Epstein-Barr Virus, Methotrexate, and Lymphoma in Patients with Rheumatoid Arthritis and Primary Sjögren's Syndrome: Case Series

THERESE M. DAWSON, GORDON STARKEBAUM, BRENT L. WOOD, ROBERT F. WILLKENS and ALLEN M. GOWN

ABSTRACT. ***Objective.*** Rheumatoid arthritis (RA) and primary Sjögren's syndrome (SS) are associated with an increased risk of lymphoma. Epstein-Barr virus (EBV), a ubiquitous herpes virus, has been linked etiologically to lymphoma in patients with RA and primary SS. Recently, methotrexate (MTX) has also been linked to the development of these lymphomas. We investigated the frequency of EBV in lymphoma tissue of patients with RA and primary SS and the association of MTX with lymphomagenesis.
Methods. Twenty-three patients with RA and 9 with primary SS with a history of lymphoma were identified by writing to all Arthritis Foundation member rheumatologists in Washington State. Formalin fixed, paraffin embedded tissue blocks were then requested from pathology laboratories. Lymph nodes from 5 RA patients without lymphoma were also studied. *In situ* hybridization using a biotinylated EBER-1 oligonucleotide probe was used to detect EBV in tissue sections. Positive and negative laboratory controls were used to ensure procedural integrity.
Results. Specimens from 21 RA patients were obtained, with 2 subsequently excluded due to specimen quality. Specimens from 6 patients with primary SS were obtained. *In situ* hybridization for EBV was positive in 5/19 (26%) RA patients and 1/6 patients with primary SS. In the nonmalignant lymph nodes no patient showed EBV. One primary SS and 12 RA patients were known to be taking MTX at the time of lymphoma diagnosis. Of the EBV positive RA lymphoma patients, 4/5 were receiving MTX at the time of diagnosis. These results show that EBV is present in lymphoma tissue of some patients with RA and very few with primary SS.
Conclusion. EBV is over-represented in the lymphomas of patients with RA, but whether MTX plays a role in predisposing patients with RA and primary SS to the development of lymphoma, perhaps by influencing behavior of EBV, remains unclear. (J Rheumatol 2001;28:47–53)

Key Indexing Terms:
EPSTEIN-BARR VIRUS RHEUMATOID ARTHRITIS LYMPHOMA
METHOTREXATE SJÖGREN'S SYNDROME

Rheumatoid arthritis (RA) and primary Sjögren's syndrome (SS) are chronic autoimmune diseases that are associated with an increased risk of developing lymphoproliferative diseases[1–8]. Controversy exists whether this increased risk is a consequence of or is independent of immunosuppressive therapy[4,7-11]. In recent times, methotrexate (MTX), the agent of choice in the treatment of RA[12], has come under close scrutiny for its potential association with lymphomas[13-30]. In particular, some lymphomas developing in patients with RA have been reported to regress following cessation of MTX[24-26,31-37]. Reflecting this concern, package inserts provided with MTX have recently been changed to include a warning of such malignant lymphomas.

An additional noteworthy feature of some lymphomas occurring in RA patients taking MTX has been the observation of Epstein-Barr virus (EBV) DNA in pathologic tissue using *in situ* hybridization. While the majority of adults are seropositive for EBV, the virus is uncommonly identified by *in situ* hybridization in lymph nodes in healthy individuals or in most forms of lymphoma[38]. However, EBV has been commonly linked through its discovery by *in situ* hybridization with some lymphomas including Burkitt's lymphoma, and in those arising in immunosuppressed patients such as posttransplant, especially those treated with cyclosporine. The recent

From Health Services Research and Development and the Arthritis Section, VA Puget Sound Health Care System, Seattle Division; the Arthritis Section, Medicine Service, Harborview Medical Center; the Division of Rheumatology and Department of Laboratory Medicine, University of Washington; and PhenoPath Laboratories and Immunochemistry Research Institute Seattle, Seattle, Washington, USA.

T.M. Dawson, B Med, MPH, Acting Chief, Rheumatology Section, VA Puget Sound Health Care System, Acting Assistant Professor of Medicine, Division of Rheumatology, University of Washington; G. Starkebaum, MD, Chief of Staff, Seattle Division, VA Puget Sound Health Care System, Professor of Medicine, Division of Rheumatology, University of Washington; R.F. Willkens, MD, Chief, Arthritis Section, Harborview Medical Center, Professor of Medicine, Division of Rheumatology, University of Washington; B.L. Wood, MD, Assistant Professor, Department of Laboratory Medicine, University of Washington Medical Center; A.M. Gown, MD, PhenoPath Laboratories and Immunocytochemistry Research Institute Seattle.

Address reprint requests to Dr. T.M. Dawson, HSR&D, Seattle Division, VA Puget Sound Health Care System, 1600 South Columbian Way, Seattle, WA 98108.

Submitted April 18, 2000 revision accepted July 4, 2000.

description of EBV in lymphomas of MTX treated patients with RA and primary SS suggests that immunosuppression in these patients could result in increased risk of lymphomagenesis[21,24-26,30,32-36,39,40]. Since EBV associated lymphomas have also been seen in RA patients not treated with MTX, the precise role of immunosuppression in this process is unclear[24]. Lymphomas in the immunosuppressed patient are often characterized by extranodal locations, polymorphous histology, and geographic necrosis in addition to the presence of clonal EBV[30]. Despite the many case reports, the prevalence of EBV in lymphomas in RA has not been firmly established. Recently, Kamel *et al*[30] in a study of 49 patients with RA associated non-Hodgkin's lymphomas reported only one case positive for EBV by *in situ* hybridization.

We investigated the frequency of EBV in lymphoma tissue of patients with RA and primary SS to explore the association of MTX with lymphomagenesis in RA.

MATERIALS AND METHODS

Patients. All rheumatologists in the state of Washington, identified with the assistance of the Arthritis Foundation, were contacted by mail or by telephone over a 3 month period. Each was asked to complete a questionnaire identifying any patients under their care with RA or SS who had developed lymphoma. Information collected on each patient included age and sex, diagnosis and duration of RA or SS, duration of RA or SS at the time of diagnosis of lymphoma, type and site of lymphoma, treatment of RA or SS (including use of MTX), treatment at the time of lymphoma diagnosis and course of RA or SS and lymphoma. In addition, the rheumatologists were asked to specify the pathology laboratory and provide laboratory accession numbers to enable specimen acquisition. The laboratory involved in the lymphoma diagnosis was then contacted, and formalin fixed, paraffin embedded tissue blocks were obtained. The investigators were kept blinded to any direct patient identifier, such as name or social security number, and patients were instead identified by a unique patient code. The study was granted exemption from Human Subjects Review under Federal Human Subjects regulations and University of Washington Human Subjects guidelines.

In situ hybridization. All analyses were performed in the Immunocytochemistry Laboratory at the University of Washington Medical Center. Microtome sections (5 µ) were cut from tissue blocks, prepared, and placed on Fisher Superfrost/Plus microscope slides and dried at 60˚C for 30 min. The lymphoma diagnosis was confirmed using hematoxylin and eosin stained slides and EBV analysis was performed by *in situ* hydridization using a biotinylated EBER-1 oligonucleotide probe for the EBER-1 oligonucleotide. This oligonucleotide is a 30 base pair sequence of the EBER-1 gene (base pairs 69–98) that is terminally biotinylated with 6 molecules of biotin at the 3′ end[41,42]. Detection and visualization were performed by applying a mouse anti-biotin antibody followed by a biotinylated anti-mouse antibody and an immunoperoxidase antigen detection procedure using a preformed complex consisting of avidin and biotinylated horseradish peroxidase[41,42]. 3,3′-diaminobenzidine was used as a chromogen.

External controls (lymph nodes) from patients with RA or primary SS but without lymphoma were obtained from local rheumatologists and the Pathology Department of the VA Puget Sound Health Care System. Internal control of the *in situ* hybridization procedure was provided by processing 2 slides from every patient but only staining one with the biotinylated probe. Using the standard laboratory protocol, further internal control of the procedure was assured by processing a known historical EBV positive and a known historical EBV negative specimen slide with each run.

Statistical analysis. Results were analyzed using descriptive methods only. Given the discrepancy between the size of the RA lymphoma positive and RA lymphoma negative (controls) cohorts, the data did not lend itself to further comparison.

RESULTS

All 65 Washington State rheumatologists were contacted, from whom 32 cases of lymphoma were identified. Five cases/specimens (2 RA, 3 primary SS) were unobtainable and 2 specimens (2 RA) were excluded due to insufficient amount of tissue or poor quality of tissue blocks, yielding 25 examinable lymphoma cases. Of those cases 19 patients had RA (Table 1) and 6 SS (Table 2). Patients had a mean age at lymphoma diagnosis of 63 years (RA patients) and 58 years (SS), with a mean duration of RA at diagnosis of 15 years and SS of 4.5 years (durations specified as "years" omitted from disease duration calculation). One patient with RA was reported to have concomitant sicca symptoms and none had Felty's syndrome. A further 5 specimens from 5 RA patients without lymphoma (controls) who had had lymph node biopsies performed for varied reasons were obtained and also examined for EBV (Table 3). All biopsies were performed between 1982 and 1996. EBV was detected by *in situ* hybridization in 5 of 19 patients with RA and one out of 6 with SS (Tables 1 and 2). No control tissue lymph nodes were positive for EBV. Representative photomicrographs showing *in situ* hybridization for EBV are shown in Figure 1.

Of the 19 patients with RA and lymphoma, 12 were taking MTX at the time of lymphoma diagnosis. Four of the 5 EBV positive RA patients were taking MTX at lymphoma diagnosis. Only one patient with SS was taking MTX at the time of diagnosis and this patient was negative for EBV. Regarding response of the lymphomas to treatment, no patient was reported to have undergone spontaneous remission following MTX withdrawal.

DISCUSSION

By contacting every rheumatologist in Washington State we believe that our cross sectional sample of RA patients diagnosed with lymphoma is representative of the general population of RA patients who have developed lymphoma. Since we did not contact oncologists or consult tumor registries, however, our sample is likely incomplete, since inclusion depended on recall by individual practicing rheumatologists.

In our RA patients 12/19 were taking MTX at the time of lymphoma diagnosis. No differences in histologic type or age at diagnosis were identifiable between those patients treated with MTX and those not, similar to patients reported by Moder, *et al*[10]. Most of our patients with RA (68%) and SS (67%) had B cell, diffuse large cell lymphomas, the most commonly reported lymphoma in immunosuppressed patients[10,23,30,43]. While the majority of our patients were either taking MTX or had a history of MTX treatment it is not clear whether this finding by itself supports a causative role of the drug or instead merely reflects current prescribing habits and MTX popularity. Several patients had been or were concomi-

Table 1. Results of *in situ* hybridization (ISH) for Epstein-Barr virus (EBV) in the lymphomas of patients with rheumatoid arthritis (RA).

Patient	Age, yrs*	Sex	RA duration, yrs*	Type of Lymphoma	Site	MTX Treatment	Time Taking MTX, yrs†	Other RA Treatment	EBV-ISH Result
1	72	M	34	Large cell	Lung	Yes	0.3	P	–
2	59	F	48**	Large cell	Groin	Yes (pre)	4†	A,H,S,P	+
3	70	F	10	Large cell	Diffuse	Yes	4	AZA	+
4	61	F	38	Large cell	Buccal	Yes	3	AZA	+
5	68	F	6	Small cell	GIT	Yes	6	P	–
6	59	F	4	Hodgkin's	Thorax	Yes	3	P	+
7	60	F	4	Large cell	Diffuse	Yes	4	AZA	–
8	62	M	2	Large cell	CNS	Yes	2	Unknown	+
9	59	F	12	Follicular	Neck	No	—	D-Pen	–
10	63	M	20	Large cell	Diffuse	Yes (post)	3†	Gold	–
11	75	M	20	Large cell	Back	Unknown	—	P	–
12	81	F	3	Large cell	Neck	No	—	P	–
13	64	F	6	Large cell	Axilla	Yes	2.5	No	–
14	40	F	30	Im/blastic	Neck	Yes	0.5	Unknown	–
15	57	F	Years	Large cell	Pharynx	Yes	5	Unknown	–
16	74	M	10	Small cell	Mediastinum	Yes	Years	P	–
17	40	F	16	Large cell	Pelvis	Yes	1	P	–
18	54	F	14	Large cell	Mantle	Unknown	—	P	–
19	76	M	10	Mixed cell	Neck	No	—	P	–

*Age and duration of RA given at time of lymphoma diagnosis. **RA with sicca syndrome. † Not taking MTX at time of lymphoma diagnosis. A: auranofin, H: hydroxychloroquine, S: sulfasalazine, P: prednisone, D-Pen: D-penicillamine, GIT: gastrointestinal tract, im/blastic: immunoblastic, MTX: methotrexate, AZA: azathioprine.

Table 2. Results of *in situ* hybridization (ISH) for Epstein-Barr virus (EBV) in the lymphomas of patients with primary Sjögren's syndrome.

Patient	Age, yrs*	Sex	Disease Duration, yrs*	Type of Lymphoma	Site	MTX Treatment	Time Taking MTX, yrs	Other Treatment	EBV-ISH Result
1	77	F	Years	Large cell	Lung	Yes	0.5	No	–
2	63	F	2	Large cell	Parotid	No	—	Pred	–
3	77	F	3	Large cell	Parotid	No	—	No	–
4	58	M	2	Large cell	Lung	No	—	Unknown	–
5	70	F	13	Follicular	Axilla	No	—	No	–
6	68	F	7	Hodgkin's	Axilla	No	—	Pred	+

*Age and duration of primary SS given at time of lymphoma diagnosis.

Table 3. Results of *in situ* hybridization (ISH) for Epstein-Barr virus (EBV) in the lymph nodes of patients with rheumatoid arthritis (RA) and no lymphoma.

Patient	Age, yrs*	Sex	RA Duration, yrs*	Biopsy Result	Biopsy Site	MTX Treatment	Time Taking MTX (yrs)	Other RA Treatment	EBV-ISH Result
1	59	F	6	Hyperplasia	Axilla	Yes	2	HCL	–
2	45	F	5	Hyperplasia	Axilla	No	—	Unknown	–
3	54	F	9	Hyperplasia	Pelvis	No	—	Gold	–
4	49	F	4	Hyperplasia	Axilla	Yes	3	Gold, c.phos	–
5	50	F	3	Hyperplasia	Axilla	No	—	Gold	–

*Age and duration of RA at time of biopsy. MTX: methotrexate, HCL: hydroxychloroquine, c.phos: cyclophosphamide.

tantly taking other immunosuppressive drugs, the importance of which is also undetermined.

Analyses were performed by *in situ* hybridization as it is both sensitive and enables the specific detection of nucleic acids. Unlike protein antigens, nucleic acids are not generally altered by routine tissue processing. In addition, unlike the polymerase chain reaction, *in situ* hybridization can localize the viral genome to a specific cell population such as lymphoma cells, negating the possibility of a positive result purely on the basis of virus present in nonlymphoma tissue[44]. This

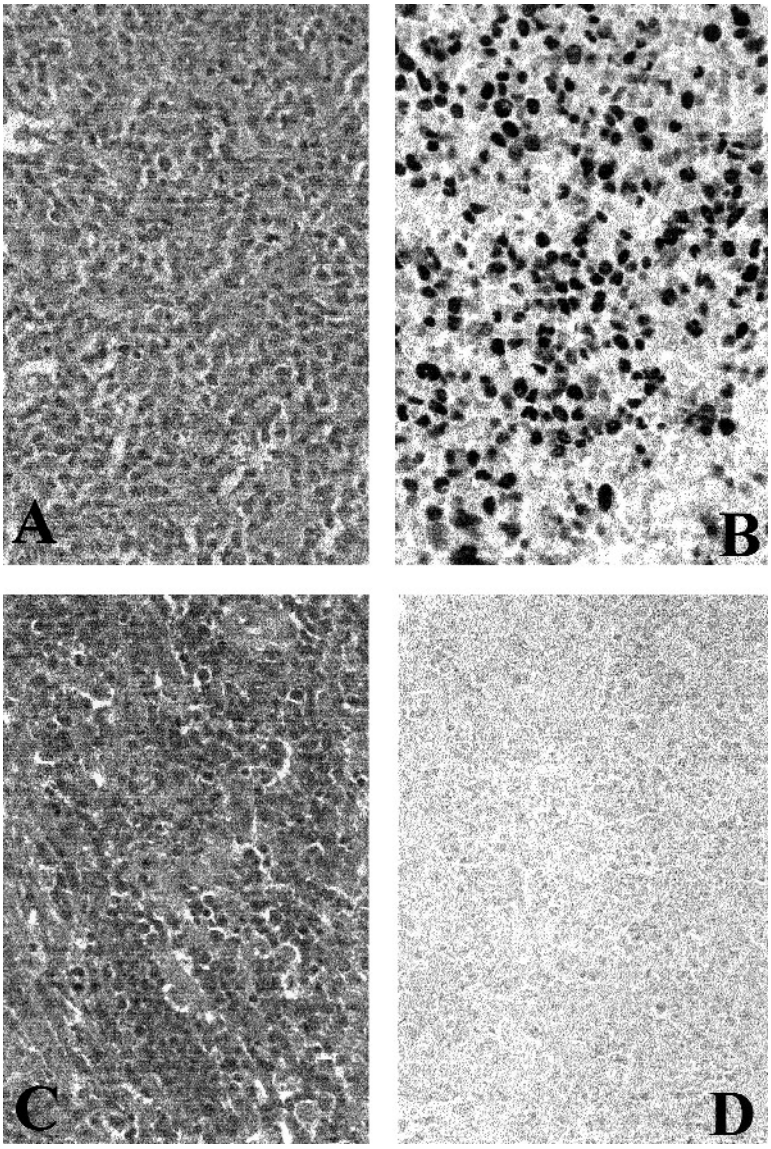

Figure 1. Photomicrographs of 2 different lymph nodes with large cell lymphoma. A. H&E stained section of large cell lymphoma. B. EBER-1 *in situ* hybridization showing strong nuclear signal in large subsets of tumor nuclei. C. H&E stained section of different node with large cell lymphoma. D. EBER-1 *in situ* hybridization showing complete absence of nuclear signal in tumor cell nuclei.

technique is recognized to be the most sensitive for localizing EBV to neoplastic lymphoid cells[26].

In our study 5/19 (26%) patients with RA were positive for EBV by *in situ* hybridization, higher than that expected in non-Hodgkin's lymphomas arising in the general population and more in keeping with the reports cited below[21,24]. In contrast to our RA lymphoma patients, none of the 5 control patients (RA but no lymphoma) had detectable EBV. Since 4 of the 5 EBV positive lymphomas developed in RA patients taking MTX (the remaining patient, although not taking MTX at the time of lymphoma diagnosis, had taken it in the recent past), our results are consistent with the notion that MTX contributes to the risk of lymphomagenesis through an EBV dependent mechanism in such patients. Clearly, other factors also contributed to the risk of lymphoma in our patients with RA since 8 lymphomas developed in patients taking MTX that were EBV negative and 3 EBV negative lymphomas developed in patients not taking MTX at diagnosis. Patients with both RA and SS appear to be at greater risk of developing lymphoma, perhaps due to altered lymphocyte activity, defects in immune surveillance, drug effects, or other unknown factors[2-4,6,45-49]. EBV may contribute to the pathogenesis of some of these lymphomas. EBV has been clearly linked to the development of lymphomas in immunosup-

pressed patients with other disorders. Examples include well described cases in posttransplant patients (posttransplant lymphoproliferative disease syndrome)[8,26,50], patients taking cyclosporine for RA or posttransplantation[39,51], and those with psoriasis treated with MTX[52]. In some of these patients, regression of EBV associated lymphomas occurs when immunosuppression is stopped[24]. Such spontaneous regressions are rare events in non-Hodgkin's lymphomas unassociated with EBV[53,54]. It is possible that in some patients with RA immunosuppression with MTX could result in development of lymphomas through a similar EBV related mechanism. Over a decade ago Tosato, *et al* described a specific defect in EBV directed suppressor T cell function in RA patients[55]. They also showed that RA patients have higher numbers of circulating EBV infected B cells compared to controls. Whether MTX immunosuppression in RA patients augments the defect in suppressor T cell function has not been established, however. Nevertheless, evidence implicating EBV in some RA lymphomas includes the reported increased frequency of EBV detected in these lymphomas (see below), tumor regression when immunosuppressive (MTX) therapy is ceased in EBV positive lymphomas[24-26,31-36], and the monoclonality of EBV genomes in lymphoma cells[26,35]. The latter data suggest that EBV incorporation into a transformed B cell occurs at an early, possibly single cell stage of lymphoma development.

In a study of lymphoma occurring in patients with RA or dermatomyositis, Kamel, *et al* reported 4/15 (27%) RA patients were positive for EBV, greater than the 4% positive rate observed in non-Hodgkin's lymphomas occurring in the general population[21,26,38]. Three of these 4 patients were taking MTX. Salloum, *et al* reported 12 of 27 (44%) of MTX treated RA lymphoma cases derived from their own studies and from the literature were positive for EBV-RNA transcripts[24]. Further support for a potential role of EBV–MTX interaction in lymphomagenesis in RA patients are reports of tumor regression with cessation of MTX therapy[24,26,31-36]. As mentioned, such spontaneous regressions are rare events in non-Hodgkin's lymphomas[52,53], although without rechallenging these patients, the role of MTX is not conclusive. Of those patients whose tumors regressed with MTX withdrawal, almost all were positive for EBV[24].

These results do not provide information regarding the magnitude of increased risk of developing lymphoma associated with MTX use in RA. The age of the patient, degree of inflammatory disease activity, and duration of disease may be more closely associated with lymphoma development than MTX use[56]. The general paucity of reports of increased lymphoproliferative disease in psoriasis, a disorder also treated with MTX, may support this view[11]. In a recent study of 1773 patients with RA from 15 countries who developed malignancy, only one patient treated with MTX who developed a lymphoma was identified[57]. In a large case-control study Kamel, *et al* recently found only one of 42 patients (2%) with RA and lymphoma had EBV by *in situ* hybridization, the same number as in the control group of 49 patients (2%). Only 11 of these patients had their self-reported RA confirmed by a rheumatologist, however, before study inclusion. Furthermore, a surprisingly small number, 5/42, reported treatment with immunosuppressive agents such as MTX. Kamel, *et al* concluded that EBV associated lymphomas represent only a small fraction of all non-Hodgkin's lymphomas in the general RA patient population and are uncommon in RA patients not taking immunosuppressive therapy[30].

Despite the variability in the reported frequency of EBV in the lymphomas of RA and other reports noting lack of tumor regression after stopping MTX[13-24,26-28,30], it would seem prudent to test for EBV by *in situ* hybridization in all RA lymphoma patients on immunosuppressive treatment since patients positive for EBV may have a greater chance of tumor regression. Regardless of whether EBV is positive or not, immunosuppression should be withdrawn and lymphoma directed therapy delayed for a short period to allow for the chance of spontaneous tumor regression[24].

Of our patients with primary SS only 1/5 was undergoing MTX therapy, probably reflecting treatment of SS, where MTX is infrequently used, rather than risks entailed with MTX treatment. This patient was negative for EBV. Patients with SS have been estimated to have a risk for the development of non-Hodgkin's lymphoma up to 44 times greater than the normal population[3], and to have a higher risk of lymphoma than RA[7]. However, because of the relative infrequency of the disease compared to RA it is perhaps not surprising that our study identified a larger number of RA lymphoma patients. In a recent series reported by Voulgarelis, *et al* 33 patients were described whose characteristics are not dissimilar to our patients in age and lymphoma histologic type[58]. Little has been published on the role of EBV in these lymphomas, although Hirose, *et al* recently reported EBV was present in 2/17 patients with primary SS and lymphoma, similar to our findings of 1/6. These authors also found 3/55 salivary glands positive for EBV in SS patients without lymphoma[40]. Hirose, *et al* concluded that EBV has no association with the lymphoma in patients with primary SS.

In summary, our study of EBV and lymphoma in patients with RA found that 5/19 patients were EBV positive, all of whom were taking or had recently been taking MTX, suggesting a possible, but no absolute association between EBV, MTX, and lymphoma in some patients with RA. In contrast, there appeared to be no association between either EBV or MTX in patients with primary Sjögren's syndrome. The role of MTX in the development of EBV negative lymphomas in RA patients is less clear. Although more than half the patients with RA were receiving MTX at the time of lymphoma diagnosis, this finding could reflect the prescribing habits of Washington State rheumatologists. Given reports of tumor regression with cessation of MTX therapy, particularly in EBV positive tumors, this practice seems an appropriate ini-

tial approach to lymphoma management in patients with RA, perhaps in conjunction with *in situ* hybridization studies to determine EBV status. Further studies are necessary to determine if EBV positive lymphomas are more likely to regress following cessation of MTX and to determine risk factors for development of lymphomas in RA.

ACKNOWLEDGMENT

The authors thank the rheumatologists of Washington State for their assistance with this study, the staff of the Immunocytochemistry Laboratory at the University of Washington Medical Center, and Sarah Conyers of the Pathology Department of the VA Puget Sound Health Care System, Seattle Division, for her kind assistance with slide preparation.

REFERENCES

1. Isomaki HA, Hakulinen T, Joutsenlahti U. Excess risk of lymphomas, leukemias and myeloma in patients with rheumatoid arthritis. J Chron Dis 1978;31:691-6.
2. Hakulinen T, Isomaki H, Kneky P. Rheumatoid arthritis and cancer studies based on linking nationwide registries in Finland. Am J Med 1985;78 Suppl 1A:29-32.
3. Kassan SS, Thomas TL, Moutsopoulus HM. Increased risk of lymphoma in sicca syndrome. Ann Intern Med 1978;89:888-92.
4. Matteson EL, Hickey AR, Maguire L, Tilson HH, Urowitz MB. Occurrence of neoplasia in patients with rheumatoid arthritis enrolled in a DMARD registry. J Rheumatol 1991;18:809-14.
5. Shokri F, Mageed RA, Maziak BR, et al. Lymphoproliferation in primary Sjogren's syndrome: evidence of selective expansion of a B cell subset characterized by the expression of cross-reactive idiotypes. Arthritis Rheum 1993;36:1128-36.
6. Gridley G, McLaughlin JK, Ekbom A, et al. Incidence of cancer among patients with rheumatoid arthritis. J Natl Cancer Inst 1993;85:307-11.
7. Kauppi M, Pukkala E, Isomaki H. Elevated incidence of hematologic malignancies in patients with Sjogren's syndrome compared with patients with rheumatoid arthritis. Cancer Causes Control 1997;8:201-4.
8. Bleyer WA. Methotrexate induced lymphoma? [editorial]. J Rheumatol 1998;25:404-7.
9. Tennis P, Andrews E, Bombardier C, Wang Y, Strand L, West R. Record linkage to conduct an epidemiologic study on the association of rheumatoid arthritis and lymphoma in the province of Saskatchewan, Canada. J Clin Epidemiol 1993;46:685-95.
10. Moder KG, Tefferi A, Cohen MD, Menke DM, Luthra HS. Hematologic malignancies and the use of methotrexate in rheumatoid arthritis: a retrospective study. Am J Med 1995; 99:276-81.
11. Kremer JM. Is methotrexate oncogenic in patients with rheumatoid arthritis? [editorial]. Semin Arthritis Rheum 1997;26:785-7.
12. Ward MM, Fries JF. Trends in antirheumatic medication use among patients with rheumatoid arthritis, 1981-1996. J Rheumatol 1998;25:408-16.
13. Willkens RF, Marks CR. Malignancies occurring during methotrexate treatment of rheumatoid arthritis. In: Pincus SH, Pisetsky DS, Rosenwasser LJ, editors. Biologically based immunomodulators in the therapy of rheumatic diseases. New York: Elsevier Science Publishing Co. Inc; 1986:127-32.
14. Sany J, Anaya JM, Lussiez V, Couret M, Combe B, Daures J-P. Treatment of rheumatoid arthritis with methotrexate: a prospective open longterm study of 191 cases. J Rheumatol 1991;18:1323-7.
15. Ellman MH, Hurwitz H, Thomas C, Kozloff M. Lymphoma developing in a patient with rheumatoid arthritis taking low dose weekly methotrexate. J Rheumatol 1991;18:1741-3.
16. Kingsmore SF, Hall BD, Allen NB, Rice JR, Caldwell DS. Association of methotrexate, rheumatoid arthritis and lymphoma: report of 2 cases and literature review. J Rheumatol 1992; 19:1462-5.
17. Donnelly S, Amos R, Norton AJ. A patient with rheumatoid arthritis and lymphoma. Br J Rheumatol 1992;31:107-12.
18. Cobeta-Garcia JC, Ruiz-Jimeno MT, Fontova-Garrofe R. Non-Hodgkin's lymphoma, rheumatoid arthritis and methotrexate [letter]. Ellman MH [reply]. J Rheumatol 1993;20:200-1.
19. Taillan B, Garnier G, Castanet J, Ferrari E, Pesce A, Dujardin P. Lymphoma developing in a patient with rheumatoid arthritis taking methotrexate. Clin Rheumatol 1993;12:93-4.
20. Morris CR, Morris AJ. Localized lymphoma in a patient with rheumatoid arthritis treated with parenteral methotrexate. J Rheumatol 1993;20:2172-3.
21. Kamel OW, van de Rijn M, Lebrun DP, Weiss LM, Warnke RA, Dorfman RF. Lymphoid neoplasms in patients with rheumatoid arthritis and dermatomyositis. Frequency of Epstein-Barr virus and other features associated with immunosuppression. Hum Pathol 1994;25:638-43.
22. Zimmer-Galler I, Lie JT. Choroidal infiltrates as the initial manifestation of lymphoma in rheumatoid arthritis after treatment with low-dose methotrexate. Mayo Clin Proc 1994;69:258-61.
23. Usman AR, Yunus MB. Non-Hodgkin's lymphoma in patients with rheumatoid arthritis treated with low dose methotrexate. J Rheumatol 1996;23:1095-7.
24. Salloum E, Cooper DL, Howe G, et al. Spontaneous regression of lymphoproliferative disorders in patients treated with methotrexate for rheumatoid arthritis and other rheumatic diseases. J Clin Oncol 1996;14:1943-9.
25. Bachman TR, Sawitzke AD, Perkins SL, Ward JH, Cannon GW. Methotrexate-associated lymphoma in patients with rheumatoid arthritis. Arthritis Rheum 1996;2:325-9.
26. Van de Rijn M, Cleary ML, Variakojis D, Warnke RA, Chang PP, Kamel OW. Epstein-Barr virus clonality in lymphomas occurring in patients with rheumatoid arthritis. Arthritis Rheum 1996;39:638-42.
27. Padeh S, Sharon N, Schiby G, Rechavi G, Passwell JH. Hodgkin's lymphoma in systemic onset juvenile rheumatoid arthritis after treatment with low dose methotrexate. J Rheumatol 1997; 24:2035-7.
28. Georgescu L, Quinn GC, Schwartzman S, Paget SA. Lymphoma in patients with rheumatoid arthritis: association with disease state or methotrexate treatment. Semin Arthritis Rheum 1997;26:794-804.
29. Bologna C, Picot M-C, Jorgensen C, Viu P, Verdier R, Sany J. Study of eight cases of cancer in 246 rheumatoid arthritis patients treated with methotrexate. Ann Rheum Dis 1997;56:97-102.
30. Kamel OW, Holly EA, van de Rijn M, Lele C, Sah A. A population based, case control study of non-Hodgkin's lymphoma in patients with rheumatoid arthritis. J Rheumatol 1999;26:1676-80.
31. Shiroky JB, Frost A, Skelton JD, Hargert DG, Newkirk MM, Neville C. Complications of immunosuppression associated with weekly low dose methotrexate. J Rheumatol 1991;18:1172-5.
32. Kamel OW, van de Rijn M, Weiss LM, et al. Brief report: reversible lymphomas associated with Epstein-Barr virus occurring during methotrexate therapy for rheumatoid arthritis and dermatomyositis. New Engl J Med 1993;328:1317-21.
33. Ferraccioli GF, Casatta L, Bartoli E, De Vita S, Dolcetti R, Boiocchi M. Epstein-Barr virus-associated Hodgkin's lymphoma in a rheumatoid arthritis patient treated with methotrexate and cyclosporin A. Arthritis Rheum 1995;38:867-8.
34. Liote F, Pertuiset E, Cochand-Priollet B, et al. Methotrexate related B lymphoproliferative disease in a patient with rheumatoid arthritis. Role of Epstein-Barr virus infection. J Rheumatol 1995;22:1174-7.
35. Thomason RW, Craig FE, Banks PM, Sears DL, Myerson GE, Gulley ML. Epstein-Barr virus and lymphoproliferation in

methotrexate treated rheumatoid arthritis. Mod Pathol 1996; 9:261-6.
36. Chevrel G, Berger F, Miossec P, et al. Hodgkin's disease and B cell lymphoproliferation in rheumatoid arthritis patients treated with methotrexate. A kinetic study of lymph node changes. Arthritis Rheum 1999;42:1773-6.
37. Le Goff P, Chicault P, Saraux A, Baron D, Valls-Bellec I, Leroy J-P. Lymphoma with regression after methotrexate withdrawal in a patient with rheumatoid arthritis. Role for the Epstein-Barr virus. Rev Rhum [Engl Ed] 1998;65:283-6.
38. Staal SP, Ambinder R, Beschorner W, Hayward GS, Mann R. A survey of Epstein-Barr virus DNA in lymphoid tissue: frequent detection in Hodgkin's disease. Am J Clin Pathol 1989;91:1-5.
39. Zijlmans JM, Van Rijthoven AW, Kluin PM, Jiwa NM, Dijkmans BA, Kluin-Nelemans JC. Epstein-Barr virus associated lymphoma in a patient with rheumatoid arthritis treated with cyclosporine [letter]. N Engl J Med 1992;326:1363.
40. Hirose Y, Sugai S, Masaki Y, et al. Epstein-Barr virus study in malignant lymphoma in Sjogren's syndrome. Int J Hematol 1999;69:174-9.
41. Gown AM, McNutt MA. Diagnostic immunocytochemistry of solid tumors. Chicago: ASCP Press; 1988.
42. Weiss LM, Mavahead LA. In situ demonstration of Epstein-Barr viral genome in viral associated B cell lymphoproliferations. Am J Pathol 1989;134:651-9.
43. Kamel OW, van de Rijn M, Hanasono MM. Immunosuppression-associated lymphoproliferative disorders in rheumatic patients. Leukemia Lymphoma 1995;16:363-8.
44. Shiroky JB, Newkirk MM. Reversible lymphomas [letter]. Kamel OW, van de Rijn M, Warnke RA, Dorfman RF [reply]. N Engl J Med 1993;329:1657-8.
45. Kinlen LJ. Incidence of cancer in rheumatoid arthritis and other disorders after immunosuppressive treatment. Am J Med 1985;78 Suppl 1A:44-9.
46. Prior P. Cancer and rheumatoid arthritis: epidemiologic considerations. Am J Med 1985;78 Suppl 1A:15-21.
47. Fries JF, Bloch D, Spitz P, Mitchell DM. Cancer in rheumatoid arthritis: a prospective long-term study of mortality. Am J Med 1985;78 Suppl 1A:56-9.
48. Kinlen LJ. Malignancy in autoimmune diseases. J Autoimmun 1992;5 Suppl A:363-71.
49. Beauparlant P, Papp K, Haraoui B. The incidence of cancer associated with the treatment of rheumatoid arthritis. Semin Arthritis Rheum 1999;29:148-58.
50. Morrison VA, Dunn DL, Manivel JC, Gajl-Peczalska KJ, Peterson BA. Clinical characteristics of post transplant lymphoproliferative disorders. Am J Med 1994;97:14-24.
51. Penn I. The changing pattern of post-transplant malignancies. Transplant Proc 1991;23:1101-3.
52. Paul C, Le Tourneau A, Cayuela JM, et al. Epstein-Barr virus-associated lymphoproliferative disease during methotrexate therapy for psoriasis. Arch Dermatol 1997;133:867-71.
53. Gattiker HH, Wiltshaw E, Galton DA. Spontaneous regression in non-Hodgkin's lymphoma. Cancer 1980;45:2627-32.
54. Horning SJ, Rosenberg SA. The natural history of initially untreated low-grade non-Hodgkin's lymphoma. N Engl J Med 1984;311:1471-5.
55. Tosato G, Steinberg AD, Blaese M. Defective EBV-specific suppressor T-cell function in rheumatoid arthritis. N Engl J Med 1981;305:1238-43.
56. Wolfe F. Inflammatory activity, but not methotrexate or prednisone use predicts non-Hodgkin's lymphoma in rheumatoid arthritis: a 25-year study of 1,767 RA patients [abstract]. Arthritis Rheum 1998;41 Suppl:S188.
57. Asten P, Barrett J, Symmons D. Risk of developing certain malignancies is related to duration of immunosuppressive drug exposure in patients with rheumatic diseases. J Rheumatol 1999;26:1705-14.
58. Voulgarelis M, Dafni UG, Isenberg DA, Moutsopoulus HM. Malignant lymphoma in primary Sjogren's syndrome. Arthritis Rheum 1999;42:1765-72.