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

	Means		
	Inmediate	*24 hours*	*p*
Group 1			
CD3	1434.78	1500.6	
CD4	476.34	489.0	
CD8	888.65	893.8	>0.1
RATIO	0.803	0.775	
Group 2			
CD3	1338.45	1342.9	
CD4	284.35	291.1	
CD8	978.75	979.5	>0.1
RATIO	0.37	0.39	

ature, although these differences were not statistically significant (p>0.1)(Table 1). According to previously established limits (10% of the mean value in all cases for SD and 8% for CV)[6] 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 4ºC. These results refer only to this specific method and may not be necessarily be extended to other techniques.[7]

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Key words

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

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References

1. Liu CM, Muirhead KA, George SP, Landay AL. Flow cytometric monitoring of human immunodeficiency virus-infected patients. Simultaneous enumeration of five lymphocyte subsets. Am J Clin Pathol 1989; 92: 721-8.
2. Turner BJ, Hecht FM, Ismail RB. CD4+ T-lymphocyte measures in the treatment of individuals infected with human immunodeficiency virus type 1. A review for clinical practitioners. Arch Intern Med 1994; 154: 1561-73.
3. Castagnola C, Lunghi M, Corso A, et al. Management of acute promyelocytic leukemia relapse in the ATRA era. Haematologica 1998; 83:714-7.
4. Orfao A, Ruiz-Argüelles A. Citometría de Flujo y su aplicación en Hematología. In: A. López Borrasca, ed. Enciclopedia Iberoamericana de Hematología, I. Salamanca: Ediciones Universidad de Salamanca; 1992. p. 161-75.
5. Strauss K, Hannet I, Engels S, et al. Performance evaluation of the FACS Count System: a dedicated system for clinical cellular analysis. Cytometry 1996; 26:52-9.
6. Johnson D, Hirschkorn D, Busch MP. Evaluation of four alternative methodologies for determination of absolute CD4+ lymphocytes counts. The National Heart, Lung, and Blood Institute Retrovirus Epidemiology Donor Study. J Acq Immun Def Synd Hum Retrovirol 1995; 10:522-30.
7. Santagostino A, Garbaccio G, Pistorio A, et al. An Italian national multicenter study for the definition of reference ranges for normal values of peripheral blood lymphocyte subsets in healthy adults. Haematologica 1999 ; 84:499-504.

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 PML/RARα was detected using reverse transcription polymerase chain reaction. The patient began induction treatment consisting of all-trans retinoic acid (ATRA) (45 mg/m^2/d) and intravenous idarubicin (12 mg/m^2/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-glutamyltranspeptidase 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 umol/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

and showed normal blood flow in portal and suprahepatic 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. Dexamethasone 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%.[1-3] ATRA liver toxicity, consisting in mild, transiently increased aminotransferases or bilirubin, is a well-described adverse effect.[1,4,5] 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.[3,6] Mechanisms of liver damage caused by ATRA are unknown, but impaired glucuronidization of its secretion resulting in cholestatic jaundice have been proposed.[7] 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.[8-10]

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, hepatotoxicity, hepatomegaly, adverse effects.

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References

1. Warrell RP Jr, de Thé H, Wang ZY, Degos L. Acute promyelocytic leukemia. N Engl J Med 1993; 329:177-89.
2. De Botton S, Dombret H, Sanz M, et al. Incidence, clinical features, and outcome of all-trans retinoic acid syndrome in 413 cases of newly diagnosed acute promyelocytic leukemia. The European APL Group. Blood 1998; 92:2712-8.
3. Frankel SR, Eardley A, Lauwers G, Weiss M, Warrell RP Jr. The "retinoic acid syndrome" in acute promyelocytic leukemia. Ann Intern Med 1992; 117:292-6.
4. Castaigne S, Chomienne C, Daniel MT, et al. All-trans retinoic acid as a differentiation therapy for acute promyelocytic leukemia. I. Clinical results. Blood 1990; 76:1704-9.
5. Adamson PC, Reaman G, Finklestein JZ, et al. Phase I trial and pharmacokinetic study of all-trans retinoic acid administered on an intermittent schedule in combination with interferon-α2a in pediatric patients with refractory cancer. J Clin Oncol 1997; 15:3330-7.
6. Shibata K, Shimamoto Y, Ishibashi S, Tominaga H, Suga K, Yamaguchi M. Life-threatening hepatic toxicity caused by all-trans-retinoic acid in a patient with acute promyelocytic leukemia. Clin Lab Haematol 1994; 16:191-5.
7. Goss GD, McBurney MW. Physiological and clinical aspects of vitamin A and its metabolites. Crit Rev Cl Lab Sci 1992; 29:185-215.
8. Mandelli F, Diverio D, Avvisati G, et al. Molecular remission in PML/RARα-positive acute promyelocytic leukemia by combined all-trans retinoic acid and idarubicin (AIDA) therapy. Gruppo Italiano-Malattie Ematologiche Maligne dell'Adulto and Associazione Italiana di Ematologia ed Oncologia Pediatrica Cooperative Groups. Blood 1997; 90:1014-21.
9. Estey EH, Thall PF, Pierce S, et al. Randomized phase II study of fludarabine + cytosine arabinoside + idarubicin ± all-trans retinoic acid ± granulocyte colony-stimulating factor in poor prognosis newly diagnosed acute myeloid leukemia and myelodysplastic syndrome. Blood 1999; 93:2478-84.
10. Sacchi S, Kantarjian HM, O'Brien S, et al. Excessive toxicity with the combination of alpha interferon (IFN-α), low dose cytosine arabinoside (LD-ARA-C) and all-trans retinoic acid (ATRA) in patients with chronic myelogenous leukemia (CML). Blood 1998; 92 (suppl 1):4067.

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*