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Predictors of infection in chronic lymphocytic leukaemia (CLL)

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SUMMARY

A group of patients with chronic lymphocytic leukaemia (CLL) were studied to determine whether particular clinical and laboratory parameters might help to identify those patients at risk of recurrent infection who would benefit from immunoglobulin replacement therapy. The case notes of 59 patients were reviewed with regard to stage and duration of disease, chemotherapy and frequency of infection over the preceding 2 years. Serum IgG levels and specific antibodies to tetanus, diphtheria and pneumococcal capsular polysaccharide were measured at the end of the 2-year period. A group of 56 healthy age-matched volunteers were used as controls. Eighteen patients had severe or multiple infections during the study period, 11 patients had recurrent infections and the remaining 30 patients had only minimal infections. Overall, serum IgG levels were low in 32 patients but in none of the control group ($P = 8{\cdot}8 \times 10^{-11}$). However, less than half of those patients with hypogammaglobulinaemia suffered from severe or multiple infections. Specific antibodies to pneumococcal capsular polysaccharide were low in 23 patients compared with six of the control group ($P = 4{\cdot}9 \times 10^{-4}$). The majority of patients with severe or multiple infections (13/18) had low levels of both total IgG and specific antibodies to pneumococcal capsular polysaccharide. However, in the groups of patients with less frequent infections, a higher proportion had low serum IgG than low pneumococcal antibody levels. Low levels of pneumococcal antibodies were particularly associated with severe or multiple infections ($P = < 0{\cdot}00001$).

Keywords chronic lymphocytic leukaemia infection specific antibodies

INTRODUCTION

Infection is a major cause of morbidity and mortality in patients with low grade B cell tumours [1]. The infections are commonly bacterial though the underlying mechanisms for this increased susceptibility to infection are not clearly defined. Hypogammaglobulinaemia is a well documented complication of chronic lymphocytic leukaemia (CLL) [2–5]. The degree of hypogammaglobulinaemia correlates with the number of infections [4] as well as with disease duration [3]. Regular i.v. immunoglobulin replacement therapy has been shown to reduce the incidence of serious bacterial infections in hypogammaglobulinaemic patients with low-grade B cell tumours [6,7]. The success of such replacement therapy suggests that impaired antibody responses result in increased susceptibility to bacterial infection and that this is reversed by administration of immunoglobulin. However, not all patients with CLL require immunoglobulin replacement [7] and it is important to be able to define which patients will benefit from this expensive therapy [8,9].

Serum IgG level is not a precise indicator of infection since a proportion of patients with low IgG levels do not suffer from recurrent bacterial infections [7]. Other factors, such as levels of specific antibodies, may be more important as indicators of infection risk and the need for immunoglobulin replacement therapy.

We report a study on a group of patients with CLL which attempts to identify possible indicators of infection risk, in particular the levels of specific antibodies to protein and carbohydrate antigens.

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PATIENTS AND METHODS

Patients

The records of patients with CLL attending the John Radcliffe Hospital, Oxford were reviewed. Patients on continuous antibiotics (with the exception of prophylactic penicillin following splenectomy) or those receiving immunoglobulin replacement therapy were excluded from the study. The stage of disease according to the Rai classification [10], and the duration of disease were recorded at the time of review, in addition to any chemotherapy or radiotherapy administered during the course of the disease. The number and severity of infections occurring over the preceding 2 years was noted. The infections were graded as either serious (infections requiring hospital admission, parenteral antibiotic therapy or both) or as moderate

(infections requiring oral antibiotic therapy). The patients were grouped according to their infection history into those who had severe or multiple infections (one serious infection or six or more moderate infections over the 2-year period), recurrent infections (between three and five moderate infections in 2 years), or minimal infections (less than three moderate infections in the previous 2 years). Blood was taken at the end of the 2-year period for the measurement of immunoglobulin levels and specific antibodies to tetanus and diphtheria toxoids and pneumococcal capsular polysaccharide. Patients were infection-free at the time of blood sampling.

Controls

A group of 56 healthy normal volunteers aged 50–83 years (26 males and 30 females) were used as controls; individuals were excluded if they had had an infection within the previous 3 months, an autoimmune disease or were receiving immunosuppressive therapy.

The project received approval from the Central Oxford Research Ethical Committee.

Methods

Serum immunoglobulins were measured by turbidimetry (Baker Encore™). Specific antibodies were measured by an ELISA as follows. Flat-bottomed microtitre plates (Dynatech) were sensitized overnight at 4°C with the appropriate antigen diluted in 0·05 M carbonate buffer (pH 9·6) to approximately 1 μg/ml, using 100 μl per well. Tetanus simple vaccine (Wellcome), diphtheria toxoid (Wellcome) or Pneumovax II (Thomas Morson) were used as antigens. Doubling dilutions of standard serum and single dilutions of test sera (1:50 or 1:100) were prepared in PBS with 0·05% Tween 20 (PBS-T). After washing the sensitized plates with PBS-T, 100 μl volumes of test and standard dilutions were added to duplicate wells. The plates were incubated at room temperature for 1 h and then washed with PBS-T. Goat anti-human IgG-Fc antibody conjugated to alkaline phosphatase (100 μl) was then added to each well, each batch of conjugate having been previously tested to determine the appropriate dilution for use. After a further incubation of 1 h at room temperature, the plates were washed again with PBS-T, 100 μl of substrate (one Sigma 104® phosphatase substrate tablet dissolved in 5 ml diethanolamine buffer, pH 9·8) was added to each well and the colour allowed to develop in the dark at room temperature. The reaction was stopped after 30 min by the addition of 50 μl 3 M sodium hydroxide, and the optical density measured at 405 nm, using a Uniskan ELISA reader. A log/lin standard curve was plotted with antibody concentration against optical density, from which the antibody concentration of the test samples could be calculated. International standards were available for tetanus and diphtheria antibodies and the results were expressed as IU/ml. In-house standards were prepared for pneumococcal antibodies using a commercial immunoglobulin preparation (Gammagard, Baxter Healthcare, Glendale, CA) diluted in negative serum obtained from a healthy blood donor and assigned arbitrary units/ml. Serum samples from 115 blood donors with no details of previous immunizations, were tested for antibodies to tetanus toxoid, diphtheria toxoid and pneumococcal capsular polysaccharide to determine levels of specific antibodies in a normal adult population (18–64 years). Tetanus and diphtheria antitoxin levels below 0·01 IU/ml are usually considered to indicate non-immunity. However, a comparison of the guinea pig neutralization test and ELISA for the measurement of antibodies to diphtheria toxoid suggested that levels below 0·1 IU/ml as measured by ELISA should be considered as non-immune [11]. In addition a WHO report recommended a minimum level of 0·1 IU/ml for individual protection [12]. Therefore 0·1 IU/ml was used as the lower limit for both tetanus and diphtheria antibodies. Ninety per cent of normal donors had tetanus antibody levels >0·1 IU/ml and 70% of normal donors had diphtheria antibody levels >0·1 IU/ml. For pneumococcal antibodies, 90% of normal donors had spontaneously occurring pneumococcal antibody levels >20 u/ml and this value was taken as the lower limit of our normal range.

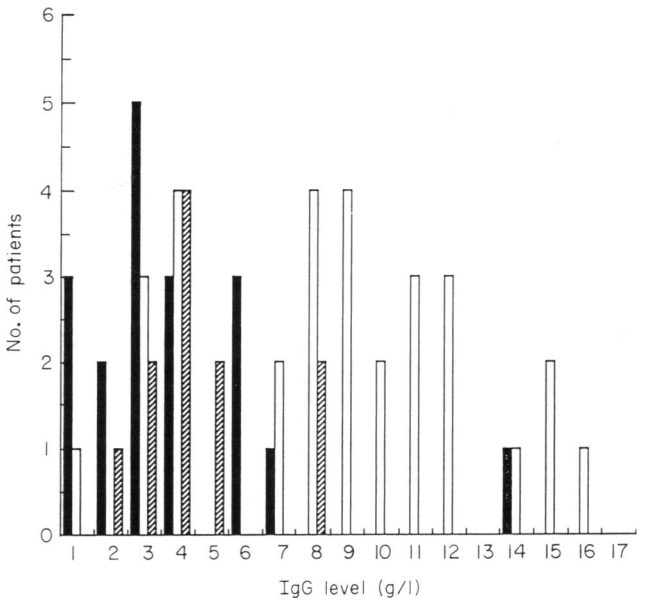

Fig. 1. Infection history related to serum IgG level in patients with chronic lymphocytic leukaemia (CLL). The lower limit of normal in the laboratory is 6·0 g/*l*. ■, Severe/multiple; ■, recurrent; □, minimal.

Statistical analysis

Comparison of serum IgG levels and pneumococcal antibody levels between groups of individuals was performed using the χ^2-test for independence. Geometric means and standard deviations were used to summarize the data. One-way analysis of variance of the log transformed data was used to determine the relationship between the numbers of infections and pneumococcal antibody units. The Student–Newman–Keul multiple comparison procedure was used to determine where specific group differences lay [13].

RESULTS

Patient details

Fifty-nine patients (40 males and 19 females) with CLL were eligible for the study. At the time of review, 10 patients were stage 0, seven were stage I, 11 were stage II, 10 were stage III and 21 were stage IV. The diagnosis had been made from 2 months to 20 years previously. In 14 patients the diagnosis was made during the study period when the disease had yet to be recognized. Eighteen patients had received no treatment for their disease, 29 had been treated with chlorambucil with or

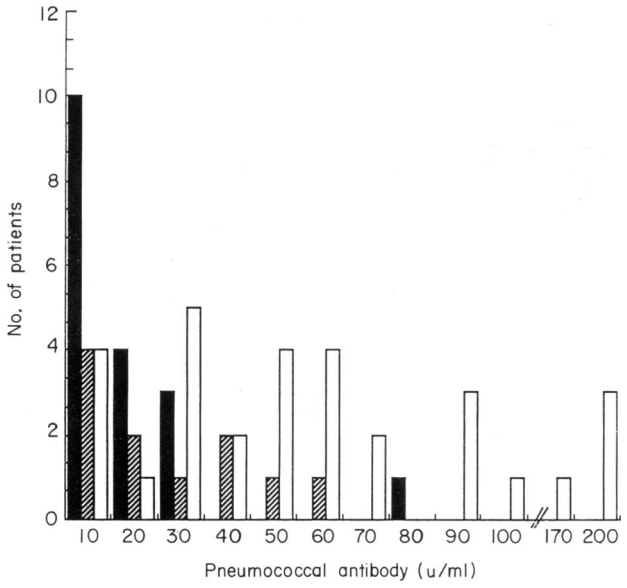

Fig. 2. Infection history related to pneumococcal antibody levels in patients with chronic lymphocytic leukaemia (CLL). The lower limit of normal in the laboratory is 20 u/ml. ■, Severe/multiple; ▨, recurrent; □, minimal.

Table 1. Number of chronic lymphocytic leukaemia (CLL) patients divided according to stage of disease and infection history.

Stage of disease	Infection history			
	Minimal	Recurrent	Severe/multiple	Total
0	10	0	0	10
I	3	2	2	7
II	8	3	0	11
III	2	3	5	10
IV	7	3	11	21
Total	30	11	18	59

without prednisolone, seven had been treated with other chemotherapy (cyclophosphamide, vincristine and prednisolone, methyl prednisolone, doxorubicin), five patients had received splenic radiotherapy in addition to chemotherapy and one patient had undergone splenectomy and was on prophylactic penicillin.

Infection history

Thirty per cent (18) of the patients studied had severe or multiple infections within the 2-year study period; 19% (11) had recurrent infections and the remaining 51% (30) had only minimal infections. The infections predominantly affected the respiratory tract (pneumonia, bronchitis) with occasional episodes of skin sepsis and pyrexia of unknown origin.

Serum IgG level

Over half the patients (32) had serum IgG levels below the lower limit of the normal range in our laboratory (<6·0 g/l), compared with none of the control group ($P=8{\cdot}8\times10^{-11}$). The majority of patients with severe or multiple infections (15/18) had low IgG levels ($P=<0{\cdot}0001$) and a further two had borderline levels. However, 27% (8/30) patients with minimal infections also had IgG levels below the lower limit of the normal range (Fig. 1). There was a tendency for long duration of disease to be associated with low serum IgG levels but this association was not significant ($P=0{\cdot}39$); nor was there a significant association between IgG levels and disease stage ($P=0{\cdot}08$).

Specific antibodies

Low levels of pneumococcal antibodies were found in 39% (23/59) of the patients studied, compared with 11% (6/56) of the control group ($P=4{\cdot}9\times10^{-4}$). Low levels were found predominantly in 14 of 18 patients with severe or multiple infections (78%), compared with five of 11 patients with recurrent infections (45%) and in only four of 30 patients with minimal infections (13%) (Fig. 2). This association between severity or frequency of infections and low pneumococcal antibody levels was highly significant ($P=<0{\cdot}00001$), greater than that for IgG.

Although low pneumococcal antibody levels showed a strong correlation with the presence of hypogammaglobulinaemia in the CLL patients ($P=<0{\cdot}0001$), there were 12 patients with low serum IgG levels but normal pneumococcal antibody levels. Only two of these patients were in the severe or multiple infection group. In contrast low pneumococcal antibody levels in the presence of a normal total IgG were found in only three patients. The majority of patients with severe or multiple infections (13/18) had low levels of both total IgG and pneumococcal antibodies. In the intermediate group of patients with recurrent infections, the incidence of low total IgG levels was higher than that of low pneumococcal antibody levels.

Antibodies to tetanus toxoid were low in almost half the patients (43% of the males and 63% of the females). This compares with low levels in 32% of the elderly normal control group (19% of the males and 43% of the females). Antibodies to diphtheria toxoid were also low in 77% of CLL patients (68% of the males and 95% of the females), compared with low levels in 34% of the control group (35% of the males and 33% of the females). Since no details about previous immunizations were available, these levels were not analysed further.

Clinical parameters

Although there was no relationship between severity or frequency of infection and duration of disease ($P=0{\cdot}854$), there was evidence for a relationship with stage of disease ($P=0{\cdot}006$). Patients with lower stages of disease tended to have fewer infections (Table 1), higher IgG levels ($P=0{\cdot}08$) and higher pneumococcal antibody levels ($P=0{\cdot}07$). However, this did not appear to be related to chemotherapy.

DISCUSSION

The incidence of infection in patients with chronic lymphocytic leukaemia in this study is similar to the incidence reported following a review of earlier studies [1]. The infections were

predominantly of the upper and lower respiratory tract, as are those seen in patients with primary hypogammaglobulinaemia, when pathogens such as *Streptococcus pneumoniae* and *Haemophilus influenzae* are commonly found [4]. Because of the similarity in type of infection in patients with CLL and primary hypogammaglobulinaemia, a common immune defect, namely low serum IgG, would appear to be responsible for the increased susceptibility to infection. Indeed total serum IgG levels were reduced in 83% of the patients with severe or multiple infections in this study. However, reduction of total IgG levels was not confined to those patients with frequent infections, since low IgG levels were also found in almost a third of patients without infections, suggesting that a low IgG alone is insufficient to account for the increased susceptibility to infection.

Specific antibodies might be more helpful in identifying those patients at risk of infection. Fourteen of 18 (78%) patients with severe or multiple infections had low pneumococcal antibody levels in this study whereas only four of 30 patients had low pneumococcal antibody levels without infection. This suggests that the degree of reduction in total IgG levels may be of less predictive value than the patient's ability to respond to micro-organisms by the production of specific antibodies.

The patients with a history of moderate infection and low IgG levels, but normal pneumococcal antibody levels, may represent an intermediate group of patients who only develop more serious or frequent infection as the specific antibody levels fall. Since pneumococcal antibodies normally persist throughout life as a result of natural exposure, the levels of these antibodies in elderly patients with CLL may predict which patients are at risk of serious or frequent infection. However, this was a retrospective study in which serum IgG and specific antibody levels were measured when infections had already occurred. A prospective trial is needed of spontaneous antibody levels and response to immunization in patients with CLL before the onset of infection. This might provide a means by which patients who will benefit from i.v. immunoglobulin can be selected.

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REFERENCES

1 Bunch C. Management of infection in chronic leukaemia. In: Gale RP, Kai, Rai KR, eds. Chronic lymphocytic leukaemia: recent progress, future advances. New York: Alan R Liss, 1987:373–81.
2 Brown RK, Read JT, Wiseman BK, France WG. Electrophoretic analysis of serum proteins of blood dyscrasias. J Lab Clin Med 1948; **33**:1523–33.
3 Fairley GH, Scott RB. Hypogammaglobulinaemia in chronic lymphocytic leukaemia. Br Med J 1961; **4**:920–4.
4 Chapel HM, Bunch C. Mechanisms of infection in chronic lymphocytic leukaemia. Semin Haematol 1987; **4**:291–6.
5 Rai KR, Montserrat E. Prognostic factors in chronic lymphocytic leukaemia. Semin Haematol 1987; **4**:252–6.
6 Griffiths H, Brennan V, Lea J, Bunch C, Lee M, Chapel H. Crossover study of immunoglobulin replacement therapy in patients with low-grade B-cell tumours. Blood 1989; **73**:366–8.
7 Co-operative Group for the Study of Immunoglobulin in Chronic Lymphocytic Leukaemia. A randomised, controlled clinical trial of intravenous immunoglobulin in chronic lymphocytic leukaemia. N Engl J Med 1988; **319**:902–7.
8 Weeks JC, Tierney MR, Weinstein MC. Cost effectiveness of prophylactic intravenous immune globulin in chronic lymphocytic leukaemia. N Engl J Med 1991; **325**:81–86.
9 Besa EC, Klumpe D. Prophylactic immune globulin in chronic lymphocytic leukaemia. N Engl J Med 1992; **326**:139.
10 Rai KR, Sawitsky A, Cronkite EP, Chanana AD, Levy RN, Pasternack BS. Clinical staging of chronic lymphocytic leukaemia. Blood 1975; **46**:219–34.
11 Melville-Smith M, Balfour A. Estimation of *Corynebacterium diphtheriae* antitoxin in human sera: a comparison of an enzyme-linked immunosorbent assay with the toxin neutralisation test. J Med Microbiol 1988; **25**:279–83.
12 Hasley N, Galazka A. The efficacy of DPT and oral poliomyelitis immunisation schedules initiated from birth to twelve weeks of age. Bull WHO 1985; **63**:1151–69.
13 Glantz SA, Slinker BK. Primer of applied regression and analysis of variance. New York: McGraw-Hill, 1990:300–2.