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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.

The patient (P) was a 90-year-old man with chronic B-cell lymphocytic leukaemia (Binet stage A, Matutes score 5/5) and VD deficiency (25(OH)D, 25 nmol/l). His lymphocyte count had been stable for 1 year (around 12×10^9/l). One patient was selected as control (C) for comparison: she was a 81-year-old woman with the same leukaemia (Binet stage A, Matutes 4/5) and VD deficiency [25(OH)D, 45 nmol/l], hospitalized consecutively after P. Both patients received oral cholecalciferol to treat their VD deficiency, but no other new treatment was given for the whole duration of their observation. They gave written informed consent for the *in vitro* studies, with approval by the ethic committee of our institution. Figure 1A illustrates the effect of cholecalciferol treatment on the lymphocyte count in P and C. In P, the lymphocyte count rapidly reduced, from $14{\cdot}6 \times 10^9$/l (89% monoclonal B-cells) to $2{\cdot}0 \times 10^9$/l (55%) on week 9 after four oral doses of cholecalciferol (100 000 units/dose), when an adequate level of VD was obtained [25(OH)D 90 nmol/l]. After 9 months of remission, the lymphocyte count increased to 5×10^9/l concomitant to relative VD insufficiency [25(OH)D, 62·5 nmol/l]. An additional dose of cholecalciferol was efficacious but, 13 months after the start of VD therapy, the lymphocyte count increased rapidly (up to 33×10^9/l) until lethal pneumonia occurred in P, after further VD therapy (reinitiated 6 weeks before death) had shown no effect on the lymphocyte count (Fig 1A). VD administration exerted no sizeable effect on the lymphocyte count in C (Fig 1A).

We studied the *in vitro* apoptotic effects of VD on lymphocytes cultured for 48 h in the presence of VD3 (100 nmol/l). Lymphocytes were isolated by density centrifugation on Ficoll-Hypaque, and then suspended in RPMI medium supplemented with 10% fetal calf serum. We used real-time quantitative polymerase chain reaction (RQ-PCR) to measure the expression of VD receptor

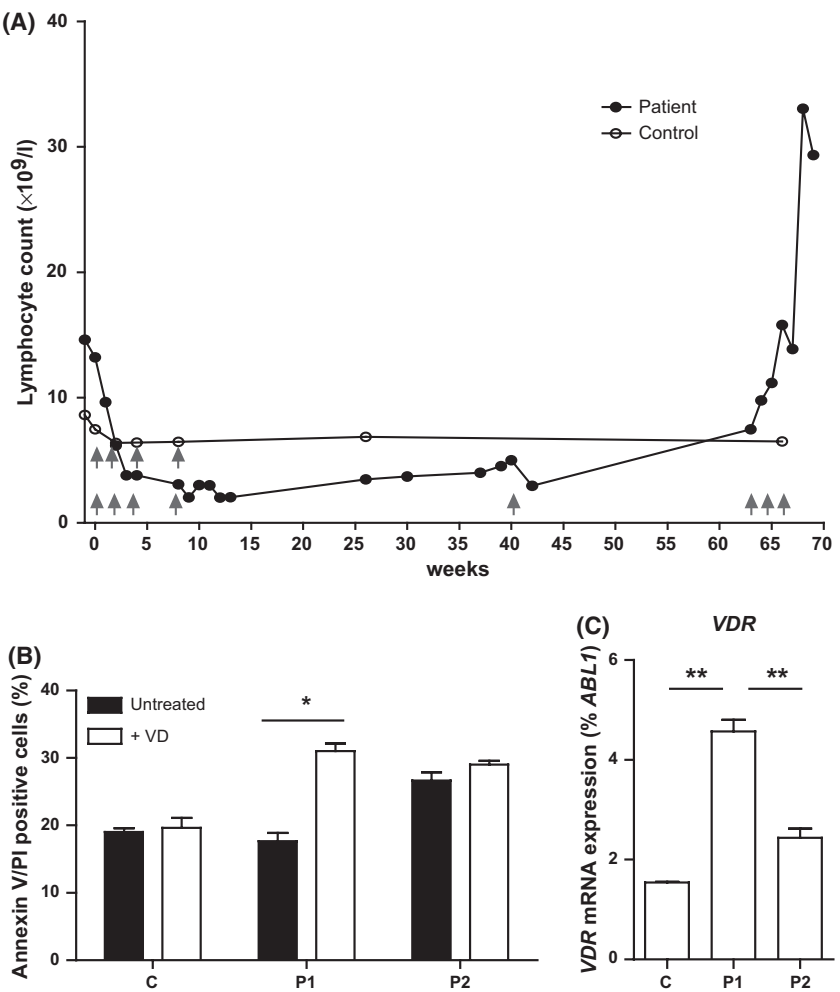

Fig 1. Antiproliferative and pro-apoptotic *in vivo* and *in vitro* effects of VD on CLL lymphocytes. (A) Evolution of blood lymphocyte count (×10⁹/l, vertical axis) with time (weeks, horizontal axis) in the patient (P, solid circles) and control (C, open circles). Arrowheads display administration of cholecalciferol (100 000 units) in P (red) and C (blue). B, C: *in vitro* studies: (B) percentage of double positive annexin V (AV) and propidium iodide (PI) cells staining by flow cytometry after 48 h of cell cultures with VD in the patient (P1 and P2, culture at the start of VD repletion and before death respectively) and control (C). (C) *VDR* mRNA expression in P1, P2 and C lymphocytes measured by RQ-PCR relative to *ABL1* transcript.

(*VDR*) relative to *ABL1* transcript, and flow cytometry to study the apoptotic effect of VD. VD induced apoptosis of cultured lymphocytes in P prior to remission, but not in C or in P at relapse (Fig 1B, C). Response to VD correlated with VDR expression (Fig 1C).

Pepper *et al* (2003) demonstrated that lymphocytes from CLL patients overexpress variable levels of VDR, and that an analog of 1-25$(OH)_2$ D_3, can induce apoptosis of these cells *in vitro*. Our observations strongly suggest that these *in vitro* concepts may hold true *in vivo*, with VD supplementation at the conventional dose. This effect seems to be related to the level of VDR expression in lymphocytes. It could be speculated that patients with higher VDR levels could have the best response to VD therapy. Our late observations in P also suggest that VD resistance may also develop. A clinical trial is now needed to determine whether there is room for VD in the treatment of CLL.

Conflict of interest

All authors declare no competing financial interests.

Author contributions

JBA, LC, JP contributed to diagnosis and management of the patient, accumulation of clinical data, and wrote the manuscript; JBA, CC, EM and OH contributed to the *in vitro* experiments and wrote the manuscript.

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References

Luong, K. & Nguyen, L.T. (2009) The beneficial role of vitamin D and its analogs in cancer treatment and prevention. *Critical Reviews in Oncology/hematology*, **73,** 192–201.

Pepper, C., Thomas, A., Hoy, T., Milligan, D., Bentley, P. & Fegan, C. (2003) The vitamin D3 analog EB1089 induces apoptosis via a p53-independent mechanism involving p38 MAP kinase activation and suppression of ERK activity in B-cell chronic lymphocytic leukemia cells in vitro. *Blood*, **101,** 2454–2460.

Shanafelt, T.D., Drake, M.T., Maurer, M.J., Allmer, C., Rabe, K.G., Slager, S.L., Weiner, G.J., Call, T.G., Link, B.K., Zent, C.S., Kay, N.E., Hanson, C.A., Witzig, T.E. & Cerhan, J.R. (2011) Vitamin D insufficiency and prognosis in chronic lymphocytic leukemia. *Blood*, **117,** 1492–1498.

Comment on Guidelines on oral anticoagulation with warfarin – 4th edition

In response to the recently updated guidance for anticoagulation with warfarin (Keeling *et al*, 2011), we were disappointed to note that there was no consideration given to the issue of left ventricular thrombus and anticoagulation. This is a not uncommonly encountered scenario, and one for which little robust evidence exists to assist with the management.

Left ventricular thrombus is commonly seen in the context of severely reduced ventricular function and low-flow states, and may occur in myocardial infarction or other non-ischaemic cardiomyopathy. Left ventricular thrombus may be asymptomatic and detected during investigations such as echocardiography or left ventriculography, or alternatively may give rise to cardio-embolic phenomena.

Once detected, there is little available evidence to guide duration of therapy in this group of patients, and the decision becomes even more complex in ischaemic cardiomyopathy with coronary artery stenting when patients are already on antiplatelet therapy.