Europ. J. Cancer Vol. 15, pp. 791–796.

0014-2964/79/0501-0791 $02.00/0

Serum β_2 Microglobulin and its Prognostic Value in Lymphomas

P. L. AMLOT* and M. ADINOLFI†

**Department of Medicine, Guy's Hospital Medical School, London SE1 9RT, United Kingdom*
†Paediatric Research Unit, The Prince Philip Research Laboratories, Guy's Hospital Medical School, London SE1 9RT, United Kingdom

Abstract—*The serum concentration of β_2 microglobulin (β_2m) was measured by radioimmunoassay in untreated patients with lymphomas. Increased levels of β_2m were found in both Hodgkin's and non-Hodgkin's lymphomas. Chromatographic analysis of sera from these patients has shown that β_2m molecules were not complexed with HLA chains or other cell membrane structures.*

The levels of β_2m were greater in patients with widespread, rather than localised, disease. The initial level of β_2m was found to be of prognostic significance in patients with lymphomas classified as diffuse, poorly-differentiated lymphoblastic (Rappaport's classification). Survival was shorter the higher the level of β_2m and vice versa. This correlation with survival was not seen in the other poor prognostic group—diffuse histiocytic lymphomas (DHL). Indeed levels of β_2m were significantly lower in DHL than PDLL. Treatment of patients with lymphomas led to a reduction in the serum levels of β_2m and normal values were often observed in treated patients even in the presence of recurrent or persistent disease.

INTRODUCTION

IN THE past few years β_2 microglobulin (β_2m) has received considerable attention, largely as a result of the demonstration of its close structural resemblance to certain domains of IgG molecules [1, 2] and of its association on cell membranes with HLA serologically defined (SD) antigens [3–5].

Although the biological function of this protein is still unknown, a variety of malignancies have been shown to be associated with high serum concentrations of β_2m. The most striking elevations of β_2m have been found in patients with plasma cell dyscrasias and some solid tumours, particularly those affecting the lung [6–8]. In contrast, it has been claimed that the β_2m concentration is normal in sera from patients with Hodgkin's disease (HD) [8]. However, in the course of an investigation on the levels of β_2m in patients with a variety of benign and malignant disorders, we have observed high levels of β_2m in most sera from untreated patients with HD and non-Hodgkin's lymphomas.

Accepted 22 November 1978.

MATERIALS AND METHODS

Patients

Blood samples were obtained from 79 patients with Hodgkin's disease (HD) and 73 with non-Hodgkin's (non-HD) lymphomas between November 1972 and October 1977, in each case before treatment was started. All patients had normal blood creatinine and urea levels at the time blood was taken for measurement of β_2m [9]. The extent of their disease was staged by the Rye Classification [10] which included staging laparotomy and splenectomy in forty-two patients with HD and only three of those with non-HD lymphomas. In the latter, splenectomy was performed because of pancytopoenia.

Histology of the non-HD lymphomas accorded with Rappaport's classification [11]. Histological types carrying a good prognosis were chronic lymphatic leukemia (CLL), diffuse well differentiated lymphocytic lymphoma (DLL), all nodular types (NLL) and mixed histiocytic/lymphocytic lymphomas (MHL). The distinction between CLL and DLL was arbitrarily set at a circulating lymphocyte count above or below 10,000/mm^3 respectively. Histological types with a bad

prognosis were diffuse poorly differentiated lymphoblastic lymphomas (PDLL) and diffuse histiocytic lymphomas (DHL). Other types of lymphoma included three primary solitary intestinal lymphomas, one immunoproliferative small intestinal disease, one angioimmunoblastic lymphadenopathy and one leukaemic reticuloendotheliosis. β_2 microglobulin measurement was by radioimmunoassay using commercial Phadebas kits kindly provided by Pharmacia Ltd., Uppsala, Sweden. Serum samples were stored at $-20°C$ until assay. The normal range for serum β_2m of 0.6–3.0 μg/ml was established on healthy laboratory personnel and blood donors and agrees with previous reports [7, 8].

In some cases, sera were fractionated on a Sephadex G100 column equilibrated with phosphate buffered saline pH 7.2, containing 0.01 mole/l edetic acid and 0.05% sodium azide. Individual fractions were assayed to assess the size of β_2m material using dextran blue, bovine serum albumin, ovalbumin, cytochrome C and lysozyme as molecular weight markers.

RESULTS

The non-Hodgkin's lymphomas were divided into good and bad prognosis groups on histopathological grounds; that this was justified is shown by the marked difference in survival between these two groups. The good prognosis group had a median survival greater than 24 months, and only 4 out of 36 died during a period of observation between 6 and 60 months. In the bad prognosis group, median survival was 5 months and 21 out of 31 died during the same period of observation.

Pre-treatment levels of β_2m in non-HD lymphomas are shown in Fig. 1. The highest levels of β_2m in the good prognosis groups were seen in CLL and DLL. These are usually indistinguishable on histological grounds, though the DLL type may show lymphocytes with cleft nuclei. The mean lymphocyte count in the CLL group was 56,000/mm^3 compared with 5260/mm^3 in the DLL group. Despite this difference in circulating lymphocyte load, there was no significant difference in β_2m between these two groups, and no correlation between β_2m and lymphocyte count. All the patients with DLL had generalised (Stage IV) disease on the basis of diffuse bone marrow infiltration by small lymphocytes.

The other two good prognosis histologies, NLL and MHL, showed more modest elevation of β_2m than CLL and DLL and the increase occurred predominantly in patients with generalised disease. So far there has been no correlation between β_2m levels and survival in any of the good prognosis group.

In the bad prognosis group, there are two histological types, PDLL and DHL, whose distinction as separate entities has been questioned in recent years [12–14]. In view of this controversy it is interesting that they differ in terms of serum β_2m. The PDLL group had significantly higher levels of β_2m than DHL (mean values: 9 and 3.4 μg/ml respectively, $P < 0.005$ by Student's 't' test).

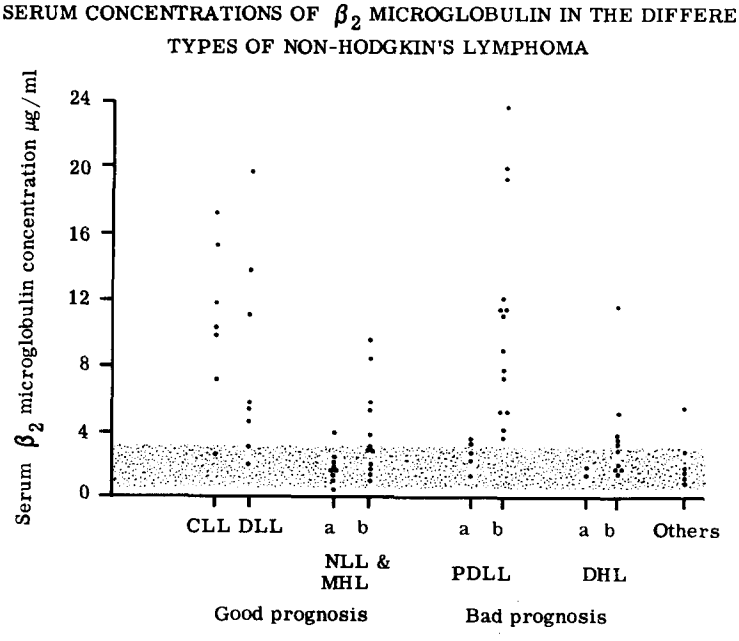

Fig. 1. For abbreviations see Materials and Methods. (a) Indicates localised disease (Stage I or II). (b) Indicates generalised disease (Stage III or IV).

Invariably patients with generalised PDLL had elevated levels of β_2m while this only occurred in half the patients with generalised DHL. Although survival is equally short in PDLL and DHL, it is only with the survival of the former that β_2m correlated. In PDLL those patients with the higher β_2m concentrations tend to have the shorter survival and vice versa (Fig. 2). No patient with PDLL and a β_2m level greater than 6 μg/ml survived longer than 10 months, while all the long survivors had β_2m below this level or in the normal range.

Among the other types of non-HD lymphomas β_2m was raised only in the patient with angioimmunoblastic lymphadenopathy. The patients with primary intestinal lymphomas all had quite extensive disease localised to the abdomen and histology ranged from diffuse lymphoblastic to lymphocytic with plasma-cytoid differentiation.

β_2m was increased in patients with untreated Hodgkin's disease (HD) and this is shown in Fig. 3. With the progressive spread of HD both the proportion of patients with abnormally high β_2m and the mean concentration of β_2m increased. Again, no clear correlation between β_2m level and survival has yet been seen.

Treatment of either the non-HD lymphomas (Fig. 4) or HD (Fig. 5) led to a fall in β_2m whether the treatment was successful in inducing complete remission or not. In the figures, unbroken lines indicate patients attaining complete clinical remission during the first 3 months of treatment, while broken lines indicate patients in whom complete remission was not achieved, and the transition from a solid to a broken line indicates clinically detected relapse of disease. From this small sequential study, monitoring β_2m does not appear useful in detecting either persistent or relapsing disease.

Sera from patients with HD, PDLL, CLL and DLL were fractionated on Sephadex G100 and β_2m then estimated in the individual fractions. β_2m was found in the same fractions as cytochrome C, and just preceded fractions in which lysozyme was found. This indicates a mol. wt around 12,000, corresponding to the mol. wt of free uncomplexed β_2m.

DISCUSSION

The reasons for the difference between the increased levels of β_2m in Hodgkin's disease (HD), observed in the present study, and the normal values reported in a previous investigation [8] are not clear. In the previous study, neither the stage of HD spread nor the treatment status of the patients were described and clarification of either of these points may help to explain the contradictory results. Therapy may be particularly relevant because we have seen that even in the presence of active HD, normal levels of β_2m can be detected in patients undergoing or having received treatment. Following treatment, a similar suppressive effect upon the serum levels of immunoglobulin has been described [15].

The raised levels of β_2m found in lymphomas cannot be attributed to renal causes, since our patients had normal creatinine levels

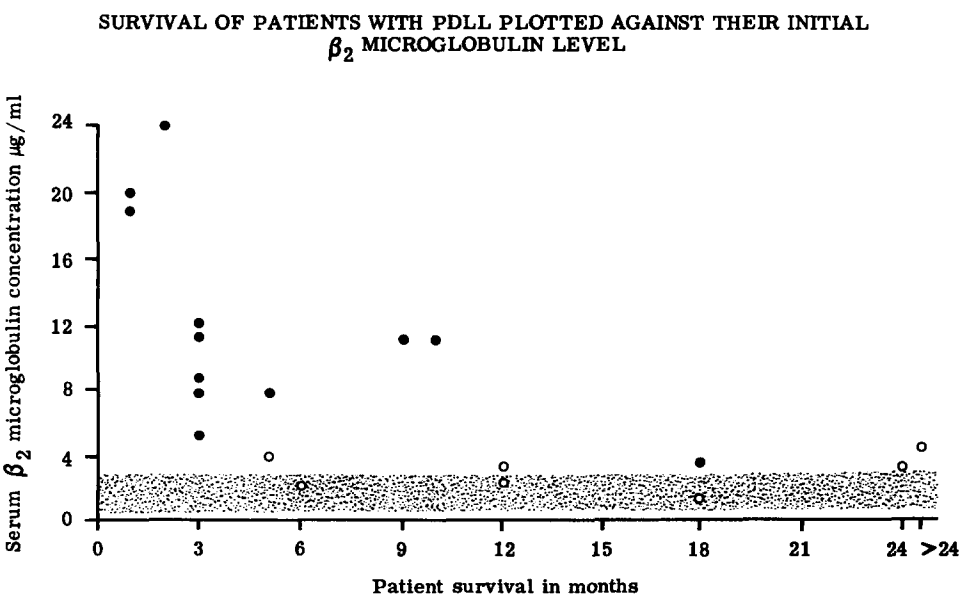

Fig. 2. Solid dots indicate patients that have died (●) and open circles patients still alive (○).

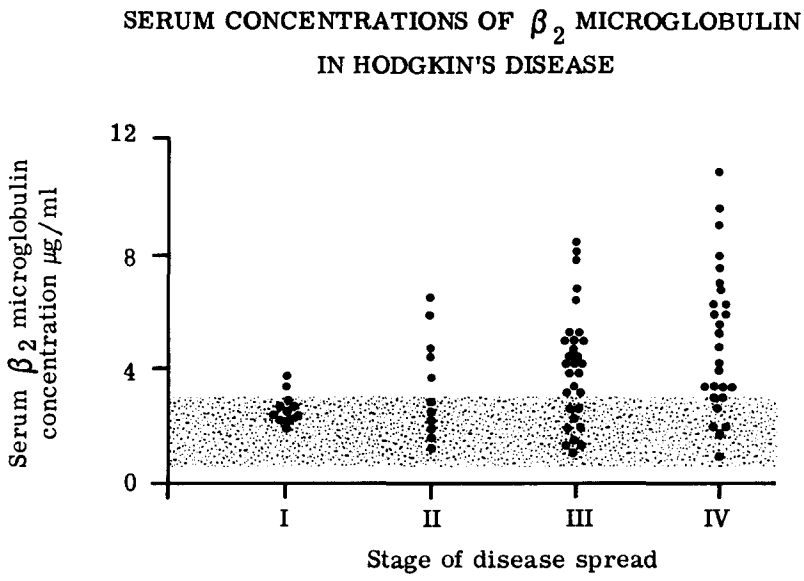

Fig. 3. Shaded area is the normal range for β_2m 0.6–3.0 μg/ml.

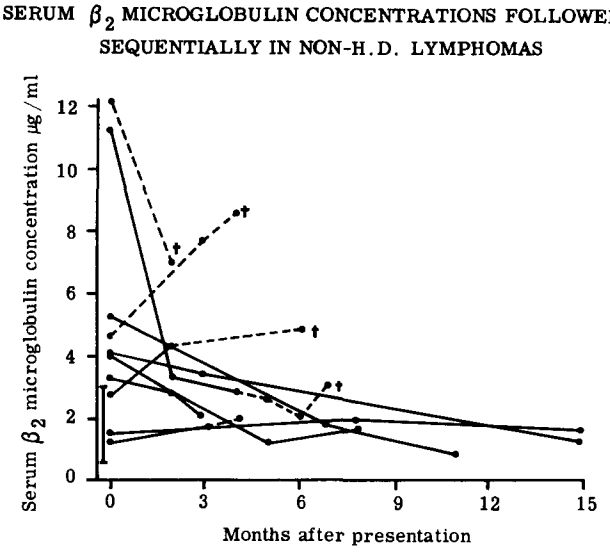

Fig. 4. Vertical bar represents normal range 0.6–3.0 μg/ml. *Unbroken line represents patients attaining complete remission (CR) within 3 months of starting treatment; broken lines are patients not attaining CR, or relapsing from CR. Crosses indicate the death of a patient.*

and serum β_2m was detected in small, uncomplexed molecular form.

Therefore we suggest that the raised β_2m level reflects an increased synthesis either indirectly from normal "reactive" cells or directly from malignant cells within involved lymphoid tissues. HD and the non-HD lymphomas may differ in this respect. In HD a chronic inflammatory cellular infiltrate predominates over abnormal malignant cells in the involved tissues and it may be that these "reactive" cells produce increased amounts of β_2m, like the raised levels of β_2m seen in Sjögren's disease, rheumatoid arthritis, systemic lupus erythematosus, viral hepatitis and infectious mononucleosus [8, 16]. Mitogen stimulated lymphocytes and lymphoblastoid cell lines secrete greater quantities of β_2m *in vitro* than unstimulated lymphocytes and HD lymphoid tissue also secretes increased quantities of β_2m [17]. This increased production of β_2m by HD tissue may be seen where less than 1% of the total cell population were abnormal cells characteristic of HD (unpublished observation).

In the non-HD lymphomas, particularly the diffuse types where normal lymphoid tissue is replaced by a proliferation of malignant cells, it seems more plausible that the increased β_2m levels derive directly from the malignant cells. This is not because malignant cells express more β_2m on the cell surface, but because it is released more rapidly than from normal cells [17]. If β_2m is a cell membrane constituent along with HLA chains, then an accelerated membrane turnover or accelerated cell division could increase the shedding of β_2m by lymphoma cells. It is interesting in this context that β_2m is released *in vitro* as the free, uncomplexed, 11,800 dalton unit [18, 19].

Prediction of prognosis can be difficult in lymphomas; the present study suggests that additional prognostic information may be provided by measurement of serum β_2m level.

Acknowledgements—We are indebted to the excellent technical assistance given by Susan Beck and Jenny Slaney in carrying out this study. We would like to thank Dr. G. A. K. Missen and Dr. D. R. Turner for their careful classification of lymphoma tissue, and the Department of Radiotherapy and Oncology and Department of Medical Oncology for allowing us to study their patients.

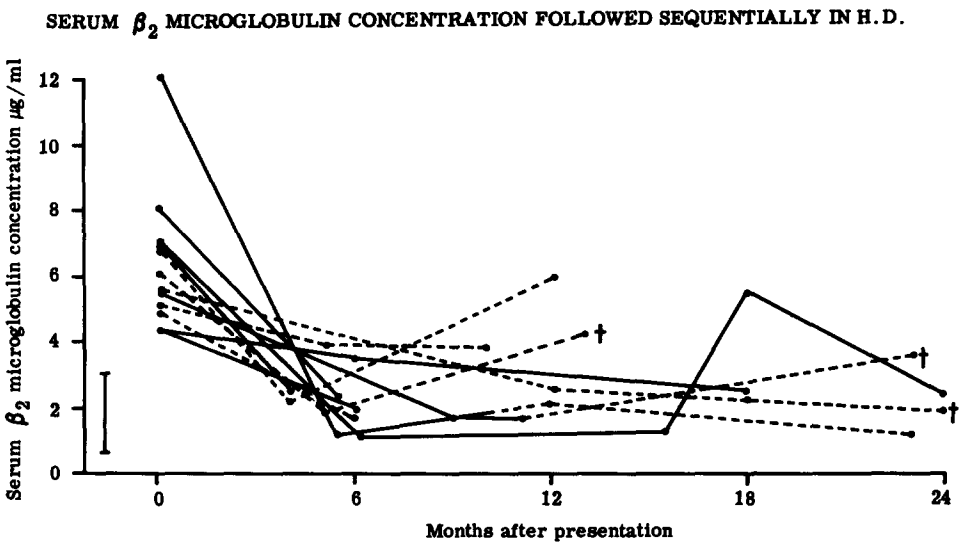

Fig. 5. Vertical bar represents normal range 0.6–3.0 µg/ml. Unbroken line represents patients attaining complete remission (CR) within 3 months of starting treatment; broken lines are patients not attaining CR, or relapsing from CR. Crosses indicate the death of a patient.

REFERENCES

1. O. Smithies and M. D. Poulik, Initiation of protein synthesis at an unusual position in an immunoglobulin gene. *Science* **175,** 187 (1972).
2. P. A. Peterson, B. A. Cunningham, I. Berggard and G. M. Edelman, β_2 microglobulin—a free immunoglobulin domain. *Proc. nat. Acad. Sci. (Wash.)* **69,** 1697 (1972).
3. K. Nakamuro, N. Tanigaki and D. Pressman, Multiple common properties of human β_2 microglobulin and the common portion fragment derived from HL-A antigen molecules. *Proc. nat. Acad. Sci. (Wash.)* **70,** 2863 (1973).
4. M. D. Poulik, M. Bernoco, D. Bernoco and R. Ceppellini, Aggregation of HL-A antigens at the lymphocyte surface induced by antiserum of β_2 microglobulin. *Science* **182,** 1352 (1973).
5. H. M. Grey, R. T. Kubo, S. M. Colon, M. D. Poulik, P. Cresswell, T. S. Springer, M. Turner and J. L. Strominger, The small subunit of HL-A antigens is β_2 microglobulin. *J. exp. Med.* **138,** 1608 (1973).
6. M. D. Poulik, D. Farrah and O. Smithies, Association of urinary β_2 microglobulin with myeloproliferative diseases. *Fed. Proc.* **31,** 741 (1972).
7. P. E. Evrin and L. Wibell, Serum β_2 microglobulin in various disorders. *Clin. chim. Acta* **43,** 183 (1973).
8. J. Shuster, P. Gold and M. D. Poulik, β_2 microglobulin levels in cancerous and other disease states. *Clin. chim. Acta* **67,** 307, (1976).
9. G. M. Bernier, R. J. Cohen and M. E. Conrad, Microglobulinaemia in renal failure. *Nature (Lond.)* **218,** 598 (1968).
10. P. P. Carbone, H. S. Kaplan, K. Musshoff, D. W. Smithers and M. Tubiana, Report of the Committee on Hodgkin's disease staging classification. *Cancer Res.* **31,** 1860 (1971).
11. H. Rappaport, *Tumors of the Hematopoietic System:* Atlas of Tumor Pathology, Section 3, Fascicle 8, Armed Forces Institute of Pathology, Washington D.C. (1966).
12. R. Gerard-Marchant, I. Hamlin, K. Lennert, F. Rilke, A. G. Stansfeld and J. A. M. van Unnik, Classification of non-Hodgkin's lymphomas. *Lancet* **ii,** 406 (1974).
13. C. D. Bloomfield, J. H. Kersey, R. D. Brunning and K. J. Gajlpeczalska, Prognostic significance of lymphocyte surface markers in adult non-Hodgkin's malignant lymphoma. *Lancet* **ii,** 1330 (1976).
14. H. Stein, E. Kaiserling and K. Lennert, Evidence for B cell origin of reticulum cell sarcoma. *Virchows Arch. path. Anat.* **364,** 51 (1974).
15. P. L. Amlot and L. Green, Atopy and immunoglobulin E concentrations in Hodgkin's disease and other lymphomas. *Brit. med. J.* **1,** 327 (1978).

16. J. P. Michalski, T. E. Daniels, N. Talal and H. M. Grey, β_2 microglobulin and lymphocytic infiltration in Sjögren's syndrome. *New Engl. J. Med.* **293,** 1228 (1975).
17. K. Nilsson, P. E. Evrin and K. I. Welsh, Production of β_2 microglobulin by normal and malignant human cell lines and peripheral lymphocytes. *Transplant Rev.* **21,** 53 (1974).
18. P. Cresswell, T. Springer, J. L. Strominger, M. J. Turner, H. M. Grey and R. T. Kubo, Immunological identity of the small subunits of HL-A antigens and β_2 microglobulin and its turnover on the cell membrane. *Proc. nat. Acad. Sci.* **71,** 2123 (1974).
19. R. T. Kubo, S. M. Colon, R. T. McCalmon and H. M. Grey, Some biological properties of β_2 microglobulin and its antibody. *Fed. Proc.* **35,** 1183 (1976).