

Parathyroid Hormone-Related Protein-Associated Hypercalcemia in a Patient with CLL Type Low Grade Leukemic B-Cell Lymphoma

Humoral hypercalcemia of malignancy is a common metabolic disturbance associated with solid tumors, but it also occurs in lymphoma patients. Among these, low grade B-cell lymphoma accounts for only few cases, in which secretion of parathyroid hormone-related protein (PTHrP) remains even exceptional. We report the very rare case of a patient with a CLL type low grade leukemic B-cell lymphoma showing PTHrP-related hypercalcemia without evidence of bone lesions. Using immunohistochemistry, we demonstrate the cytoplasmic expression of PTHrP by the lymphoma cells in the bone marrow obtained at the onset of hypercalcemia. We postulate a pathogenetic role of leukemic cell production and secretion of PTHrP in hypercalcemia in low grade leukemic B-cell lymphoma.

Haematologica 2006; 91:(9)e119-e122

Hypercalcemia is a common paraneoplastic syndrome in patients with malignant diseases and is one of the most serious metabolic disorders associated with cancer. It may occur due to osteolytic metastases in some solid tumors and on the other hand due to the secretion of humoral factors (humoral hypercalcemia of malignancy, HHM).¹ Several solid tumors such as breast, non-small cell lung, and renal cancer or advanced stage squamous cell tumors of the head and neck or esophagus have been described to lead to HHM, mainly by secretion of parathyroid hormone-related protein (PTHrP).^{2,3} Furthermore, HHM is also associated with hematologic malignancies: About 30% of myeloma patients present with elevated serum calcium levels in the course of the disease or at diagnosis. In adults with T-cell leukemia or lymphoma HHM is diagnosed in up to 40% of cases. In contrast, only up to 8.5% of patients with B-cell lymphoma (B-NHL) develop hypercalcemia.⁴ Apart from the possible coincidence of primary hyperparathyroidism with B-NHL – which has to be ruled out due to its incidence of 3% in patients more than 50 years of age⁵ – hypercalcemia is most likely to be mediated by 1,25-dihydroxyvitamin D₃ (calcitriol, 1,25(OH)₂D₃), which is secreted from lymphoma-adjacent macrophages.^{6,7} However, PTHrP-related HHM in B-NHL has been described in few case reports only^{8,9}, again in contrast with (human T-cell lymphotropic virus type 1-associated) T-cell malignancies, in which PTHrP secretion contributes to the majority of cases with hypercalcemia.¹⁰ Interestingly, with rare exemptions, the underlying B-NHL usually is high or intermediate grade according to the WHO classification. Only anecdotally, PTHrP-mediated HHM associated with low grade B-NHL has been described.^{11,12}

Here, we report a patient with CLL type low grade leukemic B-cell lymphoma presenting with symptomatic hypercalcemia. On detection of elevated serum PTHrP levels we aimed to directly attribute HHM to paraneoplastic synthesis of PTHrP by lymphoma cells. For this purpose, we performed immunohistochemical examinations of bone marrow specimens obtained at manifestation of the metabolic disorder and at diagnosis of the underlying disease.

Case report

A 69-year-old caucasian male first presented with thrombocytopenia, anemia, leukocytosis (96% lymphocytes), modest lymphadenopathy and moderate splenomegaly in November 2002. 2% of cells in the peripheral blood smear were microscopically determined to be prolymphocytoid cells. Bone marrow smears revealed 95% lymphocytic cells with prolymphocytes and paraimmunoblasts, the latter together accounting for up to 20% of bone marrow cells. Immunophenotyping of the peripheral blood showed positivity of CD19, CD23, CD27, and partially CD20 and CD11c, but negativity of CD5, matching the differential diagnosis of lymphoplasmocytic lymphoma. Pathologists of the local German reference institute of lymph node pathology, however, diagnosed CD5- B-CLL with increased prolymphocyte number from the bone marrow biopsy. Lymphoplasmocytic lymphoma was ruled out due to the diffuse rather than nodular infiltration of the bone marrow, and the absence of both plasmocytoid differentiation and cytoplasmic immunoglobuline expression of the lymphoma cells, marginal zone B-cell lymphoma due to both the cell morphology and weak or partial expression of CD20 and CD11c on the lymphoma cells obtained from peripheral blood and the absence of cyclin D expression of lymphoma cells in the bone marrow. There was no clear evidence of transformation into Richter's syndrome from the bone marrow biopsy. A lymph node biopsy was not performed. Karyotype analysis revealed multiple aberrations [45, X, -Y, del(1)(q32q42), ins(6)(q21), dup(8)(q22q24), del(9)(q21), del(11)(q21q23)]. Retrospectively, the atypical morphological, immunohistochemical, and immunophenotypical findings were summarized to the diagnosis CLL type low grade leukemic B-cell lymphoma.

From January to March 2003 the patient received 3 courses of CHOP chemotherapy (cyclophosphamide, vincristine, doxorubicin, prednisone), but transfusion-dependent anemia and leukocytosis remained unaffected, and thrombocytopenia only moderately improved. 2 courses of fludarabine and cyclophosphamide (FC) were added after exclusion of autoimmune hemolytic anemia (AIHA), which led to a partial remission. Thrombocytopenia deteriorated only 4 months later, and disease progression required treatment in February 2004. Due to the coincidental development of AIHA, a combination of cyclophosphamide, mitoxantrone, and rituximab was chosen. Because this regimen failed to achieve a remission, therapy was changed to bendamustine and rituximab which led to a good response. Low dose oral prednisone was continually administered to control AIHA. Some weeks later, a severe pneumonia resistant to antibacterial therapy and equivocal of pulmonary mycosis was treated with itraconazole. On detection of CMV DNA in the bronchoscopy and positivity of serum pp65 antigen, additional therapy with ganciclovir and later foscavir was necessary. Partial response of the lymphoma remained stable until 6 months later, he presented again with symptomatic thrombocytopenia and pulmonary lesions equivocal of a manifestation of the lymphoma. A lung biopsy histologically revealed necrotic tissue, but microbiological examination of the material did not yield any evidence for infectious disease as e. g. invasive mycosis or mycobacterial infection. 2 courses of bendamustine were given, and after a period of only 3 months disease progression again required a change of therapy to ifosfamide, etoposide, and epirubicin (IVE), but the regimen failed to induce a response. Finally, in April 2005 the patient presented with asymptomatic hypercalcemia for

Table 1. Relevant laboratory data of the patient during the course of the disease.

	normal value	November 2002	April 2005	June 20 2005	June 25 2005	June 30 2005	July 2005
lLeukocytes [G/L]	5.0 - 10.0	81.7	45.0	16.8	26.0	44.1	91.5
Lymphocytes [%]	25 - 40	96	89	70	80	79	75
Prolymphocytes [%]	0	2	7	2	3	2	2
Hemoglobin [g/dL]	14.0 - 18.0	11.0	10.7	9.0	11.1	9.3	10.1
Thrombocytes [G/L]	150 - 450	44	15	6	6	31	14
Creatinine [mg/dL]	0.5 - 1.1	1.1	1.2	1.9	1.7	1.2	1.3
LDH [U/L]	135 - 225	367	356	250	255	272	175
CRP [mg/dL]	0.0 - 0.5	2.01	2.97	9.35	6.57	3.59	0.52
sodium [mmol/L]	135 - 145	140	136	142	143	141	137
potassium [mmol/L]	3.5 - 5.0	4.6	4.1	4.3	4.0	4.3	4.1
calcium [mmol/L]	2.0 - 2.7	2.3	3.3	4.7	3.7	4.0	3.5
ALP [U/L]	40 - 129	118	153	287	308	244	200
PTHrP [pmol/L]	0.0 - 1.3		13.0				
PTH [pg/mL]	12.0 - 72.0		5.9				
25(OH)D ₃ [ng/mL]	6.5 - 54.8		10.5				
1,25(OH) ₂ D ₃ [pg/mL]	35 - 80		47				

LDH: lactic dehydrogenase, CRP: C-reactive protein, ALP: alkaline phosphatase, PTHrP: parathyroid hormone-related protein, PTH: parathyroid hormone, 25(OH)D₃: 25-hydroxy-vitamin D₃, 1,25(OH)₂D₃: 1,25-dihydroxy-vitamin D₃.

the first time. Bendamustine, rituximab, and bisphosphonate therapy with pamidronic acid (3-amino-1-hydroxypropylidene-diphosphonic acid) returned serum calcium levels to normal ranges. Scintigraphy of the skeleton and osteodensitometry did not reveal either osteolytic lesions or osteopenia. A bone marrow biopsy confirmed the initial diagnosis, again without clear evidence of transformation into Richter's syndrome. A biopsy of a lymph node was refused by the patient. Pneumonia caused by oxacilline-resistant *Staphylococcus aureus* (ORSA) was successfully treated in May 2005. The final admission in June 2005 was again due to disease progression-associated hypercalcemia (4.7 mmol/L) with neurological symptoms and typical electrocardiographic (ECG) findings. Prolymphocytes in the peripheral blood did not exceed the percentage detected at diagnosis. Serum intact PTH levels were markedly suppressed (5.9 pg/mL). Serum 25-hydroxyvitamin D₃ (25(OH)D₃) and 1,25(OH)₂D₃ levels were within low normal ranges (10.5 ng/mL and 47 pg/mL, respectively). However, serum PTHrP was 10fold elevated to 13.0 pmol/L. Intravenous hydration, furosemide, and pamidronic acid were administered. Intermediate dose prednisone was added for a minimum control of the underlying disease and reduction of intestinal calcium absorption. Neurological symptoms resolved and ECG abnormalities disappeared, but serum calcium levels did not improve sufficiently despite repeated pamidronic acid infusions. The patient was discharged some days after a final application of zoledronic acid ([1-hydroxy-2-(imidazole-1-yl)ethylidene]diphosphonic acid) and died at home few days later. Relevant laboratory findings are shown in Table 1.

Materials and Methods

Serum PTHrP was measured in a standard immunoluminescence assay (ILMA). Serum intact PTH and 25(OH)D₃ levels were determined by standard chemoluminescence immunoassays (CLIA), 1,25(OH)₂D₃ was measured in a radioimmunoassay (RIA).

For immunohistochemical detection of PTHrP, bone marrow slides were deparafinized with xylol. Xylol was washed out with alcohol in serial dilutions. Immunoepitope retrieval was performed in 10 mM citrate buffer in a microwave oven. After triton permeabi-

lization, tissue sections were incubated with PTHrP polyclonal rabbit antiserum (Abcam, www.abcam.com), directed against N-terminal PTHrP, at a concentration of 3 µg/mL overnight at 4°C. After rinsing in PBS, slides were blocked with 3% normal goat serum. Primary antibody was detected with DAKO Envision® labelled polymer-AP Fast Red System (Dakocytomation, Denmark) according to the manufacturer's protocol. Cells were counterstained with hematoxylin (Dakocytomation, Denmark). Negative controls were performed using the DAKO Envision® labelled polymer-AP Fast Red System without prior incubation with PTHrP antiserum.

Pictures were created using a Nikon Eclipse TE 2,000-U microscope, a Nikon DXM 1,200 camera, and the Nikon ACT-1 V.2.12 software.

Results and Discussion

The immunohistochemical finding of prominent cytoplasmic expression of PTHrP in the leukemic cells in the bone marrow specimens obtained at the onset of hypercalcemia strongly supports the assumption that secretion of PTHrP by the lymphoma cells was responsible for the metabolic disturbance in our patient. Normal serum calcium levels measured at diagnosis of the lymphoma coincided with the lack of PTHrP expression by lymphoma cells in a bone marrow specimen obtained at that time point (*data not shown*). Histological and immunohistochemical findings are illustrated in Figure 1.

In contrast to solid tumor diseases and T-cell malignancies, HHM is a rare complication in B-NHL. While occurrence in high or intermediate grade B-NHL has been reported to be up to 8.5% of cases,⁴ this paraneoplastic syndrome turns out to be very rare in low grade B-cell lymphoma: In one patient with cleaved cell B-NHL, HHM was described, but the mediator of hypercalcemia was not identified, possibly because only intact PTH and 1,25(OH)₂D₃ serum levels were measured, but PTHrP levels were not.¹⁵ In another report,^{11,2} patients with low grade B-NHL were described to have developed HHM, but only one of them was confirmed to have low grade B-NHL at the onset of hypercalcemia. The second one showed transformation into Richter's syndrome when elevated serum calcium levels were first detected. In none of the 2 cases mentioned, serum levels of potential medi-

leukocytosis indicated disease progression. However, from the time of diagnosis to the final admission, there was no evidence of transformation into Richter's syndrome nor did the percentage of prolymphocytoid cells in the peripheral blood increase to numbers resembling a progression into a prolymphocytoid leukemia-like state of the CLL type low grade leukemic B-cell lymphoma. In contrast with reports about lymphoma patients presenting with hypercalcemia and radiologic evidence of bone destruction^{12,15,17}, osteolytic bone lesions or osteopenia were excluded in our patient.

In conclusion, we present a very rare case of PTHrP-associated HHM in a patient with CLL type leukemic low grade lymphoma. We provide strong evidence for the correlation between synthesis and secretion of PTHrP by the malignant cells and the metabolic disorder. We believe that analysis of serum PTHrP concentrations may be helpful for differential diagnosis of hypercalcemia in patients with low grade B-cell lymphoma and postulate a pathogenetic role of leukemic cell production and secretion of PTHrP in hypercalcemia in these cases.

Björn Schöttker,¹ Werner Heinz,¹ Florian Weissinger,¹ Korkut Sözen,¹
Matthias Eck,² Jochen Seufert¹

¹Medizinische Klinik und Poliklinik II, Julius-Maximilians-Universität Würzburg, Germany, ²Institute of Pathology, Julius-Maximilians-Universität Würzburg, Germany

Keywords: parathyroid hormone-related protein, hypercalcemia, low grade leukemic B-cell lymphoma

Correspondence and reprint requests:

Björn Schöttker, M.D., Medizinische Klinik und Poliklinik II, Julius-Maximilians-Universität Würzburg, Klinikstrasse 6 - 8, 97070 Würzburg, Germany; Tel.: ++49/931/201-70800; Fax: ++49/931/201-71230; E-mail: schoettker_b@klinik.uni-wuerzburg.de

Figure 1. Bone marrow specimens from the presented patient obtained at first manifestation of hypercalcemia. A and B: Hematoxylin-eosin (H&E) staining (magnification 400* in A and 600* in B) showing an almost complete infiltration with small lymphoid cells and groups of prolymphocytoid cells with prominent nucleoli (arrow in B). C and D: Immunohistochemical staining showing strong positivity of CD23 (magnification 400* in C and 600* in D). E and F: Immunohistochemistry with anti-PTHrP antibody (magnification 400* in E and 600* in F), demonstrating cytoplasmic expression of PTHrP by the leukemic cells. G and H: Negative control (DAKO Envision® labelled polymer-AP Fast Red System) without addition of PTHrP antiserum. Pictures were created using a Nikon Eclipse TE 2,000-U microscope, a Nikon DXM 1,200 camera, and the Nikon ACT-1 V.2.12 software

ators of HHM were examined. Finally, sporadic reports of hypercalcemia associated with B-CLL are known.^{12,14,15,16} In one case, PTHrP involvement was documented by Northern Blot analysis of PTHrP mRNA expression in lymph node and organ tumor samples.¹² This patient, however, presented with osteolytic bone lesions that were attributed to the underlying disease and at least equivocal of originating hypercalcemia by direct bone destruction. In a different B-CLL patient, transformation into PLL coincided with the increase of serum calcium levels,¹⁴ but a mediator of hypercalcemia was not discovered. In one case, both osteolytic lesions and immunohistochemical detection of PTHrP expression were reported.¹⁵ Finally, in one out of 8 patients with typical B-CLL and hypercalcemia, elevated serum PTHrP levels and PTHrP expression by leukemic cells were correlated.¹⁶

In the patient presented here, recurrent increases in serum calcium levels were detected when progressive

References

- Mundy GR, Guise TA. Hypercalcemia of malignancy. *Am J Med* 1997; 103:134-45.
- Burtis WJ, Wu T, Bunch C, Wysolmerski JJ, Insogna KL, Weir EC, et al. Identification of a novel 17,000-dalton parathyroid hormone-like adenylate cyclase-stimulating protein from a tumor associated with humoral hypercalcemia of malignancy. *J Biol Chem* 1987; 262:7151-56.
- Danks J, Ebeling P, Hayman J, Chou ST, Moseley JM, Dunlop J, et al. Parathyroid hormone-related protein: immunohistochemical localization in cancers and in normal skin. *J Bone Miner Res* 1989; 4:273-8.
- Firkin F, Seymour JF, Watson A, Grill V, Martin TJ. Parathyroid hormone-related protein in hypercalcemia associated with hematological malignancy. *Br J Haematol* 1996; 94:468-92.
- Heath H, Hodgson S, Kennedy M. Primary hyperparathyroidism. *N Engl J Med* 1980; 302:189-93.
- Seymour JF, Gagel RF. Calcitriol: The major humoral mediator of hypercalcemia in Hodgkin's disease and non-Hodgkin's lymphomas. *Blood* 1993; 82:1383-94.
- Hewison M, Kantorovich V, Liker HR, van Herle AJ, Cohan P, Zehnder D, et al. Vitamin D-mediated hypercalcemia in lymphoma: evidence for hormone production by tumor-adjacent macrophages. *J Bone Miner Res* 2003; 18:579-82.
- Wada S, Kitamura H, Matsuura Y, Katayama Y, Ohkawa H, Kugai N, et al. Parathyroid hormone-related protein as a cause of hypercalcemia in a B-cell type malignant lymphoma. *Intern Med* 1992; 31:968-72.
- Uno H, Shima T, Maeda K, Katakami H, Tsubouchi H. Hypercalcemia associated with parathyroid hormone-related protein produced by B-cell type primary malignant lymphoma of the kidney. *Ann Hematol* 1998; 76:221-4.
- Senba M, Kawai K. Hypercalcemia and production of parathyroid hormone-like protein in adult T-cell leukemia-lymphoma. *Eur J Haematol* 1992; 48:278-9.
- Majumdar G. Incidence and prognostic significance of hypercalcemia in B-cell non-Hodgkin's lymphoma. *J Clin Pathol*

- 2002; 55:637-8.
12. Fain O, el M'Selmi A, Dosquet C, Meseure D, Lejeune F, Garel JM, et al. Hypercalcemia in B cell chronic lymphocytic leukaemia. *Br J Haematol* 1994; 87:856-8.
 13. Suemaru S, Kageyama J, Ota Z, Ohnoshi T, Sakamoto K, Kamura J. A case of non-Hodgkin's lymphoma with hypercalcemia. *Acta Med Okayama* 1991; 45:55-9.
 14. Lerner D, Esteves C, De Oliveira MS. B-CLL in PLL transformation associated with hypercalcemia. *Leuk Lymphoma* 1994; 12:321-5.
 15. Vlasfeld LT, Pauwels P, Ermens AAM, Aarnoudse WH, Ooms HWA, Haak HR. Parathyroid-hormone-related protein (PTHrP)-associated hypercalcemia in a patient with an atypical chronic lymphocytic leukaemia. *Neth J Med* 1999; 54: 21-6.
 16. Seymour JF, Keating MJ. Parathyroid-hormone-related protein in hypercalcaemia in CLL. *Br J Haematol* 1995; 89: 685-6.
 17. Beaudreuil J, Lortholary O, Martin A, Feuillard J, Guillevin L, Lortholary P, et al. Hypercalcemia may indicate Richter's syndrome: report of four cases and review. *Cancer* 1997; 79:1211-15.