AIL- Research Fellowship Application

Title: Study of platelet function in patients with Myelodysplastic Syndromes or Myeloid Leukemias

Abstract

Hemorrhagic complications are frequent and among the leading causes of death in patients with myelodysplastic syndromes (MDS) or Myeloid Leukemias. Not only thrombocytopenia, but also platelet function abnormalities contribute to bleeding tendency of these patients. However, no published studies comprehensively evaluated several parameters of platelet function, including the measurement of the platelet levels of cyclic AMP (cAMP), which is an effective inhibitor of platelet function.

In preliminary studies, it was reported that a patients with chronic myelomonocytic leukemia and a personal history of moderate/severe bleeding had severe abnormalities of platelet aggregation and secretion, which could not be fully explained based on the observed abnormalities of the contents of platelet granules and of thromboxane A2 production, and on the observation that major platelet surface glycoproteins were normally expressed. The levels of intraplatelet cAMP in this patient resulted dramatically increased. Another patient was referred to the Ospedale San Paolo for severe bleeding manifestations and was diagnosed with MDS. A thorough platelet function evaluation revealed that he had severe abnormalities of platelet function that were comparable to those observed in the previously reported patient with chronic myelomonocytic leukemia.

This finding suggested that increased cAMP levels may be an important determinant of the bleeding diathesis of these patients.

The aim of this case-control study is to comprehensively assess in vitro platelet function of patients with MDS of any IPSS risk category or Myeloid Leukemias, by enrolling 40 patients and 40 sex-age matched healthy subjects. Platelet aggregation and secretion induced by several agonists will be measured by lumiaggregometry; GPVI, and GPIb on the platelet membrane and the binding of PAC-1 (a monoclonal antibody binding to activated GPIIB/IIIa) will be measured by flow cytometry; platelet content of beta-thromboglobulin, ADP, ATP and serotonin will be measured by standardized assays; the intraplatelet concentration of cAMP at baseline and after stimulation by PGE1 and the serum TXB2 levels will be measured by commercial ELISA kits.

Unravelling the mechanism(s) contributing to the bleeding risk of MDS patients may help improving their management.

Background

Myelodysplastic syndromes (MDS) are a heterogenous group of clonal stem cell disorders characterized by trilineage dysplasia resulting in peripheral cytopenias and increased incidence of leukemic transformation[1].

Hemorrhagic complications can have serious outcomes in patients with MDS and are among the major causes of death in this population. In a chart review referring to the M. D. Anderson Cancer Center database, 460 patients with MDS died without progression to AML. Bleeding was the only cause of death in 48 (10%) of these patients, while it contributed to death in 90 patients (20%)[2]. Other studies reported that hemorrhagic complications account for about 14%-45% of deaths in MDS patients.

Although thrombocytopenia, which is observed in 40-65% of MDS patients, may be a contributing factor[3], bleeding complications are observed also in MDS patients with normal platelet count. As a matter of fact, the frequency of fatal bleeding was evenly distributed among all four International Prognostic Scoring System (IPSS) risk groups[4], which are defined based on 4 parameters, including the platelet count[5]. Platelet function abnormalities are likely responsible for bleeding events in MDS patients with normal platelet count[6].

Hence, several evidences support the idea that both thrombocytopenia and platelet dysfunction contribute to hemorrhagic complications observed in MDS patients[7,8].

Although some abnormalities of platelet function have been documented[9,10], to the best of our knowledge, no published studies evaluated several parameters of platelet function, including platelet aggregation, platelet secretion, the content of platelet granules, thromboxane A_2 production and the levels of cyclic AMP (cAMP), which is a strong inhibitor of platelet function[11], at baseline and after stimulation of adenylyl cyclase with increasing concentrations of prostaglandin E_1 (PGE_1).

Aim of the study

In this study we aim at thoroughly exploring platelet function in patients with MDS or Myeloid Leukemias.

Preliminary data

In 2010 a case report of a woman of 74 years, with no previous bleeding history, was presented[12]. She came to the outpatient clinic of Centro Emofilia A Bianchi Bonomi, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico for occurrence of severe mucocutaneous bleedings. Coagulation tests and VWF were normal. She had mild thrombocytopenia and normocitic anemia, and was later diagnosed with chronic myelomonocytic leukemia. Platelet aggregation and ATP secretion induced by ADP, collagen and U46619 were found to be severely impaired on two separate occasions. CD41/61 and CD42b were normally expressed on her platelets (flow cytometry). The platelet contents of delta-granules (ADP and serotonin) and of alpha-granules (fibrinogen), and serum thromboxane B2 (TXB2) levels were markedly decreased (the patient was not taking non-steroidal anti-inflammatory drugs). The concentration of cyclic-AMP in resting platelets was higher than normal and dramatically increased after exposure of platelets to 1 μmol/L prostaglandin E_1 (PGE_1).[12] Another patient was referred to the Unit at Ospedale San Paolo for severe bleeding manifestations. The patient was diagnosed with MDS and a thorough platelet function evaluation revealed that he had severe abnormalities of platelet function that were comparable to those observed in the previously studied patient with chronic myelomonocytic leukemia.

Methodology

This project will be realized at Ospedale San Paolo, Dipartimento di Scienze della Salute, Università degli Studi di Milano (Milan, Italy). Approval by the Institutional Review Board of the Institution will be requested. The medical staff of Internal Medicine (Medicina III) of Ospedale San Paolo will recruit MDS patients, who will sign and informed consent form. An electronic database for recording clinical, laboratory and experimental data will be created, stored and accessed according to current privacy laws.

Study design

This will be a case control study enrolling about 40 patients with MDS or Myeloid Luekemias and 40 sex-age matched apparently healthy subjects.

All subjects will be eligible for the study if they meet all of the following inclusion criteria:

- ≥18 years of age,
- free of drugs known to affect hemostasis for ≥10 days two before blood sampling,

- no drug or alcohol abuse.

Diagnoses of MDS will be done according to the WHO 2008 Consensus Conference criteria. Ongoing medical treatments will not be modified.
Clinical information including demographics, diagnosis classification, bleeding or thrombosis history, IPSS risk category (score), family history, as well as medication will be collected for each patient.

A venous blood sample (30 mL) will be taken from all enrolled individuals at the same time in the morning. Laboratory tests of platelet function will be performed in vitro using whole blood, platelet-rich plasma and washed human platelets.

1. Platelet aggregation and secretion induced by collagen, ADP, epinephrine, thrombin and convulxin will be measured by lumiaggregometry.
2. Serum TXB2 will be measured by ELISA.
3. The expression of P-selectin, GPVI, and GPIb on the platelet membrane and the binding of PAC-1 (a monoclonal antibody that binds to activated GPIIb-IIIa) will be measured by flow cytometry.
4. The platelet content of beta-thromboglobulin (contained in the platelet alpha-granules), ADP, ATP and serotonin (all contained in the platelet delta-granules) will be measured by standardized assays.
5. The intraplatelet concentration of cAMP will be measured at baseline and after stimulation with increasing concentrations of PGE_1, using a commercially available ELISA kit.
6. Morphological analysis of platelets will be done by electron microscopy (EM).
7. Attempts will be made to unravel the mechanism(s) by which the platelet cAMP levels are elevated in MDS patients, including the study of Gs protein, adenylate cyclase and phosphodiesterase enzymes.

Statistics

Dichotomous variables will be compared by means of Chi-squared, Fisher's Exact or the Wilcoxon Rank-sum test, as appropriate. Differences for continuous variables will be tested for statistical significance by t test, and also multiple regression techniques may be used to

adjust analyses for potential confounding factors. Logarithmic transformations will be applied to data with skewed distributions. Correlations between the studied variables and the severity of the bleeding score will be evaluated.

Milestone

The first 2 months will be employed to request the Institutional Review Board approval and to set up all the experimental procedures. During the following 8 months, recruitment of patients and platelet function studies will take place. The results will then be analyzed and a scientific report will be prepared in the following 2 months.

Facility

The project will be realized at the Laboratory of Hematology and Thrombosis of the Dipartimento di Scienze della Salute of the Università degli Studi di Milano (Milan, Italy) in collaboration with the Unit of Internal Medicine Clinic (Medicina III) of the Ospedale San Paolo (Milan, Italy) under the supervision of professor Marco Cattaneo. The operative units involved can provide facilities, instrumentation and tools necessary to perform all experiments.

Possible critical aspects

The only potential critical aspect could be the difficulty to obtain consensus from our patients to be enrolled in the study.

Relevance and impact of this project

Unravelling the mechanism(s) contributing to the bleeding risk of patients with MDS or Myeloid Leukemias may help improving their management. It has been shown that 14-45% of deaths in MDS patients are caused by pathological bleeding. The overall incidence of MDS is about 4 cases per 100 000 persons, but it dramatically increases to 40-50 per 100 000 persons older than 70 years. Therefore, its social impact is very relevant and will likely increase in the future.

References

1. Bennett JM, Catovsky D, Daniel MT, et al. Proposals for the classification of the acute leukaemias. French-American-British (FAB) co-operative group. British journal of haematology 1976; 33(4): 451-8.
2. Kantarjian H, Giles F, List A, et al. The incidence and impact of thrombocytopenia in myelodysplastic syndromes. Cancer 2007; 109(9): 1705-14
3. Hofmann WK, Ottmann OG, Ganser A, et al. Myelodysplastic syndromes: clinical features. Seminars in hematology 1996; 33(3): 177-85.
4. Neukirchen J, Blum S, Kuendgen A, et al. Platelet counts and haemorrhagic diathesis in patients with myelodysplastic syndromes. European journal of haematology 2009; 83(5): 477-82.
5. Greenberg PL, Tuechler H, Schanz J, et al. Revised international prognostic scoring system for myelodysplastic syndromes. Blood 2012; 120(12): 2454-65.
6. Doll DC, List AF. Myelodysplastic syndromes. The Western journal of medicine 1989; 151(2): 161-7.
7. Maldonado JE, Pierre RV. The platelets in preleukemia and myelomonocytic leukemia. Ultrastructural cytochemistry and cytogenetics. Mayo Clinic proceedings 1975; 50(10): 573-87.
8. Lintula R, Rasi V, Ikkala E, et al. Platelet function in preleukaemia. Scandinavian journal of haematology 1981; 26(1): 65-71.
9. Mittelman M, Zeidman A. Platelet function in the myelodysplastic syndromes. International journal of hematology 2000; 71(2): 95-8.
10. Manoharan A, Brighton T, Gemmell R, et al. Platelet dysfunction in myelodysplastic syndromes: a clinicopathological study. International journal of hematology 2002; 76(3): 272-8.
11. Smolenski A. Novel roles of cAMP/cGMP-dependent signaling in platelets. J Thromb Haemost 2012; 10(2): 167-76.
12. Lecchi A, Artoni A, Mantovani G, Gelain F, Motta M, Cattaneo M. An acquired hemorrhagic disorder associated with a complex phenotype of platelet dysfunction in a patient with chronic myelomonocytic leukemia. Blood Transfusion 2010; 8 (Suppl. 4). XXI Congresso nazionale SISET 2010.