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Chronic lymphocytic leukaemia (CLL) is characterised by a clonal expansion of CD5+B cells in the bone marrow and secondary lymphoid organs. More than 75% of patients with CLL develop hypogammaglobulinemia, and this may contribute to the excess mortality related to infection which is the commonest cause of death. The heterogeneity of hypogammaglobulinemia and infection susceptibility suggests a complex interplay of genetic factors including disease and treatment-related immunosuppression affect overall survival [1].

Current treatment approaches include chemoimmunotherapy, non-receptor Bruton tyrosine kinase (BTK)-inhibition and BCL-2 inhibitors such as venetoclax. Ibrutinib irreversibly inhibits BTK via covalent binding and disrupts downstream signals of B-cell receptor (BCR) activation [2-4]. It has shown to be remarkably effective in the treatment of relapsed/refractory CLL and is now licensed in the United Kingdom as first-line single-agent in CLL with deletion 17p13.1/TP53 mutations and relapsed disease. As low antibody levels is a common feature of untreated CLL, the use of B-cell depletion chemotherapeutic agents or ibrutinib is likely to exacerbate hypogammaglobulinemia and thereby increase infection risk. To test this hypothesis, we analysed longitudinal data with respect to serum immunoglobulin levels and the frequency/type of infections in ibrutinib-treated CLL patients.

We report a retrospective analysis on 26 ibrutinib-treated relapsed/refractory CLL patients and 3 CLL patients who received ibrutinib as first line therapy from January 2016 to May 2020. All patients were under regular follow-up with the Department of Hemato-Oncology and had microbiological culture/sensitivity studies, serum immunoglobulin levels as per clinical requirements. Patients on immunoglobulin replacement therapy (IgRT) were also under Department of Immunology and investigated according to United Kingdom-Primary Immunodeficiency Network (UK-PIN) guidelines.

Our primary objective was to determine the degree of hypogammaglobulinemia related to ibrutinib therapy, including documenting the frequency and type of infections during the follow-up period. An infectious episode was defined as a microbiologically-proven infection and categorised as: Grade 1 (treatment at home with oral antimicrobials), Grade 2 (in-hospital admission for intravenous agents), Grade 3 (sepsis requiring intensive care admission) [5]. Serum immunoglobulin levels (Beckman Coulter AU turbidimetry), specific antibody studies (The Binding Site enzyme immunoassay) were performed at Hull University Teaching Hospitals NHS Trust, while fluorescent in-situ hybridisation (FISH) including high-throughput sequencing (HTS) studies done at Leeds University Teaching Hospitals NHS Trust. Statistical analysis for descriptive and Kaplan-Meier survival graphs were performed using GraphPad Prism Version 8.4.3 for Windows (GraphPad Software).

The median age of the patients (18 men, 11 women) at diagnosis was 66 years (range, 44-87 years) and median duration of ibrutinib use was 22 months (range, 4-69 months). 17 of 29 patients (59%) had evidence of high-risk CLL of which 7 were identified using FISH (17p del [n=2], 11q del [n=5]), 3 patients using HTS (*SF3B1, TP53* mutations in two patients including extra copies of *TP53, ATM,* and *IGH* in one patient) and 7 identified on *IGHV* mutation tests. Nine patients had normal FISH studies for chromosomal abnormalities but were started on ibrutinib therapy due to disease progression.

15 of 29 ibrutinib-treated patients (51.7%) had at least one infective episode, with a preponderance of bacterial infections [12 of 15 patients (80%); See Table 1]. One of 3 patients who had ibrutinib as first line had significant infections (Grade 2). Seven of 18 patients who had ibrutinib as second line had significant infections (Grade 2). Two of 3 patients on ibrutinib as 3rd line therapy had infections (Grade 1 & 2). Nine of 15 patients (60%) had more than one infection episode, predominantly the affecting chest or urinary tract. *Escherichia coli* caused urinary tract infections in 7 patients (47%). Six of 15 patients (40%) had *candida albicans* isolated on sputum culture studies (likely from oropharynx) during ibrutinib therapy. Three of 15 (20%) patients had recurrent and severe infections that necessitated starting immunoglobulin replacement therapy (IgRT). One patient on IgRT who died had required 5 lines of therapy for CLL, received immunoglobulin for 6.5 years and ibrutinib for 16 months with one documented infection (Grade 1) on ibrutinib therapy. No patients required IgRT after starting ibrutinib therapy.

Analysis of the serum immunoglobulin levels in 28 patients prior to the initiation of ibrutinib therapy showed median IgG at 6.0 g/L (IQR25-75, 3.73-8.28 g/L), IgA 0.78 g/L (IQR25-75, 0.34-1.15 g/L) and IgM 0.2 g/L (IQR25-75, 0.20-0.37 g/L). Overall, 21 of 28 patients had IgG <5g/L (75%). Serum IgG level between4-5 g/L was noted in 11 of 28 patients (39%), very low IgG (<4 g/L) in 8 of 28 patients (29%) and 2 patients had completely undetectable IgG <0.75 g/L (7%). Seventeen patients (60.7%) had undetectable IgM before ibrutinib therapy. These values were used as baseline levels to calculate the percentage change in immunoglobulin levels. 5 of 29 (17%) of patients had paraprotein detected during CLL treatment. Antibody levels were measured in 24 patients after initiation of ibrutinib therapy (median time frame, 7 months). Overall mean percentage change analyses showed IgG levels were reduced by 24.5% ((SD 19.12; 95%CI of median -32.8 to -13.2), IgA increased by 6.3% ((SD 45.5; 95%CI of median -16.7 to 17.1) and IgM increased by 3.9% ((SD 38.5; 95%CI of median 0.0-5.0) [See Supplemental file 1]. Only median IgG level post-ibrutinib therapy showed a significant change (decline) on ibrutinib therapy [two-tailed paired *t-test*, p=0.03]. Specific antibody levels showed adequate protection against tetanus and pneumococcal antigens even after chemotherapy for CLL, including evidence of immune response against Hemophilus influenzae type B in all 3 patients that remained stable on dual IgRT and ibrutinib therapy. IgRT prevented recurrent infections in all 3 patients [See Supplemental file 2].

Apart from infections, ibrutinib at 420mg/day had a favourable toxicity profile with only 4 patients (13.7%) requiring dose reduction (140mg/day) for easy bruising (2 patients), worsening chronic kidney disease (one patient) and for headache, nausea, and muscle pains (one patient).

Seven patients died in this cohort with four patients dying due to progressive disease or having developed other malignancies [Table 1]. Non-parametric Kaplan-Meier survival analysis estimators used showed no difference in survival curves for age at

presentation, gender or high-risk genetic changes identified on CLL cells. Survival plots of patients with documented infections versus no infections with duration of ibrutinib therapy showed no significant difference between the curves [Figure 1]. Similarly, survival plots of patients with IgG level <4g/L (significant hypogammaglobulinemia) before start of ibrutinib therapy versus IgG>4g/L showed no significant difference between the curves [Figure 1].

Lowering the risk of infections on cancer chemotherapy is important for the continuation of therapy and maintenance of dose-intensity. Although in our study 51% of the patients developed an infective episode during follow-up, the frequency of life-threatening infections was low. Even heavily pre-treated patients did not have serious infections on long-term BTK inhibition, and survival plots show contracting infections on ibrutinib therapy did not affect overall survival. Survival plots also did not show a difference with IgG level pre-ibrutinib therapy. This is contrary to expectation as patients with inherited germ-line BTK mutations (X-linked agammaglobulinemia, XLA) develop serious, frequent sometimes life-threatening infections. Sun C et al showed that patients on ibrutinib who had a 50% increase in IgA from baseline had lower frequency of infections [6]. A recent paper by Cassin R et al showed IgA level >0.7g/L after 6 months of ibrutinib therapy including increase of IgA levels over time were associated with lower infection rates [7].

Our study did not look at peripheral blood T/B cell numbers to understand immune recovery, but in untreated CLL patients T cell populations are oligoclonal due to CLL-antigen-driven expansions. While ibrutinib therapy led to decline and normalization of elevated T cell numbers and Th1, Th2, Th17 cytokine levels in some studies [4, 8, 9], other studies showed it improves CD4+ and CD8+ T cell numbers including effector memory subsets [10], with effects on natural killer cell function and Th17 cell expansion [11]. Ibrutinib and acalabrutinib lowered IL-10 induced immunosuppressive cytokine landscape [11], improved T-cell proliferative function and cytokine secretion [12]. BTK-inhibition in CLL therefore allows T cells to escape an immunosuppressive environment. T-cell escape from tumour-associated antigen signalling may therefore explain improved immune responses and why heavily pre-treated CLL patients on long-term ibrutinib monotherapy remain relatively infection-free (particularly invasive fungal infections) even with very low to undetectable IgG levels.

Use of replacement immunoglobulin therapy in CLL has been extensively debated [13]; and generally agreed that CLL patients are candidates for long-term IgRT with documented evidence of recurrent or severe bacterial infection despite antibiotic prophylaxis for 6 months, IgG<4g/L and failure of antibody response to polysaccharide vaccine challenges [13-15]. All patients in our cohort who required IgRT had several infection episodes and 2 of 3 patients had autoimmune haemolytic anemia secondary to CLL with no further recurrence on IgRT.

In conclusion, our study did not identify severe, unusual or recurrent infections with long-term ibrutinib use. Ibrutinib lowered IgG levels in almost all patients but did not have a consistent effect on IgA and IgM levels. Minimising side effects while maintaining durable anti-tumour response remains a challenge with the newer generation highly selective BTK-inhibitors.

Data availability statement: Raw data were generated at Hull University Teaching Hospitals NHS Trust. Derived data supporting the findings of this study are available from the corresponding author on request.

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Disclosure statement: None

Author contributions:

Sujoy Khan designed the research study, collected and analyzed the data, wrote the first draft of the manuscript.

Senthilkumar Durairaj collected the data and critically revised the manuscript.

Punyarat Phumphukhieo collected infection data and critically revised the manuscript.

SH analyzed, interpreted the data and critically revised the manuscript.

DA interpreted the data and critically revised the manuscript.

All authors verified and approved the final version of the manuscript.

References:

1. Crassini K, Stevenson WS, Mulligan SP, et al. Molecular pathogenesis of chronic lymphocytic leukaemia. *Br J Haematol.* 2019;186(5):668-684.

2. Dubovsky JA, Beckwith KA, Natarajan G, et al. Ibrutinib is an irreversible molecular inhibitor of ITK driving a Th1-selective pressure in T lymphocytes. *Blood*. 2013;122(15):2539-2549.

3. Patel V, Balakrishnan K, Bibikova E, et al. Comparison of acalabrutinib, a selective Bruton Tyrosine kinase inhibitor, with ibrutinib in chronic lymphocytic leukemia cells. *Clin Cancer Res*. 2017;23(14):3734-3743.

4. Burger JA, Keating MJ, Wierda WG, et al. Safety and activity of ibrutinib plus rituximab for patients with high-risk chronic lymphocytic leukaemia: a single-arm, phase 2 study. *Lancet Oncol.* 2014;15(10):1090-9.

5. Cordonnier C, Maury S, Ribaud P et al. A grading system based on severity of infection to predict mortality in allogeneic stem cell transplant recipients. *Transplantation.* 2006 Jul 15;82(1):86-92.

6. Sun C, Tian X, Lee YS, et al. Partial reconstitution of humoral immunity and fewer infections in patients with chronic lymphocytic leukemia treated with ibrutinib [published correction appears in Blood. 2016;128(7):1020]. *Blood*. 2015;126(19):2213-2219.

7. Cassin R, Visentin A, Giannarelli D et al. Increase of immunoglobulin A during ibrutinib therapy reduces infection rate in chronic lymphocytic leukemia patients. *Hematol Oncol.* 2020 Oct 9. doi: 10.1002/hon.2814. Epub ahead of print. PMID: 33034902.

8. Niemann CU, Herman SE, Maric I, et al. Disruption of in vivo Chronic Lymphocytic Leukemia Tumor-Microenvironment Interactions by Ibrutinib--Findings from an Investigator-Initiated Phase II Study. *Clin Cancer Res.* 2016;22(7):1572-82.

9. Yin Q, Sivina M, Robins H, et al. Ibrutinib Therapy Increases T Cell Repertoire Diversity in Patients with Chronic Lymphocytic Leukemia. *J Immunol.* 2017;198(4):1740-7.

10. Long M, Beckwith K, Do P, et al. Ibrutinib treatment improves T cell number and function in CLL patients. *J Clin Invest*. 2017;127(8):3052-3064.

11. Natarajan G, Terrazas C, Oghumu S, et al. Ibrutinib enhances IL-17 response by modulating the function of bone marrow derived dendritic cells. *Oncoimmunology*. 2015;5(1):e1057385.

12. Solman IG, Blum LK, Hoh HY et al. Ibrutinib restores immune cell numbers and function in first-line and relapsed/refractory chronic lymphocytic leukemia. *Leuk Res.* 2020;97:106432.

13. Dhalla F, Lucas M, Schuh A et al. Antibody deficiency secondary to chronic lymphocytic leukemia: Should patients be treated with prophylactic replacement immunoglobulin? *J Clin Immunol.* 2014;34(3):277-82.

14. Sewell WA, Kerr J, Behr-Gross ME et al; Kreuth Ig Working Group. European consensus proposal for immunoglobulin therapies. *Eur J Immunol.* 2014;44(8):2207-14.

15. Na IK, Buckland M, Agostini C et al. Current clinical practice and challenges in the management of secondary immunodeficiency in hematological malignancies. *Eur J Haematol.* 2019;102(6):447-456.

16. Döhner H, Stilgenbauer S, Benner A et al. Genomic aberrations and survival in chronic lymphocytic leukemia. *N Engl J Med.* 2000;343(26):1910-6.