& Speroff, 1994; Pulmonary Embolism Prevention (PEP) Trial Collaborative Group 2000). This case adds strength to this observation because the presence of a severe platelet disorder has not protected the proband suffering recurrent VT. Two previous reports have also shown the occurrence of VT in patients with a severe platelet defect (Gruel et al, 1997; Ten Cate et al, 2003). Interestingly, in the present report, the presence of F5 G1691A and/or other still unknown thrombophilia seemed to attenuate the clinical manifestations of such a severe platelet disorder in the proband. This is illustrated by the severe clinical phenotype of his brother with GT, who died from complications of bleeding compared to the mild bleeding phenotype of the proband, who was diagnosed at 36 years of age. Previous studies have shown that co-inheritance of F5 G1691A with haemophilia A (Nichols et al, 1996) and GT (D'Andrea et al, 2003) can moderate bleeding severity, as seems to be the case here.

In this particular family, the risk factor for thrombosis is not clear, as the proband's brother, who had a CVT, was not carrier of the F5 G1691A mutation, which was present in the proband. It is likely that another risk factor for thrombosis segregates in this family due to the high frequency of venous and arterial thrombotic events in the family members.

To date, recommendations for long-tem anticoagulation in situations in which VT coexists with a severe bleeding disorder, such as GT, are still lacking (Jayanthi & Liesner, 2010). This adds a major challenge for physicians when long-term secondary prophylaxis of thrombosis is indicated. As far as we know, the only report available used low molecular weight heparin for maintenance after the third thrombotic event in a patient with GT, F5 G1691A mutation and thrombosis (Ten Cate et al, 2003). In the present report, the proband has been successfully treated with long-term warfarin at a lower dose and has presented minor bleeding. In conclusion, lower dose warfarin to maintain the INR between 1·5 and 2·0 seems to be safe to prevent recurrent thrombosis in patients with GT.

Suely Meireles Rezende

Department of Internal Medicine, Faculdade de Medicina,
Universidade Federal de Minas Gerais, Avenida Alfredo Balena,
190 – 2nd floor – room 243, 30130–110 Belo Horizonte,
Minas Gerais, Brazil.
E-mail: srezende@medicina.ufmg.br

References

Bertina, R.M., Koeleman, B.P.C., Koster, T., Rosendaal, FR, Dirven, RJ, de Ronde, H, van der Velden, PA & Reitsma, PH. (1994) Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature, 369, 64–67.

Coller, B.S. & Shattil, S.J. (2008) The GPIIb/IIIa (integrin alphaIIbbeta3) odyssey: a technology-driven saga of a receptor with twists, turns, and even a bend. Blood, 112, 3011–3025.

D'Andrea, G., Margaglione, M. & Glansmann's Thrombasthemia Italian Team (GLATIT). (2003) Glanzmann's thrombasthenia: modulation of clinical phenotype by alpha2C807T gene polymorphism. Haematologica, 88, 1378–1382.

Gruel, Y., Pacouret, G., Bellucci, S. & Caen, J. (1997) Severe proximal deep vein thrombosis in a Glanzmann thrombasthenia variant successfully treated with a low molecular weight heparin. Blood, 90, 888–890.

Imperiale, T.F. & Speroff, T. (1994) A meta-analysis of methods to prevent venous thromboembolism following total hip replacement. JAMA, 271, 1780–1785.

Jayanthi, A. & Liesner, R. (2010) Modern management of severe platelet function disorders. British Journal of Haematology, 149, 813–823.

Nichols, W.C., Amano, K., Cacheris, P.M., Figueiredo, M.S., Michaelides, K., Schwaab, R., Hoyer, L., Kaufman, R.J. & Ginsburg, D. (1996) Moderation of hemophilia A phenotype by the factor V R506Q mutation. Blood, 88, 1183–1187.

Pulmonary Embolism Prevention (PEP) Trial Collaborative Group (2000) Prevention of pulmonary embolism and deep vein thrombosis with low dose aspirin: Pulmonary Embolism Prevention (PEP) trial. Lancet, 355, 1295–1302.

Ten Cate, H., Brandjes, D.P., Smits, P.H. & van Mourik, J.A. (2003) The role of platelets in venous thrombosis: a patient with Glanzmann's thrombasthenia and a factor V Leiden mutation suffering from deep venous thrombosis. Journal of Thrombosis and Haemostasis, 1, 394–395.

Salvage therapy of Autoimmune Thrombocytopenic Purpura revealing non-Hodgkin Lymphoma by the thrombopoietin receptor agonist romiplostim

Immune Thrombocytopenic Purpura (ITP) is an autoimmune disease that mainly results from a rapid splenic and hepatic clearance of autoantibody-loaded platelets from the circulation (Koehrer et al, 2010). Secondary causes of ITP must be

excluded at diagnosis, including autoimmune conditions, chronic infection (e.g. Hepatitis C virus, human immunodeficiency virus) and lymphoproliferative disorders (LPD), particularly B-cell chronic lymphocytic leukaemia (B-CLL) (Cines et al, 2009). Treatment options for idiopathic ITP are well defined, including steroids, intravenous immunoglobulins (IVIG) in acute ITP, and the anti-CD20 monoclonal antibody rituximab, splenectomy and immune suppressive drugs in chronic ITP (George, 2010). Recently, a breakthrough in chronic ITP treatment has been achieved with the use of the thrombopoietin receptor (TPO-R) agonists, romiplostim (Nplate; Amgen, Thousand Oaks, CA, USA) and eltrombopag (Promacta; GlaxoSmithKline, Research Triangle Park, NC, USA), which produce rapid dose-dependent increases in the platelet count, even in patients with refractory ITP. Moreover, responses to these new agents are generally sustained during therapy and their long-term use appears to be associated with a good safety profile (Nurden et al, 2009). In B-CLL patients, the occurrence of an autoimmune disorder (AID) negatively impact on the prognosis (Zent et al, 2009). Although no therapeutic guidelines are available in this situation, recent reports emphasized the use of rituximab alone (D'Arena et al, 2010) or in combination with chemotherapy (Kaufman et al, 2009; Bowen et al, 2010) or TPO-R agonist (Koehrer et al, 2010; D'Arena & Cascavilla, 2011). However, other causes of thrombocytopenia, including disease progression, hypersplenism and therapy-related thrombocytopenia, must be ruled out in this situation and this generally requires a bone marrow examination (Zent et al, 2009). In addition to ITP in B-CLL, ITP has occasionally been reported in association with Hodgkin lymphomas or non-Hodgkin lymphomas (NHL) of T or B cell origin. However in those single-case reports, treatment was variable and mostly focused on the lymphoma. We thus report here four cases of life-threatening ITP, diagnosed simultaneously to a B-cell NHL, and primarily managed by using the TPO-R agonist, romiplostim.

This retrospective study was approved by our local Review Board. There were four patients, two males and two females, with a median age of 76 (70–83) years (Table I). Three of them (Patients 1, 3 and 4) had no history of haematological malignancy. Patient 2 had been previously diagnosed with B-CLL, but with an initially normal platelet count. The median

platelet count at presentation was 3 (3–17) × 10⁹/l. The diagnosis of ITP was based on bone marrow smear in three patients and bone marrow biopsy in one, after the exclusion of other causes of peripheral thrombocytopenia. In all cases, the bone marrow examination showed increased megakaryopoiesis and also showed moderate infiltration (30%) by B-CLL in Patient 2 and refractory cytopenia with multilineage dysplasia (RCMD) in Patient 3. The diagnosis of ITP was thus confirmed in Patient 3 by the positivity of specific anti-platelets antibodies; this test was not performed for the other patients. All patients received 1 mg/kg per d intravenous methylprednisolone at the onset of ITP. Two patients received IVIG 0·5 g/kg per d for 2 d (Patients 1 and 3) while a third (Patient 2) was already receiving monthly IVIG treatment as supportive therapy for immune deficiency in the context of B-CLL. Patients 1, 2 and 3 were treated with romiplostim 5 µg/kg per w s.c. after failure of initial treatment; Patient 4 received romiplostim upfront for acute gastrointestinal bleeding. Patient 3 also received rituximab as a salvage therapy.

The mean follow-up was 8·5 (1–18) months. The evolution of the patients' platelet count upon treatment is detailed in Fig 1. In Patient 1, steroids and IVIG treatment failed and romiplostim was used as salvage therapy, which rapidly normalized the platelet count. This enabled a diagnosis of mantle cell lymphoma (MCL) through a laparoscopic abdominal lymph node biopsy, and treatment by rituximab plus CHOP (cyclophosphamide, adriamycin, vincristine and prednisone), then with RDHAP (rituximab, dexamethasone, cytarabine and cisplatin) and finally, high-dose chemotherapy by BEAM (BCNU, etoposide, cytarabine and melphalan) followed by autologous stem-cell transplantation. This patient is still in complete remission of both MCL and ITP. Patient 2 was diagnosed with DLBCL and was treated by three cycles of R-CHOP and then four cycles of cisplatin and navelbin, due to the concomitant diagnosis of lung epidermoid carcinoma. This patient relapsed from B-CLL, but not from DLBCL, and required further treatment by the anti-CD20 monoclonal antibody Ofatumumab. Patient 3, who had a concomitant diagnosis of RCMD, was diagnosed with marginal-zone lymphoma (MZL) and died from cerebral bleeding due to refractory thrombocytopenia. For Patient 4, romiplostim was initiated immediately after the diagnosis of ITP and resulted in a rapid normalization of the platelet count. An attempt to

Table I. Evolution of the four patients with NHL-related ITP treated by romiplostim in this study.

Patients	Age	Sex	Plt	Lymphoma	CTS	IG	R	TF	Plt/R	Treatment	Outcome	FU
1	70	M	5	MCL	+	+	−	−	100	RCHOP, RDHAP, ASCT	A	18
2	76	M	17	DLBCL	+	+	+	−	90	RCHOP, navelbin, cisplatin	A	12
3	81	F	3	MZL	+	+	+	+	36	–	D	1
4	83	F	3	MZL	+	−	+	−	202	Rituximab	A	3

Plt, platelet count at diagnosis (×10⁹/l); CTS, corticosteroids; IG, intravenous immunoglobulin; R, rituximab; TF, platelet transfusion; Plt/R, platelet count after romiplostim (×10⁹/l, maximum); FU, follow-up (months); M, male; F, female; MCL, mantle-cell lymphoma; DLBCL, diffuse large B-cell lymphoma; MZL, marginal zone lymphoma; ASCT, autologous stem cell transplantation; RCHOP, rituximab, cyclophosphamide, adriamycin, vincristine, prednisone; RDHAP, rituximab, dexamethasone, cytarabine and cisplatin; A, alive; D, dead.
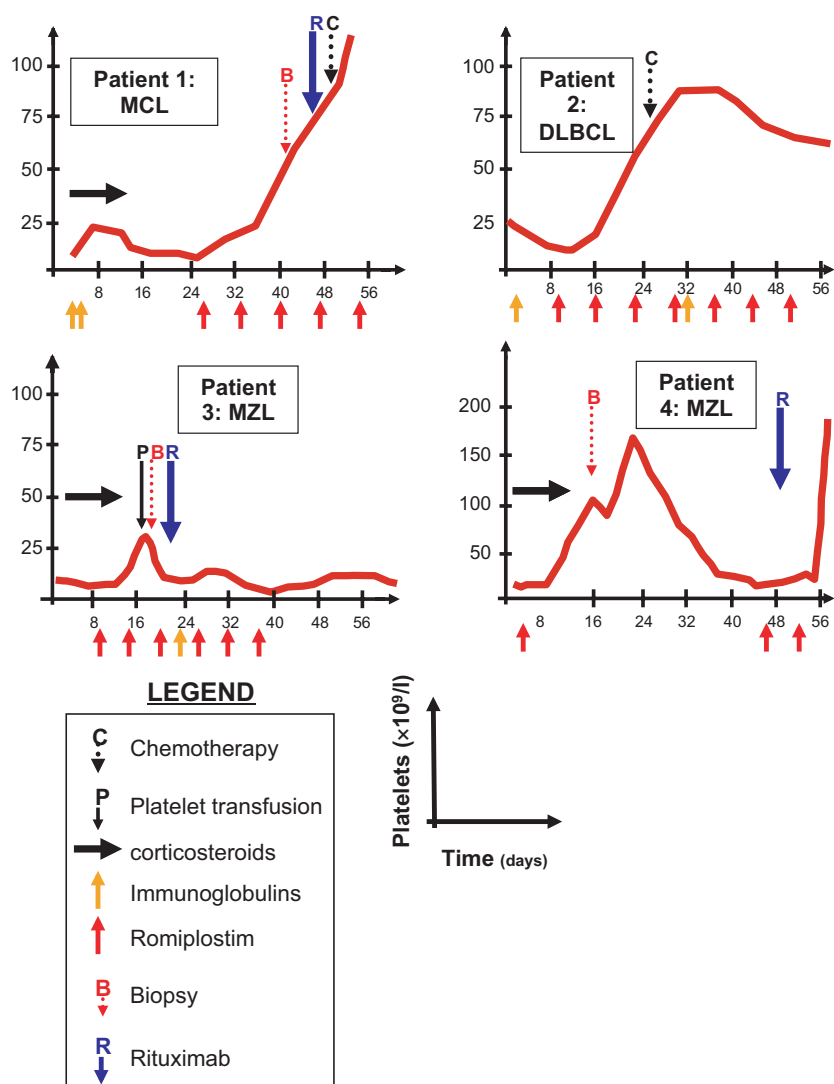
Fig 1. Evolution of the platelet count following treatment in the four study patients. MCL, Mantle Cell Lymphoma; DLBCL, Diffuse Large B-Cell Lymphoma; MZL, Marginal Zone Lymphoma.

discontinue romiplostim led to a rapid relapse, which was rapidly corrected after the reintroduction of romiplostim (Fig 1).

Lymphoma-associated ITP is an uncommon condition for which no consensual therapeutic approach is currently available. The TPO-R agonists' romiplostim and eltrombopag are generally well tolerated and their immediate side effects are moderate. In particular, an increase in marrow reticulin was reported in a few patients, which generally resolved upon treatment discontinuation (Nurden *et al*, 2009). However in the case of our patients, treatment with romiplostim had multiple goals: (i) to overcome a life-threatening ITP refractory to conventional treatment, (ii) to obtain the histological diagnosis of the lymphoma through a safe and unmodified (e.g. by steroids and/or rituximab) tissue sample biopsy, (iii) to eventually postpone splenectomy and/or to limit the use of steroids in severely immunocompromised patients and (iv) to

avoid excessive toxicity of the megakaryocyte lineage after the initiation of chemotherapy. Treatment of the underlying lymphoma was required for two patients, and appeared to be essential for the long-term control of ITP in those cases, as reported (Zent *et al*, 2009). However, both patients received rituximab, vincristine and cyclophosphamide, which are also used in chronic ITP and may explain the improved platelet count (George, 2010). Importantly, the cost/benefit balance should also be considered when using a TPO-R agonist. However, we considered only a time-limited use of those molecules and the cost may be even less than that of IVIG, although this assertion should be verified in future studies.

Author contributions

Study design: Jerome Tamburini and Didier Bouscary; Data analysis: Chadi Al-Nawakil, Sophie Park, Nicolas Chapuis,

Correspondence

Francois Dreyfus, Tali-Anne Szwebel, Laure Gibault, Thierry Molina, Olivier Hermine, Didier Bouscary and Jerome Tamburini; Manuscript writing: Chadi Al-Nawakil, Didier Bouscary and Jerome Tamburini; Patient enrolment: Sophie Park, Olivier Hermine, Didier Bouscary and Jerome Tamburini. All co-authors approved the final version of the manuscript.

Chadi Al-Nawakil[1,2]
Sophie Park[1,2]
Nicolas Chapuis[1,3]
Francois Dreyfus[1,2]
Tali-Anne Szwebel[1,4]
Laure Gibault[1,5]
Thierry Molina[1,6]
Olivier Hermine[1,7]
Didier Bouscary[1,2]
Jerome Tamburini[1,2]

[1]Université Paris Descartes, [2]Service d'Hématologie Clinique, Groupe Hospitalier Cochin-Hôtel-Dieu, Assistance Publique-Hôpitaux de Paris (AP-HP), [3]Service d'Hématologie Biologique, Groupe Hospitalier Cochin-Hôtel-Dieu, (AP-HP), [4]Service de Médecine Interne, Groupe Hospitalier Cochin-Hôtel-Dieu, Site Hôtel-Dieu (AP-HP), [5]Service d'anatomie pathologique, Groupe Hospitalier Cochin-Hôtel-Dieu, site Cochin, (AP-HP), [6]Service d'anatomie pathologique, Groupe Hospitalier Cochin-Hôtel-Dieu, site Hôtel-Dieu, (AP-HP), and [7]Service d'Hématologie Clinique, Groupe Hospitalier Necker-Enfants Malades (AP-HP), Paris, France.
E-mail: jerome.tamburini@cch.aphp.fr

References

Bowen, D.A., Call, T.G., Shanafelt, T.D., Kay, N.E., Schwager, S.M., Reinalda, M.S., Rabe, K.G., Slager, S.L. & Zent, C.S. (2010) Treatment of autoimmune cytopenia complicating progressive chronic lymphocytic leukemia/small lymphocytic lymphoma with rituximab, cyclophosphamide, vincristine, and prednisone. *Leukaemia & Lymphoma*, **51**, 620–627.

Cines, D.B., Liebman, H. & Stasi, R. (2009) Pathobiology of secondary immune thrombocytopenia. *Seminars in Hematology*, **46**, S2–S14.

D'Arena, G. & Cascavilla, N. (2011) Romiplostim for chronic lymphocytic leukemia-associated immune thrombocytopenia. *Leukaemia & Lymphoma*, **52**, 701–704.

D'Arena, G., Capalbo, S., Laurenti, L., Del Poeta, G., Nunziata, G., Deaglio, S., Spinosa, G., Tarnani, M., De Padua, L., Califano, C., Ferrara, F. & Cascavilla, N. (2010) Chronic lymphocytic leukemia-associated immune thrombocytopenia treated with rituximab: a retrospective study of 21 patients. *European Journal of Haematology*, **85**, 502–507.

George, J.N. (2010) Management of immune thrombocytopenia – something old, something new. *New England Journal of Medicine*, **363**, 1959–1961.

Kaufman, M., Limaye, S.A., Driscoll, N., Johnson, C., Caramanica, A., Lebowicz, Y., Patel, D., Kohn, N. & Rai, K. (2009) A combination of rituximab, cyclophosphamide and dexamethasone effectively treats immune cytopenias of chronic lymphocytic leukemia. *Leukaemia & Lymphoma*, **50**, 892–899.

Koehrer, S., Keating, M.J. & Wierda, W.G. (2010) Eltrombopag, a second-generation thrombopoietin receptor agonist, for chronic lymphocytic leukemia-associated ITP. *Leukemia*, **24**, 1096–1098.

Nurden, A.T., Viallard, J.F. & Nurden, P. (2009) New-generation drugs that stimulate platelet production in chronic immune thrombocytopenic purpura. *Lancet*, **373**, 1562–1569.

Zent, C.S., Ding, W., Reinalda, M.S., Schwager, S.M., Hoyer, J.D., Bowen, D.A., Jelinek, D.F., Tschumper, R.C., Call, T.G., Shanafelt, T.D., Kay, N.E. & Slager, S.L. (2009) Autoimmune cytopenia in chronic lymphocytic leukemia/small lymphocytic lymphoma: changes in clinical presentation and prognosis. *Leukaemia & Lymphoma*, **50**, 1261–1268.

Chronic lymphocytic leukaemia responsive to vitamin D administration

Since the 1980s, the extra-osseous effects of vitamin D (VD) have attracted growing attention, mainly related to immunomodulation and tumour inhibition. Many normal tissues, as well as some tumour cells (colon, pancreas, breast, prostate, leukaemias, etc.), display nuclear receptors specific for VD (VDR). Several epidemiological studies have suggested an inverse association between serum 25-hydroxyvitaminD [$25(OH)_2 D_3$] concentration, and the incidence of several solid cancers (Luong & Nguyen, 2009). More recently, Shanafelt *et al* (2011) reported, for the first time, that vitamin D (VD) insufficiency is associated with lower survival in chronic lymphocytic leukaemia (CLL) patients. Nevertheless, the therapeutic efficiency of VD in CLL has not been reported to date.

We report on an unexpected observation of a spectacular 13-month remission of CLL after the administration of cholecalciferol in an elderly patient with VD deficiency. To further explore this observation, we studied the *in vivo* and *in vitro* effects of VD on the lymphocytes of this and one other VD-deprived CLL patient.