

Methotrexate-associated mantle-cell lymphoma in an elderly man with myasthenia gravis

Huy Tran, Catherine Cheung, Devinder Gill, Ujjwal Dua, Jamie Nourse, Richard Boyle and Maher K Gandhi*

SUMMARY

Background A 75-year-old man on methotrexate immunosuppression for myasthenia gravis presented with a 2-month history of lymphocytosis and bilateral inguinal adenopathy. There were no constitutional symptoms of fever, night sweats, or weight loss.

Investigations Physical examination, blood tests, flow cytometry, fluorescent *in situ* hybridization, immunoglobulin gene sequencing, viral load quantification by real-time polymerase chain reaction, excisional lymph-node biopsy, bone-marrow biopsy, tumor morphology and immunohistochemistry, sequential CT and PET scans.

Diagnosis Methotrexate-associated mantle-cell lymphoma.

Management Cessation of methotrexate, anthracycline-based combination chemo-immunotherapy, and maintenance rituximab.

KEYWORDS Epstein-Barr virus, mantle-cell lymphoma, methotrexate, myasthenia gravis, rituximab

CME

Vanderbilt Continuing Medical Education online

This article offers the opportunity to earn one Category 1 credit toward the AMA Physician's Recognition Award.

Competing interests

MK Gandhi has declared associations with Roche. See the article online for full details of the relationship. The other authors declared no competing interests.

THE CASE

A 75-year-old man was referred to a tertiary teaching hospital with a 2-month history of peripheral lymphocytosis and bilateral inguinal nodes. Previous blood counts were normal. He was diagnosed with myasthenia gravis involving the limbs and bulbar muscles 30 months before presentation and was initially treated with intravenous immunoglobulin, prednisolone and pyridostigmine. Methotrexate was added to this regimen at an average weekly dose of 17.5 mg 1 month later. At the time of presentation, he had bilateral inguinal nodes measuring 2 cm but no other palpable lymphadenopathy or organomegaly and was receiving a weekly regimen of pyridostigmine and 25 mg methotrexate. He described no systemic symptoms such as weight loss, fevers or night sweats. There was mild bilateral ptosis and muscle fatigability with normal bulbar function and mild limb weakness graded as 2a on the Myasthenia Gravis Foundation of America Clinical Classification. Eastern Cooperative Oncology Group (ECOG) performance status was 1. Peripheral blood count revealed hemoglobin at 127 g/L (normal value 135–180 g/L), platelets at $105 \times 10^9/L$ (normal value $140\text{--}400 \times 10^9/L$), leukocytes at $12.6 \times 10^9/L$ (normal value $4\text{--}11 \times 10^9/L$) and lymphocytes at $9.2 \times 10^9/L$ (normal value $1\text{--}4 \times 10^9/L$). Lymphocytes were medium sized, with indented nuclei and inconspicuous nucleoli. Serum lactate dehydrogenase was 255 U/L (upper limit of normal 250 U/L). Peripheral blood flow cytometry revealed a κ -restricted monoclonal B-cell population that was positive for CD5, CD19 and CD20 and negative for CD10 and CD23. A t(11;14) (q13;q32) translocation was noted

H Tran is a trainee in Hematology, C Cheung is a Senior Scientist, D Gill is Head of Hematology, and R Boyle is Head of Neurology, all at the Princess Alexandra Hospital, and U Dua and J Nourse are Research Assistants and MK Gandhi is Head of Clinical Immunohematology, all at the Queensland Institute of Medical Research, Brisbane, Australia. MK Gandhi is also a Consultant Hematologist at Princess Alexandra Hospital, Brisbane, Australia.

Correspondence

*Queensland Institute of Medical Research, Bancroft Centre, 300 Herston Road, Brisbane, Qld 4006, Australia
maher.gandhi@qimr.edu.au

Received 24 April 2007 Accepted 17 September 2007 Published online 19 February 2008

www.nature.com/clinicalpractice
doi:10.1038/ncponc1071

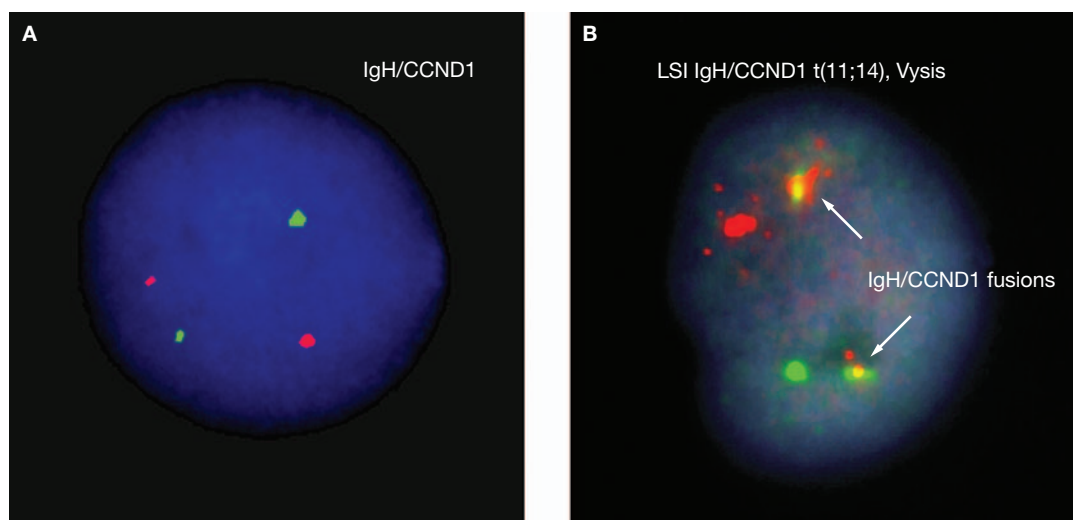


Figure 1 Fluorescent *in situ* hybridization of peripheral blood imprints for t(11;14) translocation was performed using Vysis LSI IgH/CCND1 dual color, dual fusion translocation probe. **(A)** A normal lymphoid nucleus containing two chromosome 11 (red) and two chromosome 14 signals (green). **(B)** Mantle-cell lymphoma t(11;14) nucleus containing one red, one green and two red/green fusion signals, indicated by arrows (1000× original magnification).

by fluorescent *in situ* hybridization (Figure 1). Right inguinal lymph-node excisional biopsy showed an expanded mantle zone of medium-sized lymphocytes and prominent hyalinized blood vessels surrounding atrophied residual germinal centers. Lymphocytes were positive for CD5, CD20 and cyclin D1 (Figure 2), confirming the diagnosis of mantle-cell lymphoma (MCL). Epstein–Barr virus (EBV)-encoded RNA *in situ* hybridization (EBER-ISH) was negative. CT staging revealed axillary, mediastinal, hilar, retroperitoneal, mesenteric, para-aortic, iliac and inguinal lymphadenopathy measuring up to 36 mm in maximal dimension with no hepatosplenic enlargement. [¹⁸F]-2-fluoro-2-deoxy-D-glucose-PET avidity was present in all enlarged nodal groups. Bone-marrow morphology and flow cytometry showed heavy MCL involvement. The patient was diagnosed as having Ann Arbor clinical stage IVA MCL with a high–intermediate International Prognostic Score.

Methotrexate treatment was ceased and the patient received eight cycles of fortnightly cyclophosphamide, doxorubicin, vincristine and prednisolone (CHOP) chemotherapy¹ with pegylated granulocyte colony-stimulating factor and 375 mg/m² rituximab at each cycle. Empirically, vincristine was omitted to prevent neuropathic damage. The patient tolerated chemotherapy well. Restaging after three cycles

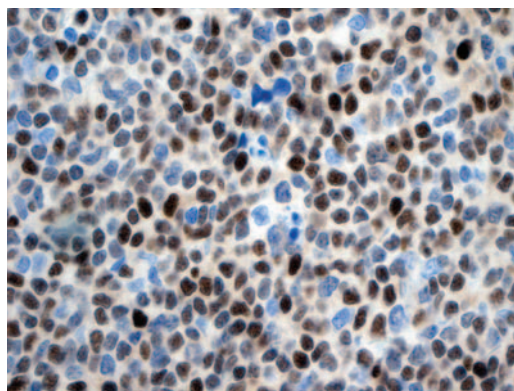


Figure 2 Cyclin D1 immunostaining of the patient's right inguinal lymph node. The cyclin D1 staining reveals intense nuclear positivity (brown; 800× original magnification).

showed partial response, with complete remission after six cycles, and ongoing complete remission at completion.² Repeat marrow examination and flow cytometry were negative and peripheral lymphocyte count was normal. The patient remains in complete remission 12 months later and receives bimonthly rituximab at 700 mg, which will be continued for another year. Despite the fact that pyridostigmine is one-third the dose at MCL diagnosis, the patient's myasthenia gravis is substantially

Box 1 Methotrexate-associated lymphomas.**Epidemiology**

One study estimated 16–33 cases of lymphoma per year per 10⁵ rheumatoid arthritis patients treated with methotrexate, which was similar to the lymphoma risk in rheumatoid arthritis patients in the absence of methotrexate.⁷ Mean duration of methotrexate treatment to diagnosis of lymphoma is 3 years. Overall, 85% of lymphoma cases are seen in rheumatoid arthritis, 6% in dermatomyositis, and 6% in psoriasis. To our knowledge, no cases have been previously reported in patients with myasthenia gravis.

Clinical presentation

In contrast to other immunodeficiency-associated lymphomas, the frequency of extranodal presentation is identical to that of lymphomas of similar histological subtypes in patients not receiving methotrexate. There are no distinctive clinical features except for the administration of methotrexate.

Histopathology

Histopathological analyses of methotrexate-associated lymphomas revealed the following phenotypes: DLBCL, 35%; HL, 25%; lymphoplasmacytic infiltrates, 14%; HL-like, 8%; FL, 10%; BL, 4% and PTCL, 4%. No cases of MCL have been previously reported.

Immunopathogenesis

Overall, approximately 50% of methotrexate-associated lymphoma cases are EBV-associated. Mechanisms by which EBV might induce transformation remain speculative. The WHO places methotrexate-associated lymphomas within the category of immunosuppressed lymphomas, but data as to whether low-dose methotrexate impairs immunity is lacking.

Therapy

On diagnosis of methotrexate-associated lymphoma, methotrexate cessation is mandatory. EBV-positive HL-like lesions seem most sensitive to methotrexate withdrawal. Combination chemotherapy is frequently indicated, depending on the histological subtype and clinical setting. In B-cell lymphomas rituximab may, in addition to its antilymphoma activity, permit control of autoimmunity despite methotrexate termination.

Abbreviations: BL, Burkitt's lymphoma; DLBCL, diffuse large B-cell lymphoma; EBV, Epstein-Barr virus; FL, follicular lymphoma; HL, Hodgkin's lymphoma; MCL, mantle-cell lymphoma; PTCL, peripheral T-cell lymphoma.

better controlled than before the antilymphoma therapy (graded as 1 on the Myasthenia Gravis Foundation of America Clinical Classification), with increased muscle strength on testing for

fatigability and an anti-acetylcholine-receptor antibody titer of 1.84 (>8 at diagnosis).

DISCUSSION OF DIAGNOSIS

MCL accounts for 5% of all non-Hodgkin's lymphomas (NHL).³ The median age at diagnosis is 63 years and patients usually present in advanced stages. Extranodal involvement occurs in up to 30% of patients with MCL. Poor prognostic factors include age, poor performance status, splenomegaly, nodal (versus non-nodal leukemic) disease and anemia. Adverse biological parameters include blastoid morphology, cyclin D1 and c-myc overexpression.³ Gene-expression profiling is of prognostic value, but was not performed in this case because freshly frozen biopsy material was not obtained.

The diagnosis of MCL is based on histologic, immunophenotypic, cytogenetic and molecular criteria. The t(11;14) (q13;q32) translocation is pathognomonic, occurring in virtually all cases.³ The juxtaposition of the *cyclin D1* gene with the immunoglobulin heavy chain variable gene (IgVH) leads to increased production of cyclin D1, which promotes cell-cycle progression from G1 through to S phase. MCL is generally regarded as aggressive and incurable, with one of the poorest long-term outcomes among all B-cell lymphomas; however, case series suggest that a more indolent leukemic disorder may exist within a proportion of patients.⁴ This subtype has the same genotypic features as MCL except for the presence of mutated IgVH genes, which indicate a post-germinal-center origin of the malignant B cells.⁴ These data await confirmation in appropriately designed prospective studies. Of note, this patient had unmutated IgVH genes.

Strictly, this case fits the criteria for a methotrexate-associated lymphoproliferative disorder (LPD; Box 1), which, although uncommon, is a recognized entity in the WHO classification.⁵ Most cases are observed in patients undergoing immunosuppression as treatment for rheumatoid arthritis.⁶ Approximately 50% of the cases are EBV-associated; however, there are conflicting epidemiological data as to whether methotrexate increases the risk of lymphoma *per se*. Studies have been insufficient in demonstrating an excessive risk of a rare event such as lymphoma after methotrexate treatment, and the lymphoma risk in patients with rheumatoid arthritis in the absence of methotrexate has been estimated as 2–20-fold greater than in the

normal population. A prospective study found a significant increase in Hodgkin's lymphoma (HL) but not in NHL in patients with rheumatoid arthritis treated with methotrexate.⁷ There are case reports of partial regression of lymphoma after methotrexate withdrawal in approximately 60% of cases, but reporting bias may lead to overestimation.⁸ Intriguingly, most regressing cases are EBV-positive.⁹

To our knowledge, there are no reported cases of MCL in methotrexate-associated LPD. There is no definitive laboratory test to distinguish between lymphomas in which methotrexate is causally implicated and those in which the development of lymphomas after methotrexate administration is more probably coincidental. This patient exhibited a number of features that would imply the latter. Firstly, at the time of myasthenia gravis diagnosis, the patient was noted to have several small retroperitoneal, mesenteric and mediastinal nodes. None met pathological size criteria, but their multiplicity and clustering raise the possibility of indolent MCL before the development of lymphocytosis. Secondly, the diagnostic specimen was EBER-ISH-negative. It is plausible that cases of EBER-ISH-positive methotrexate-associated LPD represent the principal subset in which methotrexate is a contributory etiological factor. Methotrexate is able to activate EBV viral replication of EBV-immortalized B cells *in vitro*, and rheumatoid arthritis patients treated with methotrexate have higher circulating viral loads than do similar patients treated with other immunosuppressive regimens.¹⁰ Interestingly, this patient had a modestly elevated EBV DNA of 303 copies/10⁶ peripheral blood mononuclear cells (typically 0–50 copies/10⁶ peripheral blood mononuclear cells in healthy EBV carriers).

DIFFERENTIAL DIAGNOSIS

Generalized low-volume lymphadenopathy is usually caused by a malignant LPD encompassing various subtypes of HL and NHL; however, benign and other malignant causes must also be excluded.¹¹ The principal infective causes include HIV and mononucleosis illness caused by EBV, cytomegalovirus, toxoplasma and bartonella. Infections are usually suspected clinically and are diagnosed by culture and/or antibody tests. In this patient, all infection tests were negative except for those in relation to past exposure to EBV and cytomegalovirus. Other benign causes include sarcoidosis, amyloidosis

and storage disorders such as Gaucher's disease, although the latter would be highly atypical in this age group.¹² Autoimmune syndromes might rarely cause generalized lymphadenopathy, although this would be an unlikely feature of myasthenia gravis. Patients receiving medications such as phenytoin and carbamazepine can also present with nodal masses. Advanced solid tumors might cause widespread lymphadenopathy, but additional features would be expected. In most cases, generalized lymphadenopathy is diagnosed as a malignant lymphoid disorder following histopathological examination of an excisional biopsy.¹²

The Lambert–Eaton syndrome (LES) is an autoimmune myasthenia affecting the trunk and proximal muscles because of deficient release of acetylcholine at the neuromuscular junction. In contrast to myasthenia gravis, power is initially increased with exercise. LES is classically associated with small-cell carcinoma of the bronchus; however, cases secondary to lymphoma are well recognized. In this case, both the neurological features and anti-acetylcholine-receptor antibodies rule out LES in association with MCL.¹³

TREATMENT AND MANAGEMENT

MCL remains an incurable lymphoma, with no plateau seen in survival curves. There is no consensus regarding the optimum standard of care.¹⁴ Anthracycline-based strategies such as CHOP are generally used, although unproven by randomized controlled trials, and provide a median overall survival of approximately 3 years.¹⁴ Dose-intense regimens have their advocates, but are generally not suitable for older patients. The addition of *de novo* rituximab to the CHOP regimen (R-CHOP) increases response but not survival rates.¹⁵ R-CHOP has, however, been shown to prolong survival in patients with relapsed/refractory disease.¹⁶ A nonsignificant trend towards increased event-free survival has been noted with rituximab maintenance therapy, and factors predictive of prolonged event-free survival with rituximab include Fc gamma receptor IIIA position 158-valine homozygosity.¹⁷ In this case, the patient was heterozygous (valine/phenylalanine) at position 158. Investigational approaches include high-dose chemotherapy with stem-cell support, allogeneic stem-cell transplantation, the proteasome inhibitor bortezomib, the cyclin-dependent kinase modulator flavopiridol and radioimmunotherapy.¹⁴

Acknowledgments

We would like to thank Dr RA Seaton for critically reading the manuscript. Written informed consent for release of clinical information was obtained from the patient.

Competing interests

MK Gandhi has declared associations with Roche. See the article online for full details of the relationship. The other authors declared no competing interests.

Cessation of methotrexate is mandatory in patients with methotrexate-associated LPD, but could have resulted in exacerbation of myasthenia, particularly following recovery from the immunosuppressive effects of chemotherapy. The fact that myasthenia gravis is better controlled off methotrexate may be attributable to the B-cell-depleting antibody rituximab, which has been beneficial in cases of refractory myasthenia gravis.¹⁸ Its mechanism is postulated to involve reduction in anti-acetylcholine-receptor antibodies, which inflict damage at the neuromuscular junction.

CONCLUSIONS

To our knowledge, this is the first reported case of methotrexate-associated LPD with MCL histology. We wish to highlight the difficulties in ascribing this label to individual patients, and the need for further research to identify those cases in which methotrexate is genuinely causally implicated. Given the rarity of methotrexate-associated LPD, trials can only be conducted in the multicenter clinical setting. Hemato-oncologists treating these patients should publish their findings for the benefit of future patients.

References

- Pfreundschuh M *et al.* (2004) Two-weekly or 3-weekly CHOP chemotherapy with or without etoposide for the treatment of elderly patients with aggressive lymphomas: results of the NHL-B2 trial of the DSHNHL. *Blood* **104**: 634–641
- Cheson BD *et al.* (2007) Revised response criteria for malignant lymphoma. *J Clin Oncol* **25**: 579–586
- Bertoni F *et al.* (2006) Update on the molecular biology of mantle cell lymphoma. *Hematol Oncol* **24**: 22–27
- Orchard J *et al.* (2003) A subset of t(11;14) lymphoma with mantle cell features displays mutated IgVH genes and includes patients with good prognosis, nonnodal disease. *Blood* **101**: 4975–4981
- Harris NL and Swerdlow SH (2001) Methotrexate-associated lymphoproliferative disorders. In *Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues*, 270–271 (Eds Jaffe ES *et al.*). Lyon: International Agency for Research on Cancer Press
- Gandhi MK (2006) Epstein-Barr virus-associated lymphomas. *Expert Rev Anti Infect Ther* **4**: 77–89
- Mariette X *et al.* (2002) Lymphomas in rheumatoid arthritis patients treated with methotrexate: a 3-year prospective study in France. *Blood* **99**: 3909–3915
- Kamel OW *et al.* (1993) Brief report: reversible lymphomas associated with Epstein-Barr virus occurring during methotrexate therapy for rheumatoid arthritis and dermatomyositis. *N Engl J Med* **328**: 1317–1321
- Khanna R *et al.* (2005) Technology Insight: applications of emerging immunotherapeutic strategies for Epstein-Barr virus-associated malignancies. *Nat Clin Pract Oncol* **2**: 138–149
- Feng WH *et al.* (2004) Reactivation of latent Epstein-Barr virus by methotrexate: a potential contributor to methotrexate-associated lymphomas. *J Natl Cancer Inst* **96**: 1691–1702
- Hoffman R *et al.* (2005) *Haematology: Basic Principles and Practice*, edn 4. Philadelphia: Elsevier Churchill Livingstone
- Ghirardelli ML *et al.* (1999) Diagnostic approach to lymph node enlargement. *Haematologica* **84**: 242–247
- Sanders DB (1994) Lambert-Eaton myasthenic syndrome: pathogenesis and treatment. *Semin Neurol* **14**: 111–117
- Zelenetz AD (2006) Mantle cell lymphoma: an update on management. *Ann Oncol* **17** (Suppl 4): iv12–14
- Lenz G *et al.* (2005) Immunochemotherapy with rituximab and cyclophosphamide, doxorubicin, vincristine, and prednisone significantly improves response and time to treatment failure, but not long-term outcome in patients with previously untreated mantle cell lymphoma: results of a prospective randomized trial of the German Low Grade Lymphoma Study Group (GLSG). *J Clin Oncol* **23**: 1984–1992
- Forstpointner R *et al.* (2004) The addition of rituximab to a combination of fludarabine, cyclophosphamide, mitoxantrone (FCM) significantly increases the response rate and prolongs survival as compared with FCM alone in patients with relapsed and refractory follicular and mantle cell lymphomas: results of a prospective randomized study of the German Low-Grade Lymphoma Study Group. *Blood* **104**: 3064–3071
- Ghielmini M *et al.* (2005) Single agent rituximab in patients with follicular or mantle cell lymphoma: clinical and biological factors that are predictive of response and event-free survival as well as the effect of rituximab on the immune system: a study of the Swiss Group for Clinical Cancer Research (SAKK). *Ann Oncol* **16**: 1675–1682
- Takagi K *et al.* (2005) Anti-CD20 antibody (Rituximab) therapy in a myasthenia gravis patient with follicular lymphoma. *Ann Hematol* **84**: 548–550