Pseudothrombocytopenia after Allogeneic Non-Myeloablative Stem Cell Transplantation

Shmuel Gillis MD[1*], Michal E. Eisenberg BA[2], Michael Y. Shapira MD[2] and Reuven Or MD[2]

Departments of [1]Hematology and [2]Bone Marrow Transplantation, Hadassah University Hospital, Jerusalem, Israel
Affiliated to Hebrew University-Hadassah Medical School, Jerusalem, Israel

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Pseudothrombocytopenia is an artifact of electronic blood-counting machines that sometimes cause erroneous low platelet counts due to their *in vitro* clumping [1]. Such in-the-tube platelet clumping occurs in blood specimens anticoagulated with EDTA at room temperature at a frequency of 1/1,000 to 1/10,000 [2]. The identification of pseudothrombocytopenia is critical in order to avoid misdiagnosis and mismanagement of a patient's alleged low platelet count. To the best of our knowledge there are no reports to date of pseudothrombocytopenia in a bone marrow transplant setting. We present a case of pseudothrombocytopenia in an adolescent patient after allogeneic non-myeloablative stem cell transplantation.

Patient Description

A 14 year old male (UPN 1461) had been diagnosed at age 3 in 1989 as suffering from acute lymphoblastic leukemia and treated with a standard German ALL protocol. In May 1992 he suffered testicular relapse without bone marrow or cerebral spinal fluid involvement. He was treated with a chemotherapy protocol (RE2-BFM87) and irradiation to the testicles. The patient developed liver damage in 1993 secondary to maintenance methotrexate treatment and the drug was stopped. In 1997 the bone marrow showed CALLA-positive ALL relapse. The patient was treated with a re-induction protocol that included etoposide, cyclophosphamide, vincristine, prednisone, methotrexate, 6MP, vetniporid and Ara-C, and remission was achieved.

In 1999 he suffered a second bone marrow relapse. He was treated with mitoxantrone and Ara-C and was scheduled for non-myeloablative stem cell transplantation from a fully matched unrelated female donor. The patient is a carrier of hepatitis B and C viruses. The conditioning regimen included intravenous fludarabine (30 mg/m^2/day during day -10 to -5), oral busulfan (4 mg/kg/day on days -6 and -5) and anti-T lymphocyte globulin (Fresenius, 10 mg/kg/day during days -4 to -1). On 11 November 1999 the patient was transplanted with 6.46 x 10^8/kg marrow cells. He engrafted on 30 November 1999, having a white blood cell count of 1.0 x 10^9/L and a platelet count of 28 x 10^9/L with normal peripheral blood morphology. Because of the development of graft-versus-host disease grade II with involvement of the skin and gastrointestinal tract, treatment that included cyclosporine and methylprednisone was instituted, leading to a marked improvement. After engraftment, because the patient is a carrier of hepatitis B, he was treated with lamuvidine (150 mg/day).

He was discharged in December 1999 for outpatient follow-up while placed on a drug treatment regimen (cyclosporine per os 200 mg x 2/day, prednisone per urethra 40 mg/day, bactrim twice weekly, and intravenous gancyclovir 5 mg/kg/day 3 times a week). He was consistently negative for cytomegalovirus on polymerase chain reaction for 6 months after NST until he tested positive on 28 June 2000. PCR for both amelogenin and VNTR showed invariably female genotype in peripheral blood and marrow samples, indicating 100% donor chimera.

After NST, the patient's platelet counts showed a steady rise, reaching 152 x 10^9/L. However, a severe platelet drop to approximately 10 x 10^9/L began on 9 July 2000 [Figure 1]. Examination of a peripheral blood smear showed large platelet clumps [Figure 2A and B]. To confirm the diagnosis of pseudothrombocytopenia, counts were performed immediately upon blood withdrawal on specimens anticoagulated with EDTA, citrate and heparin. The platelet count performed on the sample with EDTA was 43 x 10^9/L at core body temperature.

NST = non-myeloablative stem cell transplantation
PCR = polymerase chain reaction

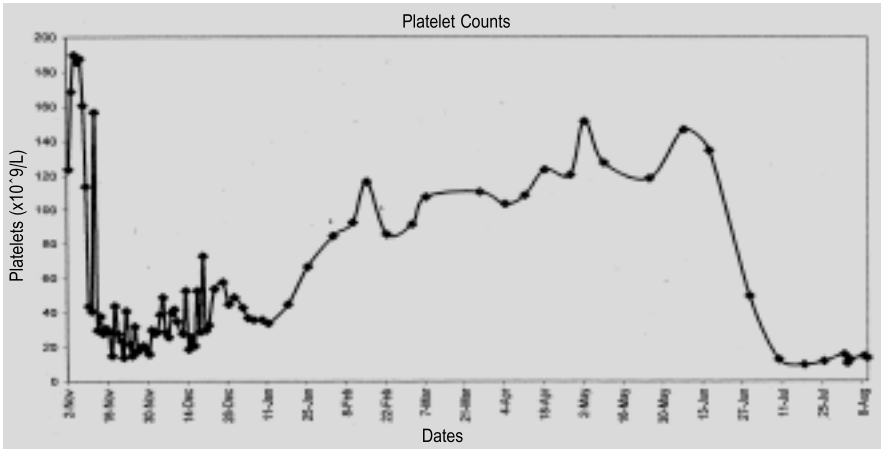

Figure 1. Course of platelet counts over time. Note the steady rise in these counts after NST in November 1999 and the abrupt fall in the apparent counts in June 2000.

* Deceased
ALL = acute lymphoblastic leukemia

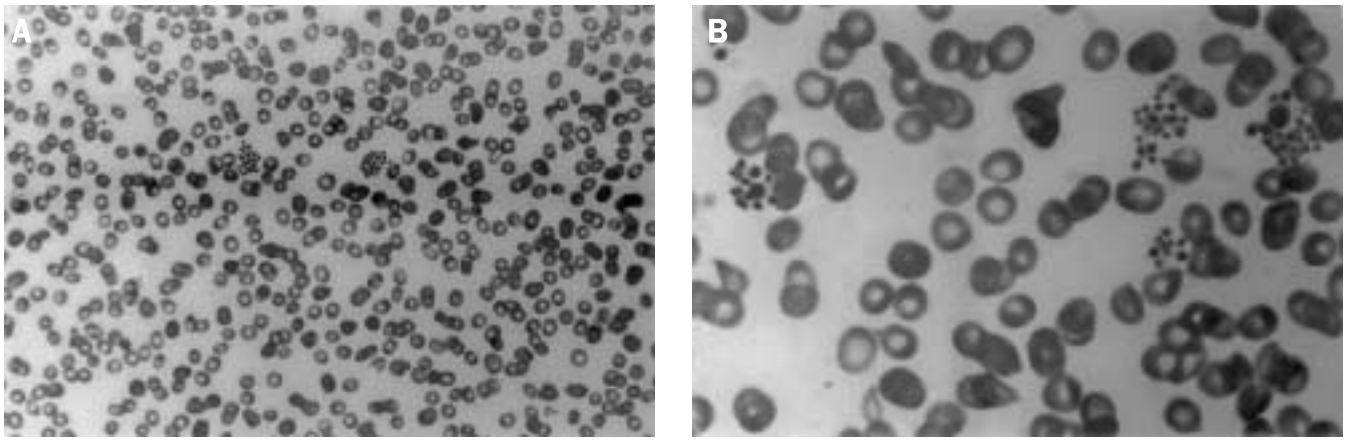

Figure 2 [A]. Peripheral blood smear. Note the numerous large platelet clumps (arrows, magnification x 10). **[B]** Magnification (x 40) of typical appearance of pseudothrombocytopenia in the peripheral blood smear.

After 20 minutes, the count decreased to 13 x 10^9/L platelets and 60 minutes later to 11 x 10^9L, demonstrating a time-dependent progressive decline of the in-the-tube platelet counts. In addition, the automated blood counts anticoagulated with citrate and heparin also showed higher values when counted immediately upon blood drawing (38 x 10^9/L and 51 x 10^9/L, respectively). There was no evidence of any bleeding tendency that would possibly have been present if the patient actually had a low platelet count, further confirming the diagnosis of pseudothrombocytopenia. The phenomenon lasted for 4 months and dissolved spontaneously. At present the patient is free of disease with a Karnofsky score of 100%.

Comment

Pseudothrombocytopenic events have been reported in hospitalized patients and in those treated on an outpatient basis [3], although none of them were associated with bone marrow transplantation. Pseudothrombocytopenia is caused by agglutination of platelets that results from immunoglobulin G or A autoantibodies or from monoclonal proteins, which bind to a cryptic antigen usually on the platelet glycoprotein IIb-IIIa complex that is exposed by ionized calcium *in vitro* [4].

There are three ways to diagnose pseudothrombocytopenia. Firstly, blood specimens anticoagulated with heparin or citrate may show a more accurate platelet count as compared with EDTA. It is noted that these anticoagulants do not always give accurate results, and they are therefore not suited for routine complete blood count; also, anticoagulation with heparin typically provides a lower platelet count than with EDTA. Secondly, immediate automated blood counting – within 5 minutes of obtaining the blood sample – may also produce a more veritable platelet count. Lastly, the most accurate method of identifying pseudothrombocytopenia is evaluation of the blood smear and detection of the platelet clumping.

It is unclear what caused the pseudothrombocytopenia in this acute lymphoblastic leukemia patient after NST. Some studies propose a correlation between infection, such as mumps, rubella, CMV and pseudothrombocytopenia [2]. It is possible that in our patient CMV infection precipitated the development of pseudothrombocytopenia, as the PCR-CMV test converted to positive 1 week prior to the drop in the platelet count. Although we have no evidence for this, it is also possible that reactivation of the hepatitis B or C was associated with the pathogenesis of the pseudothrombocytopenia.

The object of this presentation is to highlight the importance of considering pseudothrombocytopenia when evaluating low platelet counts in the bone marrow transplant setting. Common causes of *de novo* thrombocytopenia post-transplant include relapse of the basic disease, graft rejection, exacerbation of graft-vs-host disease, infection, thrombotic thrombocytopenic purpura, immune mediated thrombocytopenic purpura and overwhelming sepsis. In addition, post-transplant immune thrombocytopenic purpura and thrombotic thrombocytopenic purpura should be considered. Thrombotic thrombocytopenic purpura is a severe and potentially fatal syndrome, which may be associated with cyclosporine administration and occurs in up to 13.6% of allograft recipients [5]. All of these complications would require different approaches and rigorous treatments, and it is therefore vital to rule out pseudothrombocytopenia in these patients in order to avoid misdiagnosis and mismanagement.

It is questionable whether pseudothrombocytopenia is useful as a prognostic tool, and whether it has any effect on long-term outcome on the primary disorder [2,3]. There seem to be no grounds to assume a correlation between the underlying and secondary causes of pseudothrombocytopenia and the progression of the basic disease. Pseudothrombocytopenia is a benign cause of low platelet counts and does not require any treatment, meaning that it has no significance, even in post-bone marrow transplant patients – an observation that is prompted also by the uneventful post-pseudothrombocytopenia course in our transplanted patient.

This report wishes to stress that there are many potential reasons for low platelet counts in the bone marrow transplant setting. The most telling indicator of pseudothrombocytopenia in such a patient is the characteristic platelet clumping in the blood smear. It must be mentioned, however, that since pseudothrombocytopenia is a rare manifestation in bone marrow

CMV = cytomegalovirus

transplant patients, it is not emphasized in the pertinent literature. Nevertheless, in bone marrow transplant patients it should be suspected and ruled out in all cases of low platelet counts, especially when there is no observed bleeding tendency.

References

1. Cohen AM, Cycowitz Z, Mittelman M, Lewinski UH, Gardyn J. The incidence of pseudothrombocytopenia in automatic blood analyzers. *Haematologia Budap* 2000;30:117–21.
2. Berkman N, Michaeli Y, Or R, Eldor A. EDTA-dependent pseudothrombocytopenia: a clinical study of 18 patients and a review of the literature. *Am J Hematol* 1991;36: 195–201.
3. Gschwandtner ME, Siostrzonek P, Bodinger C, et al. Documented sudden onset of pseudothrombocytopenia. *Ann Hematol* 1997;74:283–5.
4. Fiorin F, Steffan A, Pradella P, Bizzaro N, Potenza R, De-Angelis V. IgG platelet antibodies in EDTA-dependent pseudothrombocytopenia bind to platelet membrane glycoprotein IIb. *Am J Clin Pathol* 1998;110: 178–83.
5. Kolker O, Sviri S, Linton DM, Or R, Nagler A, Shapira MY. Plamapheresis for thrombotic thrombocytopenic purpura following bone marrow transplantation. *Acta Haematol* 2001;105(3):156–58.

Correspondence: Dr. R. Or, Dept. of Bone Marrow Transplantation, Hadassah-University Hospital, P.O.Box 12000, Jerusalem 91120, Israel
Phone: (972-2) 677- 8357
Fax: (972-2) 677-7540
email: reuvenor@hadassah.org.il