Eur J Cancer Clin Oncol, Vol. 19, No. 3, pp. 327–331, 1983.
Printed in Great Britain.

0277-5379/83/030327-05$03.00/0

Serum β_2-Microglobulin in Patients with Non-Hodgkin's Lymphoma

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Abstract—*Eighty-one patients with non-Hodgkin's lymphoma had serum β_2-microglobulin (β_2M) estimated at presentation. A significant association was found between β_2M levels and stage of disease ($P < 0.001$), presence of hepatomegaly ($P < 0.001$) and bone marrow involvement ($P < 0.05$). No association was found between the level of β_2M and histological group, presence of splenomegaly, lymph-node masses greater than 5 cm in diameter, lymphocyte count or the presence of systemic B symptoms. Pretreatment levels of β_2M did not help predict response to treatment and achievement of a complete remission, the length of remission obtained or survival.*

INTRODUCTION

β_2-MICROGLOBULIN (β_2M) is a low-molecular-weight protein of 11,800 daltons first isolated from urine in patients with Wilson's disease in 1968 [1]. It is found on all nucleated cell membranes, and forms the light chain moiety of HL-A. Cell membrane turnover is the principal source of free β_2M in blood, plasma and body fluid [2]. Increased levels of β_2M have been reported with increasing age, renal impairment and a variety of malignancies, notably myeloma, chronic lymphocytic leukaemia and lymphoma [3].

Three studies of pretreatment serum β_2M levels in patients with non-Hodgkin's lymphoma (NHL) have demonstrated higher levels in patients with advanced disease [4–6]. Child *et al.* reported that high β_2M levels at presentation were common in patients with unfavourable prognosis lymph-node histology [5]. Amlot and Adinolfi [4] found high levels, particularly in patients with well-differentiated or poorly differentiated diffuse lymph-node histologies as classified by Rappaport [7]. The pretreatment level of β_2M did not help predict survival in patients with a favourable prognosis or diffuse histiocytic lymph-node pathology. Patients with poorly differentiated diffuse lymphoma, however, survived longer if they presented with a low β_2M level. No details of treatment and response were given, and the median survival of only 5 months was very short.

If β_2M levels at presentation reflect tumour mass, these levels may be a useful prognostic factor. This study was performed with the aim of assessing the possible prognostic role of pretreatment serum β_2M levels in patients with NHL uniformly staged and treated. The pretreatment β_2M levels were correlated with other clinical and laboratory variables at presentation, response to treatment, remission length and survival.

MATERIALS AND METHODS

Eighty-one patients with NHL (aged between 16 and 68 yr) had serum stored at −20°C when they presented to hospital. The level of β_2M in these serum samples was estimated using a radio-immunoassay technique (Phadebas kits, Pharmacia Ltd, Sweden) in a single batch.

Patients were staged using the Ann Arbor classification for Hodgkin's disease [8]. Staging laparotomies were not performed, but intensive staging, including marrow aspiration and trephine, computerised axial tomography, percutaneous liver biopsy and CSF examination were used as clinically indicated. All the lymph-node histologies were reviewed and classified according to the Rappaport classification [7], which is used routinely in all the Manchester Lymphoma Group trials. Patients were divided for treatment purposes on the basis of previously reported studies into a favourable prognosis histology

Accepted 5 October 1982.

group (all nodular lymphomas, plus well-differentiated diffuse lymphoma), with a median survival of 5–7 yr, and an unfavourable prognosis histology group (all diffuse lymphomas, except well-differentiated diffuse lymphoma), with a median survival of less than 2 yr [9].

There were six patients with stage I disease, 10 with stage II, 16 with stage III and 49 patients with stage IV non-Hodgkin's lymphoma. Patients with stage I and II disease presenting outside the abdomen were treated with radiotherapy at a dose of 3000 rads (30 Gy) to the involved field over three weeks, and in some cases adjuvant chemotherapy. Patients with stage III and IV disease, and stage II intra-abdominal presentations whose lymph-node pathology fell into the favourable prognosis group, were treated with a 'CVP' regimen, consisting of vincristine 1.4 mg/m^2 i.v. on day 1, and cyclophosphamide 400 mg/m^2 and prednisolone 40 mg orally on days 1–5 inclusive every 3 weeks. Patients with stage III and IV disease, and stage II intra-abdominal presentation whose lymph-node pathology was of an unfavourable prognostic type, were treated using a 'VAP' regimen, consisting of vincristine 1.4 mg/m^2 i.v. weekly, adriamycin 50 mg/m^2 i.v. on alternate weeks and prednisolone 50 mg daily for 6 weeks [10]. Induction therapy was followed by oral maintenance for 2 yr using 6-mercaptopurine, methotrexate and cyclophosphamide. Remission status was assessed after 6 weeks of VAP therapy or 6 courses of CVP.

A complete remission was defined as a normal performance status, associated with complete resolution of all clinical, radiological, biochemical and bone-marrow biopsy evidence of disease. A serum β_2M value of 4 mg/l was used as the upper range of normal in this study.

Statistical analysis

Any association of β_2M levels with stage of lymphoma or histology was assessed using a Krasker–Wallis one-way, non-parametric analysis of variants [11]. In the other analyses the χ^2 test or Mann-Whitney U test was used where applicable. Life-table survival and remission duration curves were compared using the log rank test [12].

RESULTS

The serum β_2M levels of the 81 patients studied ranged from 1.3 to 27 mg/l, with a median of 3.7 mg/l and a mean of 5 mg/l. Thirty-six patients had a β_2M level above the upper normal limit of 4 mg/l. Renal function was normal (serum creatinine <0.11 mmol/l) in 80 of the patients studied. One patient with stage IV B poorly differentiated diffuse lymphocytic lymphoma had a serum creatinine level of 0.16 mmol/l and a serum β_2M level of 27 mg/l at presentation. Renal function returned to normal after rehydration and treatment of a septicaemia, but unfortunately the patient died 7 days after admission of advancing disease, despite chemotherapy.

Stage

Serum β_2M levels of patients increased with increasing stage of the Ann Arbor classification (P <0.001). (See Fig. 1.)

Clinical features

An enlarged liver was palpable 3 cm or further below the costal margin in 17 (47%) of 36 patients with a β_2M level of >4 mg/l and in only 5 (11%) of 45 patients whose β_2M levels were normal (P <0.001). There were similarly more patients with bone-marrow involvement with lymphoma whose β_2M levels were raised above 4 mg/l. Only 13 (28%) of 45 patients with a normal β_2M compared with 20 (55%) of 36 patients whose β_2M level was raised had bone-marrow involvement (P <0.05). An enlarged spleen palpable 3 cm or further below the costal margin was found in 5 (11%) of 45 patients with a normal β_2M and in 11 (31%) of 36 with a raised β_2M. This difference just failed to reach statistical significance (P = 0.052). No significant correlation was found between a

Table 1. Non-Hodgkin's lymphoma patients: β_2M value related to liver size, bone-marrow involvement, spleen size, the presence of bulky disease ≥5 cm at any one site, lymphocyte count and the presence of A or B symptoms

	Normal β_2M (≤4 mg/l)	High β_2M (>4 mg/l)	
Normal liver size	40	19	P <0.001
Hepatomegaly >3 cm	5	17	
Bone marrow			
Involved	13	20	
Equivocal	4	3	P < 0.05
Not involved	28	13	
Normal spleen size	40	25	P = 0.052
Splenomegaly >3 cm	5	11	
No bulk disease	25	18	P >0.7
Bulk disease ≥5 cm	20	18	
Lymphocytes			
<1000	13	11	
1000–5000	30	23	P >0.9
>5000	2	2	
Symptom A	29	22	P >0.9
Symptom B	16	14	

raised β_2M level and the presence of lymph-node masses larger than 5 cm in diameter, increasing lymphocyte count, or the presence of B symptoms. (See Table 1.)

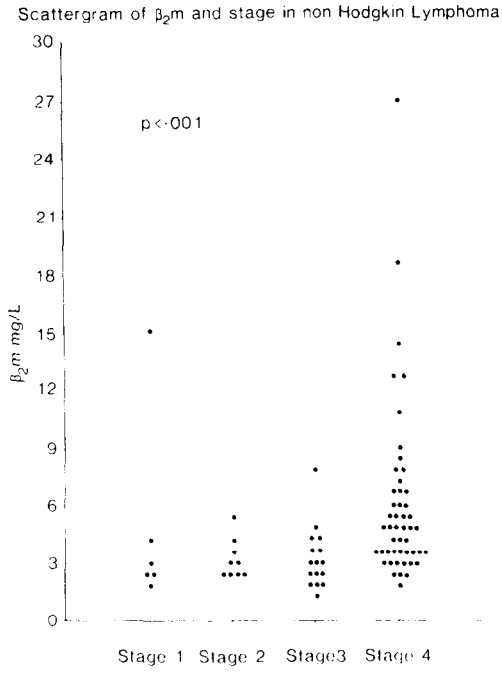

Fig. 1. Scatterogram of β_2M and stage in NHL.

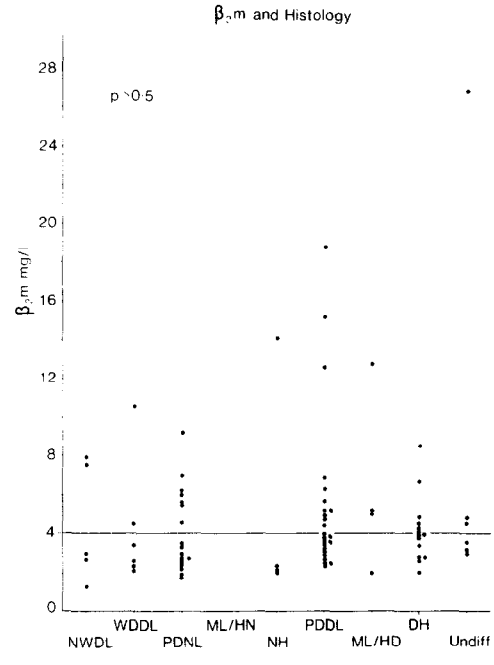

Fig. 2. Scatterogram of β_2M and histological group of NHL. NWDL, nodular well-differentiated lymphocytic lymphoma; WDDL, well-differentiated diffuse lymphoma; PDNL, poorly differentiated nodular lymphoma; ML/HN, mixed lymphocytic/histiocytic nodular lymphoma; NH, nodular histiocytic lymphoma; PDDL, poorly differentiated diffuse lymphocytic lymphoma; ML/HD, mixed lymphocytic/histiocytic diffuse lymphoma; DH, diffuse histiocytic lymphoma; Undiff, undifferentiated lymphoma.

Histology

There was a wide range of serum β_2M levels within each of the histological groups of the Rappaport classification (Fig. 2). The serum β_2M levels in the different histological subtypes were compared and no statistically significant difference was found.

Serum levels of β_2M of 33 patients with stage IV non-Hodgkin's lymphoma whose lymph-node histology was considered of 'unfavourable' histological type had a wider range of β_2M levels than 17 patients with favourable prognosis histology, but the difference was not statistically significant.

Response to treatment

All six patients with stage I and II disease not presenting in the abdomen achieved a complete response with radiotherapy. Only two patients had raised serum β_2M levels. No correlation between β_2M and response could therefore be made. Twenty-eight patients with favourable prognosis histology NHL were treated with 6 courses of the CVP regimen before reassessment. Eight (47%) of the 17 patients with normal β_2M levels and 4 (36%) of the 11 patients with raised β_2M levels achieved a complete remission (Table 2). This difference was not statistically significant ($P > 0.8$).

Forty-seven patients with unfavourable prognosis histology NHL were treated with the VAP regimen and were evaluable for assessment. Sixteen (67%) of 24 patients with normal β_2M achieved a complete remission and 11 (48%) of the 23 patients who had a raised β_2M achieved a complete remission. This difference was not statistically significant ($P > 0.3$). When all 75

Table 2. β_2M value related to the number of patients with NHL treated by VAP or CVP achieving a complete remission at reassessment

	Complete remission	Total	
Unfavourable pathology			
Normal β_2M	16	24	
High β_2M	11	23	$P > 0.8$
Total	27	47	
Favourable pathology			
Low β_2M	8	17	
High β_2M	4	11	$P > 0.3$
Total	12	28	
Combined favourable and unfavourable			
Low β_2M	24	41	
High β_2M	15	34	$P > 0.3$
Total	39	75	

patients treated with chemotherapy were assessed, 24 (58%) of 41 patients with normal β_2M achieved a complete remission in comparison with 15 (44%) of 34 patients with raised β_2M levels. There was therefore a tendency for patients with normal serum β_2M levels at presentation to have a higher complete remission rate than patients in whom the level was raised, but this was not statistically significant ($P > 0.3$).

Length of first remission

Following their reassessment, 3 patients with favourable histology were converted from good partial remission to complete remission with additional chemotherapy and/or radiotherapy. Fifteen patients with favourable prognosis histology and 27 with unfavourable prognosis histology treated primarily with chemotherapy were available for analysis. The pretreatment β_2M levels were not of prognostic significance for length of first remission for the whole group (Fig. 3; $P = 0.3$), the 15 patients with favourable prognosis ($P = 0.2$) not the 27 patients with unfavourable prognosis histology ($P > 0.3$).

Survival

When all the patients treated by chemotherapy were considered, the overall survival for the 41 patients whose initial β_2M levels were within normal range was identical to the 34 patients in whom the level was raised greater than 4 mg/l (Fig. 4; $P = 0.38$). Pretreatment β_2M levels were not of prognostic significance when the subgroups of favourable and unfavourable histologies were analysed separately. Similarly, there was no prognostic significance when only patients who achieved a complete remission or who had stage III and IV disease were assessed.

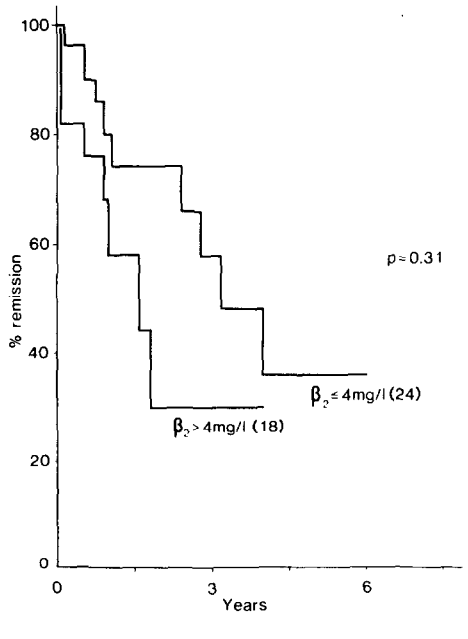

Fig. 3. Disease-free interval of the 42 patients with NHL achieving a complete remission with chemotherapy related to β_2M level at presentation.

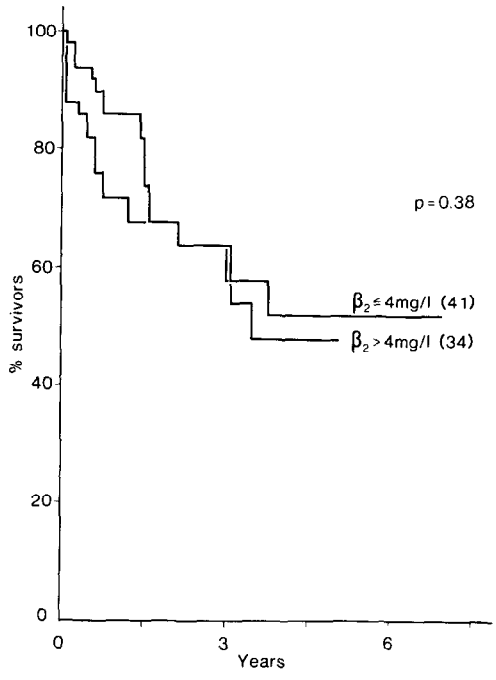

Fig. 4. Survival curves of the 75 patients treated with chemotherapy in relation to level of β_2M at presentation.

DISCUSSION

Serum β_2M levels were not corrected for deteriorating renal function, as described by Cassuto and co-workers, since only one patient had a raised serum creatinine [6].

A strong association of pretreatment serum β_2M with advancing stage of disease was found. This finding was confirmed by the correlation of raised β_2M levels and the presence of hepatomegaly and bone-marrow involvement. This association with stage has been previously described for patients with non-Hodgkin's lymphoma, Hodgkin's disease and multiple myeloma [3–6, 13].

Amlot and Adinolfi reported that β_2M levels were significantly higher in patients whose lymph-node histology was classified as poorly-differentiated diffuse rather than diffuse histiocytic lymphoma. High levels were also reported in patients with chronic lymphocytic leukaemia and well-differentiated diffuse lymphoma [4]. No association with histological sub-group was found in the present study, but the number of patients in each sub-group was small. Child *et al.* reported high levels of β_2M in patients whose lymph-node histology was classified as 'un-

favourable' rather than 'favourable' histological type [5]. This difference between 'unfavourable' and 'favourable' histological type was not confirmed in this report, even if only patients with stage IV disease were compared to make allowance for the association of β_2M levels with stage of disease.

There was no evidence in this study to support the hypothesis that patients with raised β_2M levels at presentation, because of a high tumour burden, gain less complete remissions and that the remissions obtained are shorter. The relationship of pretreatment β_2M levels and response to treatment has not previously been examined, but Amlot and Adinolfi reported that survival was poorer in patients with poorly differentiated diffuse lymphoma who presented with raised β_2M levels [4]. Unfortunately no details of treatment or response were given and the median survival of only 5 months was very short.

It has been suggested that β_2M levels could be used to monitor response to treatment. Serial levels were not measured in this study, but only 36 of the 81 patients had a raised level at presentation to use as a marker. It is doubtful that a fall to normal level would equate with a complete remission, since 11 of the 15 patients with stage I and II disease had normal levels of β_2M at presentation when definite tumour was present.

Pretreatment serum β_2M level has been confirmed to be associated with the stage of disease for patients with non-Hodgkin's lymphoma, but not to be of prognostic significance.

Acknowledgements—We would like to thank Professor E. H. Cooper of Leeds University for measuring the β_2M levels, and Pauline Willett for typing this paper.

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