>1434.78</td>
<td>1500.6</td>
<td></td>
</tr>
<tr>
<td>CD4</td>
<td>476.34</td>
<td>489.0</td>
<td></td>
</tr>
<tr>
<td>CD8</td>
<td>888.65</td>
<td>893.8</td>
<td></td>
</tr>
<tr>
<td>CD3/CD4</td>
<td>0.803</td>
<td>0.775</td>
<td></td>
</tr>
</tbody>
</table>

Key words
Flow cytometry, fluorescence gating, HIV monitoring, lymphocyte subsets, monoclonal antibodies.

Correspondence
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Acute hepatomegaly with severe liver toxicity due to all-trans-retinoic acid

All trans-retinoic acid (ATRA) has improved the outcome of patients with acute promyelocytic leukemia (APL). Despite the fact that ATRA is usually well tolerated, major adverse effects can be observed in a minority of cases. We report here a case of acute life-threatening hepatic toxicity caused by ATRA in a patient with APL.

Sir,

A 40-year old male was admitted to hospital because of gingival and nasal bleeding. His leukocyte count was 15.3×10^9/L (89% blastic forms), hemoglobin level 70 g/L and platelet count 14×10^9/L. His serum lactate dehydrogenase was 2,244 U/L. All other biochemical parameters were in normal ranges (NR) and markers for hepatitis virus were negative. A prolonged prothrombin time and hypofibrinogenemia were noted. Microscopic examination of a bone marrow aspirate revealed acute promyelocytic leukemia (APL). Cytogenetic analysis showed the t(15;17)(q22;q21) and the presence of a transcript corresponding to the PML/RARα fusion gene. The patient began induction treatment consisting of all-trans retinoic acid (ATRA) (45 mg/m²/d) and intravenous idarubicin (12 mg/m²/d on days 2, 4, 6 and 8). He also started prophylaxis against a potential ATRA syndrome with dexamethasone for ten days.

Twenty-one days later, the patient’s general condition worsened. Alkaline phosphatase and γ-glutamyltransferase rose to 370 U/L (normal range, NR: 82-198 U/L) and 198 U/L (NR: 7-43 U/L) respectively, followed by an elevation of direct bilirubin to 39 µmol/L (NR: <10 µmol/L). Aminotransferases levels were normal. At that time, a fast-growing painful hepatomegaly was noted without splenomegaly. Abdominal Doppler ultrasound examination did not find intra- or extrahepatic biliary tract injury.

References
and showed normal blood flow in portal and supra-hepatic veins. Percutaneous liver biopsy was performed and revealed intracellular cholestasis, preservation of hepatic architecture, microbiologic cultures were negative. Renal function was secondarily impaired with oliguria followed by hypotension that required management with vasoactive drugs. Dex-amethasone treatment was begun and, because a bone marrow aspirate confirmed complete remission, ATRA was discontinued. After three days, the clinical symptoms resolved, and there was a reduction in the hepatomegaly; normalization of hepatic and renal function was achieved in the following two weeks. At present, the patient remains in complete morphologic, cytogenetic and molecular remission.

Although ATRA is generally well tolerated, serious adverse effects have been reported, the ATRA syndrome being the most important of them. This syndrome occurs with an incidence ranging from 15 to 27%. ATRA liver toxicity, consisting in mild, transiently increased aminotransferases or bilirubin, is a well-described adverse effect. In our case, the patient developed life-threatening hepatic toxicity consisting of acute hepatomegaly with severe elevation of cholestatic enzymes and secondary renal failure, but without other signs of the ATRA syndrome. We ruled out other causes of acute hepatomegaly and because of the suspicion of hepatic injury by ATRA, this drug was discontinued. The clinical condition of the patient improved dramatically in the following days with normalization of parameters of liver function. The biopsy findings observed were consistent with our hypothesis of ATRA-induced hepatic toxicity.

To our knowledge, only two other cases of severe acute toxicity due to this drug have been reported. Mechanisms of liver damage caused by ATRA are unknown, but impaired glucuronidization of its secreted metabolites and secondary renal failure, but without other signs of the ATRA syndrome. The expression of CBF/MyH11 by means of non-quantitative detection of CBF/MyH11 mRNA has been proposed. Addition of ATRA to other chemotherapeutic drugs, including idarubicin, does not necessarily result in an increase of hepatic toxicity, although other adverse effects could be increased.

Because ATRA therapy can, in some cases, induce a life-threatening hepatic complication, as in the case reported here, liver enzymes should be monitored carefully. If hepatic toxicity becomes apparent, the drug should be withdrawn.

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Key words
Acute promyelocytic leukemia, all-trans-retinoic acid, hematotoxicity, hepatomegaly, adverse effects.

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References

Long-term disease-free acute myeloblastic leukemia with inv(16) is associated with PCR undetectable CBFβ/ MYH11 transcript

This study investigates the benefits of minimal residual disease in a subset of AML patients with inv(16) by means of non-quantitative detection of CBFβ/MYH11 fusion transcript. We found that CBFβ/MYH11 RT-PCR negativity is associated with cure of disease.

Sir,

The expression of CBFβ/MYH11 fusion mRNA provides a potential molecular marker that can be detected in leukemic cells taken from a subset of patients with acute myelocytic leukemia (AML)1-4 (Gene Bank