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Malignant Lymphoma: Monitoring of Tumour Status in 273 Patients Using a Monoclonal Antibody, 'B5', Reacting with Autologous Erythrocytes

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Abstract—*The value of the B5 antigen haemagglutination test has been assessed in 273 patients with malignant lymphoma—141 with non-Hodgkin's lymphoma (NHL) and 132 with Hodgkin's disease (HD). Of those patients who were in remission after treatment, 41/155 (26%) showed B5 positivity: this compares to an incidence of 20% (122/551) in a wide range of non-tumour bearing individuals. In contrast, those patients with persistent disease, or in relapse, showed a much higher incidence of B5 positivity (84%; 99/118). Serial monitoring of 113 patients showed that B5 status often changed as tumour status changed, becoming more negative with remission, and more positive in relapse. Use of the B5 test in conjunction with erythrocyte sedimentation rate (ESR) gave an increased specificity for active disease in that 87% (35/40) of those who were B5-positive together with a raised ESR had active disease: of those patients who were both B5-negative and had normal ESR, 9 out of 108 (8%) had active disease. These findings suggest a role for the B5 test, combined with the ESR, in the monitoring of patients with malignant lymphoma.*

INTRODUCTION

PATIENTS with Hodgkin's disease and non-Hodgkin's lymphoma attending the lymphoma clinic, Weston Park Hospital, Sheffield, are routinely monitored by relevant clinical and radiological assessments. In addition, erythrocyte sedimentation rate (ESR) values are measured. The ESR provides prognostic information at the time of staging [1, 2] and is a crude but useful follow-up marker. In the absence of more specific markers, ESR is of value where there is a persistent elevation or progressive increase, this being suggestive of residual or recurrent lymphoma.

The report [3] of a new tumour marker, non-specific for tumour type, led us to investigate its value in monitoring patients with malignant lymphoma. This marker is on erythrocytes and is detected by 'B5', a rat monoclonal IgG antibody, using a simple haemagglutination test. Since the incidence of B5 haemagglutination in the normal population is around 20% (122/551), compared to 80% in patients with cancer [3, 4], the relationship between B5 status and malignant disease is being investigated in detail. To date research has shown that: (a) patients with tumour tend to be B5-positive regardless of tumour type; (b) B5 status is unrelated to therapy, blood group, age or sex [3]; (c) for bladder, the extent of B5 haemagglutination does not appear to correlate with the size of the tumour [5]; and (d) monitoring individual patients with teratoma and seminoma has revealed a change in status from B5-positive towards B5-negativity associated with removal of tumour [6].

Here we report on a prospective study restricted to lymphoreticular tumours where B5 status is compared with the status of the disease, and with the ESR.

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PATIENTS AND METHODS

The patients studied were attending the lymphoma clinic at Weston Park Hospital, Sheffield, for treatment or follow-up of malignant lymphoma. Consecutive non-selected patients were entered into the study. These patients were clinically staged according to Ann Arbor criteria [7] and the histological grade of the tumour based on the British

National Lymphoma Investigation criteria [8, 9]; Grade I and II simplistically indicate low- and high-grade lymphoma respectively. Serial blood samples were taken, beginning at presentation where possible, and at follow-up visits to the lymphoma clinic.

The ESR (mm/hr) was measured by the Westergren method. Values above 20 mm/hr were regarded as abnormal. For B5 assays blood (2 ml) was collected in lithium–heparin tubes and stored at + 4 until tested (up to 7 days). The first 20 samples were assayed twice, both locally, and in Cambridge: the results were found to be in good agreement. Thereafter, all tests were carried out locally. Briefly, the test was as follows: a small aliquot of blood was washed free of serum and diluted to 1% erythrocytes. Washing buffer was phosphate buffered saline (PBS), and the final diluent was 4% foetal calf serum in PBS. Culture supernatant (25 μl) containing the B5 antibody was mixed with 25 μl of the diluted erythrocytes in a 'U' well microtitre plate. After at least 2 hr at room temperature haemagglutination was scored by microscopic viewing after resuspension and transfer of the erythrocytes to a glass slide; estimation of the percentage free cells and the size of the haemagglutinated clumps was recorded. Samples containing less than 70% free cells and clumps of more than 20 cells were considered positive.

RESULTS

(*a*) *New patients*

During this study 53 new patients presented—30 with NHL and 23 with HD. At diagnosis 66% (20/23) of NHL patients and 87% (20/23) of HD patients were scored positive in the B5 test. One patient subsequently died; his erythrocytes were totally agglutinated by B5, both at diagnosis and upon a second test prior to death. Overall, however, there was no clear evidence of a correlation between the strength of B5 positivity at diagnosis and clinical stage, histology grade, or subsequent repsonse to treatment.

Figure 1 summarises the progress of 47 new patients who have been monitored for 6 months or more: 31 patients (10 NHL and 21 HD) have now entered a clinical state of unequivocal remission. These include 6 NHL patients who were B5-positive at diagnosis, 3 of whom are now B5-negative with remission: 4 NHL patients now in remission were B5-negative at diagnosis. Sixteen HD patients who subsequently achieved remission were B5-positive at diagnosis: here, 11 patients switched from B5-positive to B5-negative. Three other HD patients who are now in remission were B5-negative at diagnosis. Any change in B5 status associated with remission was always from positive to negative; 2 of the patients changed at times prior to clinical assessment of remission being made.

(*b*) *Long-term patients*

In addition to new patients, 155 patients already in remission, and 66 others with active lymphoma, were included in this study. Patients with active NHL showed an 88% (42/48) B5 positivity. Seventeen of the 18 patients with active HD were B5-positive (94%). For those in remission, most patients were B5-negative; a positive B5 test was found in 16/60 (27%) of NHL patients and 24/85 (28%) of HD patients.

Many patients were tested 3 or more times and it was found that B5 status changed with a change in disease status for most patients. For example, those entering remission switched from B5-positive to B5-negative. Importantly, no patient (either new or long-term) entering remission showed increasing B5 positivity.

(*c*) *B5 test with ESR*

Patients with active disease were often found to be B5-positive and have a raised ESR. This is shown in Fig. 2(a), from which it can also be seen that 10 patients who subsequently died fell within the B5-positive/ESR-raised domain of the plot.

These findings were in marked contrast to those for patients in clinical remission, where a cluster of B5-negative/ESR-normal individuals was found (Fig. 2b). The remaining patients in remission were scattered due to *either* a raised ESR value, *or* to a positive B5 result. Only 4 patients in apparent remission (4/155) were B5-positive together with a raised ESR.

DISCUSSION

The mechanism by which B5 reacts with autologous erythrocyte is unknown. However it has been shown [3] that B5 is not a major blood group antigen, nor is it the precursor of the N and M antigens (i.e. the Thomsen-Freidenreich, T, or Tn antigens). Antibodies against carcinoembryonic antigen (CEA) and Lewis antigen do not mimic the B5 haemagglutination. Also, blocking experiments, and cross incubations, with serum or plasma have failed to demonstrate 'free' B5 antigen, or any enzyme activity causing a change in the B5 status of erythrocytes [3]. Thus it is likely that the B5 antigen is an intrinsic component of the red cell membrane, rather than acquired. A wide range of tumour types are associated with a change in B5 status, including tumours in 'privileged' sites such as brain and testis. It therefore seems possible that increased expression of the B5 antigen reflects a fundamental physiological or pathological change occurring with neoplastic transformation.

In this assessment of the B5 test in 273 patients

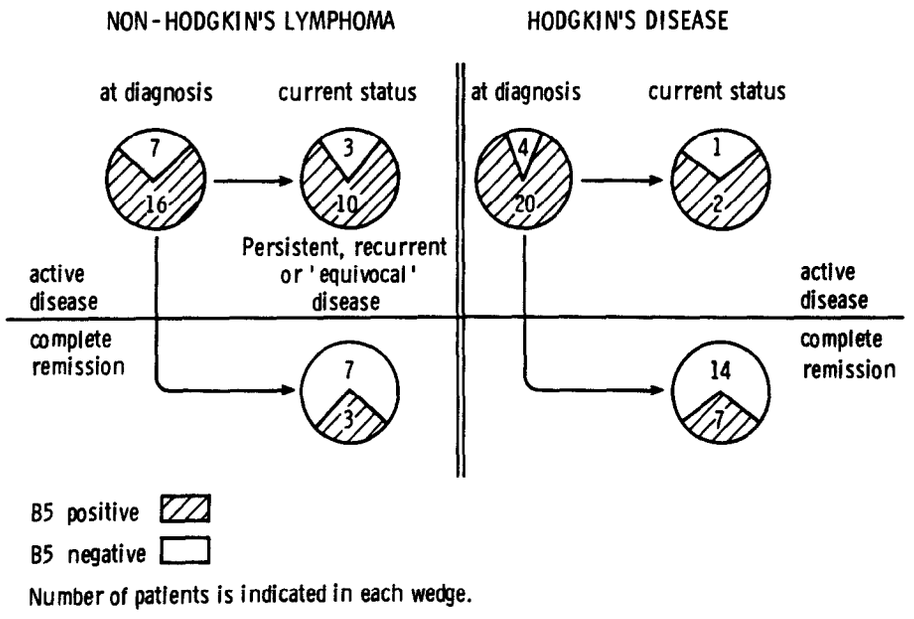

Fig. 1. New patients: distribution of B5 positivity at diagnosis and after at least 6 months follow-up.

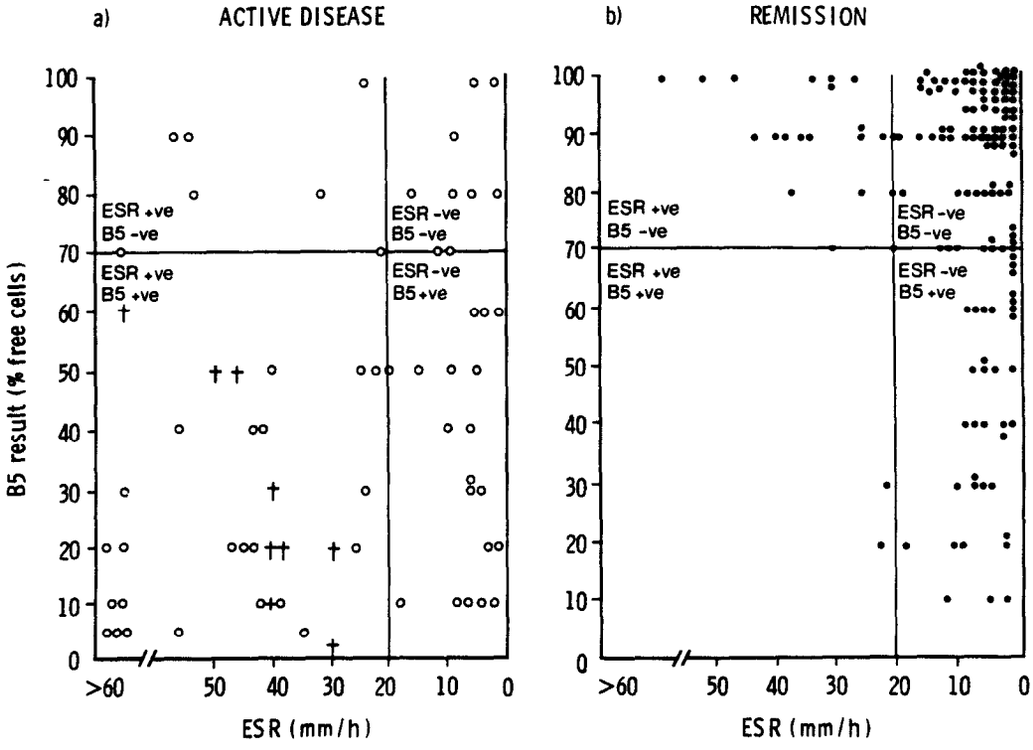

Fig. 2. B5 status plotted againt ESR for patients with active disease (a) or in clinical remission (b). Abnormal values (ESR > 20 mm/hr, B5 < 70% free cells) are indicated as positive. Each symbol represents a single patient. †Patient subsequently died.

with malignant lymphoma, we have found the assay to provide useful additional information of disease activity. An unexpected finding was the relatively low (66%) correlation of B5 positivity with NHL patients at diagnosis, compared to a significantly higher correlation (88%) with NHL patients with persistent disease or in relapse. Although preliminary, this data may indicate that patients who are B5-negative at diagnosis are less likely to suffer persistent or recurrent disease. On the other hand, the analysis of patients at presentation is complex due to the considerable heterogeneity of the lymphomas, particularly for NHL. There is also difficulty in determining complete remission in this latter group. Thus, a clearer indication of any prognostic value of the B5 test awaits a larger data base of patients monitored from their first presentation.

In conclusion, the main role for the B5 assay in malignant lymphoma is to provide additional information in monitoring patients—particularly when combined with ESR.

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