Parry, Helen Marie and Birtwistle, Jane and Whitelegg, Alison and Hudson, Christopher D. and McSkeane, Tina and Hazlewood, Peter and Mudongo, Nyasha and Pratt, Guy and Moss, Paul and Drayson, Mark T. and Murray, Jim and Richter, Alex G. (2015) Poor functional antibody responses are present in nearly all patients with chronic lymphocytic leukaemia, irrespective of total IgG concentration, and are associated with increased risk of infection. British Journal of Haematology, 171 (5). pp. 887-890. ISSN 1365-2141

Poor functional antibody responses are present in nearly all patients with Chronic Lymphocytic Leukaemia, irrespective of total IgG concentration, and are associated with increased risk of infection

Patients with chronic lymphocytic leukaemia (CLL) suffer considerable morbidity and mortality from infectious disease (Francis, *et al* 2006, Itala, *et al* 1992, Molica, *et al* 1993). This risk has been attributed to development of a secondary immunodeficiency which has a multi-factorial aetiology including the effects of the underlying disease, the age of patient and the influence of therapy (Thurmes, *et al* 2008). The most commonly recognized measure of immunodeficiency associated with CLL is hypogammaglobulinaemia, which becomes more common as the disease progresses (Ben-Bassat, *et al* 1979).

Total IgG concentration is the most commonly used indicator of antibody deficiency but the magnitude of the humoral response against specific pathogens is also of considerable importance. Specific antibody deficiency refers to a state characterized by normal immunoglobulin concentrations but poor functional antibody levels and recurrent infections.

We undertook a cross sectional study to examine the incidence of specific antibody deficiency in 56 patients with CLL over 3 weeks at Queen Elizabeth Hospital Birmingham and Birmingham Heartlands Hospital Hematology clinics in June 2013. Clinical data was obtained from electronic records. Vaccination history was sourced from 53 of the 56 patients from primary care records.

IgG antibody levels to 19 vaccine antigens were examined for evidence of specific antibody deficiency using a 19plex luminex assay. This measured 12 pneumococcal (Pn) polysaccharides (serotypes 1,3,4,5,6B,7F,9V,14,18C,19A,19F,23F), four meningococcal polysaccharides (serogroups Men A,C,W,Y), *Haemophilus influenza*–b (Hib), tetanus toxoid and diphtheria toxoid. Results were considered protective at values recommended from WHO (Pn ≥0.35μg/ml in 8/12 serotypes, Men ≥2μg/ml, tetanus ≥0.1IU/ml, diphtheria ≥0.1IU/ml, Hib≥1μg/ml).

As reported in previous studies, a high incidence of infection was observed even in patients with early stage disease {Hamblin, 2008}. Thirty one patients (55%) had one or more documented infections and 15 patients (27%) had at least one hospital admission due to infection. Within the total cohort, the median IgG concentration was 7.6g/l (IQR. 5.08-9.01) and 22 (39%) of patients had IgG concentrations below the normal lower limit of 6g/L. Hypogammaglobulinaemia was associated with significantly more hospital-recorded infection (p=0.036). Amongst untreated patients with Binet stage A disease who are on 'watch and wait' management, those with one or more infection(s) had significantly lower IgG concentrations than patients who did not suffer infections (6.3 g/l v 9.0 g/l, p = 0.037). Patients with an IgG <6g/l at diagnosis also had a shorter time to first infection (p=0.01) and more commonly reported symptoms of cough (p = 0.05) and sputum production (p = 0.05).

There were significantly lower functional antibody concentrations against 16 of the 19 serotypes measured in CLL patients compared to an age-matched control group of 162 unvaccinated healthy patients with a median age of 74.6 years (66.2-83.0) (Phillips*, et al* 2006). For pneumococcal serotypes, protective levels were demonstrated in only 3 of 12 serotypes compared with 9 out of 12 within the healthy control group. This indicates that the specific antibody deficiency seen in CLL is related to the disease and not simply a reflection of immunosenescence secondary to age. Patients with IgG<6g/l had a more marked specific antibody deficiency and were protected against a median of only 2 serotypes compared to 5 in those with IgG within the normal range (p=0.002). However, 79% (27/34) of patients with a normal IgG concentration still had suboptimal specific antibody responses to pneumococcus, demonstrating that IgG testing alone is not sufficient to identify patients at risk of infection (Figure 1). Similarly, specific antibodies against the other antigens tested were also found to be below protective levels in patients with a normal IgG; Men A: n=13 (38%), Men C: n=33 (97%); Men W n=28 (82%), Men Y n= 32 (94%), Tetanus n=13 (38%), Diphtheria n= 26 (96%) and Hib n=14 (41%).

Current BCSH guidelines for the management of B-CLL recommend screening for total immunoglobulin levels as a means of identifying patients at risk of infection. Specific antibody testing is currently recommended only after vaccination as a means to assess immune response (Oscier*, et al* 2012). However, this strategy will fail to identify those patients with a normal IgG that have poor functional antibody concentrations. This has clinical importance as functional antibody concentration was found to be lower against all pneumococcal serotypes in patients with a history of infection (p=0.04).

Surprisingly, despite the average age of the cohort being above 65 years, and with an underlying diagnosis of CLL, only 74% of patients had been vaccinated against Pneumococcus. 3 patients received Prevenar13 and the remainder had been given Pneumovax23. This suggests that a more robust system of vaccination is required with clear guidelines on whether this should occur in primary or secondary care. At the time of study the Joint Committee on Vaccination and Immunisation (JCVI) had recently changed their guidance for haematological malignancy and now recommend that patients should be immunized with the conjugated vaccine Prevenar13 followed, at least 2 months later, by the previously recommended vaccine Pneumovax23. This study supports this decision in that we found patients who had received Pneumovax23 polysaccharide vaccine (n= 37) had protective levels against only 2 of 12 compared with 4 of 12 pneumococcal serotypes for unvaccinated patients. The time from vaccination did not affect antibody concentrations. One study has found that the use of a single dose of Prevenar13 yields protective antibodies in 47% in CLL patients at 6 weeks (Sinisalo, *et al* 2007). **To achieve higher rates of protection it may be necessary to utilize other vaccine schedules such as booster doses, as is routinely recommended in infants (Jodar*, et al* 2003, Rennels*, et al* 1998). In adult HIV patients, response rates almost double in those who received a second Prevenar vaccination (response rate 32% for one vaccine; 63.6% in those receiving a further booster dose) (Lu*, et al* 2014). A further consideration is the appropriate dose in patients with immunodeficiency; Jackson et al examined a dose range of Prevenar vaccination finding that a double dose was more immunogenic in an elderly**

population with presumed immunosenescence (Jackson*, et al* 2007). Evidence for alternative schedules in adults is limited and is the focus of ongoing research in haematological patients with secondary immunodeficiency.

This cross sectional study highlights the importance of investigating for antibody deficiency even in the early stages of CLL and supports a strategy of examining both whole and specific antibodies. Vaccination status should be checked on an annual basis. Enhanced vaccine regimens and additional strategies, such as prophylactic antibiotics or immunoglobulin replacement therapy, are required to reduce the high morbidity and mortality of infection in CLL.

Acknowledgement: HP contribution to this study was supported by a Wellcome Trust Fellowship Grant.

Conflict of interest: MD and AR have received speaker fees from Pfizer, there are no other conflicts of interest. Ethical approval was obtained for this study from West Midlands regional ethics committee (10/H1206/58).

Authorship: HP and AR designed the study. HP, JB and AR wrote the manuscript. AW, TM, and PH recruited patients and along with MN and HP collected patient data. CH performed data analysis. GP, PM, MD and JM collected samples and revised the manuscript.

References

Ben-Bassat, I., Many, A., Modan, M., Peretz, C. & Ramot, B. (1979) Serum immunoglobulins in chronic lymphocytic leukemia. *Am J Med Sci,* **278,** 4-9.

Francis, S., Karanth, M., Pratt, G., Starczynski, J., Hooper, L., Fegan, C., Pepper, C., Valcarcel, D., Milligan, D.W. & Delgado, J. (2006) The effect of immunoglobulin VH gene mutation status and other prognostic factors on the incidence of major infections in patients with chronic lymphocytic leukemia. *Cancer,* **107,** 1023-1033.

Hamblin, A.D. & Hamblin, T.J. (2008) The immunodeficiency of chronic lymphocytic leukaemia. *British medical bulletin,* **87,** 49-62.

Itala, M., Helenius, H., Nikoskelainen, J. & Remes, K. (1992) Infections and serum IgG levels in patients with chronic lymphocytic leukemia. *Eur J Haematol,* **48,** 266-270.

Jackson, L.A., Neuzil, K.M., Nahm, M.H., Whitney, C.G., Yu, O., Nelson, J.C., Starkovich, P.T., Dunstan, M., Carste, B., Shay, D.K., Baggs, J. & Carlone, G.M. (2007) Immunogenicity of varying dosages of 7-valent pneumococcal polysaccharide-protein conjugate vaccine in seniors previously vaccinated with 23-valent pneumococcal polysaccharide vaccine. *Vaccine,* **25,** 4029-4037.

Jodar, L., Butler, J., Carlone, G., Dagan, R., Goldblatt, D., Kayhty, H., Klugman, K., Plikaytis, B., Siber, G., Kohberger, R., Chang, I. & Cherian, T. (2003) Serological criteria for evaluation and licensure of new pneumococcal conjugate vaccine formulations for use in infants. *Vaccine,* **21,** 3265-3272.

Lu, C.L., Chang, S.Y., Chuang, Y.C., Liu, W.C., Su, C.T., Su, Y.C., Chang, S.F. & Hung, C.C. (2014) Revaccination with 7-valent pneumococcal conjugate vaccine

elicits better serologic response than 23-valent pneumococcal polysaccharide vaccine in HIV-infected adult patients who have undergone primary vaccination with 23-valent pneumococcal polysaccharide vaccine in the era of combination antiretroviral therapy. *Vaccine,* **32,** 1031-1035.

Molica, S., Levato, D. & Levato, L. (1993) Infections in chronic lymphocytic leukemia. Analysis of incidence as a function of length of follow-up. *Haematologica,* **78,** 374-377.

Oscier, D., Dearden, C., Eren, E., Fegan, C., Follows, G., Hillmen, P., Illidge, T., Matutes, E., Milligan, D.W., Pettitt, A., Schuh, A. & Wimperis, J. (2012) Guidelines on the diagnosis, investigation and management of chronic lymphocytic leukaemia. *Br J Haematol,* **159,** 541-564.

Phillips, A.C., Carroll, D., Burns, V.E., Ring, C., Macleod, J. & Drayson, M. (2006) Bereavement and marriage are associated with antibody response to influenza vaccination in the elderly. *Brain Behav Immun,* **20,** 279-289.

Rennels, M.B., Edwards, K.M., Keyserling, H.L., Reisinger, K.S., Hogerman, D.A., Madore, D.V., Chang, I., Paradiso, P.R., Malinoski, F.J. & Kimura, A. (1998) Safety and immunogenicity of heptavalent pneumococcal vaccine conjugated to CRM197 in United States infants. *Pediatrics,* **101,** 604-611.

Sinisalo, M., Vilpo, J., Itala, M., Vakevainen, M., Taurio, J. & Aittoniemi, J. (2007) Antibody response to 7-valent conjugated pneumococcal vaccine in patients with chronic lymphocytic leukaemia. *Vaccine,* **26,** 82-87.

Thurmes, P., Call, T., Slager, S., Zent, C., Jenkins, G., Schwager, S., Bowen, D., Kay, N. & Shanafelt, T. (2008) Comorbid conditions and survival in unselected, newly diagnosed patients with chronic lymphocytic leukemia. *Leuk Lymphoma,* **49,** 49-56.