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RESEARCH ARTICLE

Chen and Wu: Biomarkers of infection in iBCL

Serum Flt3L, sIL-2R, and Gas6 are associated with infection in indolent B-cell lymphoma

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ABSTRACT

Infection poses a significant clinical challenge for patients with indolent B-cell lymphoma (iBCL), yet its assessment in this immunocompromised population remains difficult. We investigated the associations of serum FMS-like tyrosine kinase 3 ligand (Flt3L), soluble interleukin-2 receptor (sIL-2R), and growth arrest-specific 6 (Gas6) with infection status. Our goal was to evaluate their individual and combined discriminatory performance in iBCL. This retrospective, single-center cohort study included 95 iBCL patients with infection, 152 iBCL patients without infection, and 70 age- and sex-matched healthy controls. Serum Flt3L, sIL-2R, and Gas6 were quantified using enzyme-linked immunosorbent assay (ELISA), while procalcitonin (PCT) was measured via chemiluminescent immunoassay. We used multivariable logistic regression to examine independent associations with infection and assessed discrimination using the area under the receiver operating characteristic curve (AUC). Correlations with PCT were evaluated using Spearman rank correlation. Infections were predominantly community-acquired (71.6%), respiratory (50.5%), and bacterial (63.2%). Infection status was independently associated with age ≥65 years, Ann Arbor stage III–IV, chemotherapy within the preceding 3 months, hypogammaglobulinemia, diabetes mellitus, and elevated Flt3L, sIL-2R, and Gas6 levels. The combined three-biomarker model demonstrated superior apparent discrimination compared to individual biomarkers (AUC 0.89, 95% confidence interval 0.85–0.94, vs 0.73–0.76), achieving 80.0% sensitivity and 84.9% specificity. Among infected patients, all three biomarkers positively correlated with PCT (r = 0.44–0.51; all $p < 0.001$). Our findings indicate that higher Flt3L, sIL-2R, and Gas6 levels are associated with infection status in this pooled iBCL cohort. However, the three-marker model requires independent validation in homogeneous, subtype- and treatment-specific cohorts before clinical implementation.

Keywords: Indolent B-cell lymphoma, infection, biomarker panel, Flt3L, sIL-2R.

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INTRODUCTION

Indolent B-cell lymphomas (iBCL) are slow-growing non-Hodgkin lymphomas, including follicular lymphoma (FL), chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) and marginal zone lymphoma (MZL) [1]. Although iBCLs generally follow an indolent clinical course, "indolent" and "low-grade" are not synonymous across all subtypes. FL comprises distinct histological categories, and FL grade 3B has different biological characteristics from classic FL [2]. FL is one of the most common indolent lymphomas and accounts for a substantial proportion of newly diagnosed non-Hodgkin lymphoma in Western populations [3]. Because disease kinetics are typically slow, active surveillance is appropriate for selected patients, and prolonged survival is common even with relapsing disease [4]. However, iBCL is a biologically heterogeneous group, with substantial differences in molecular features, immune dysfunction, and treatment exposure among subtypes [5]. CLL/SLL is particularly distinct, with genetic features such as TP53 abnormalities and IGHV mutation status influencing disease biology and treatment selection [6,7]. In addition, CLL/SLL management has shifted toward targeted therapies, including BTK and BCL2 inhibitors, with decreasing reliance on conventional chemoimmunotherapy [8].

Despite durable disease control, infection remains a major clinical challenge in iBCL. Immune dysfunction is intrinsic to iBCL and is often exacerbated by treatment-related immunosuppression [9, 10]. In CLL, infections drive substantial morbidity and are a leading cause of death, underscoring the impact of impaired humoral immunity and immune dysregulation [3,9]. Anti-CD20 therapy can further increase susceptibility, and a high burden of infections has been reported across lymphoproliferative disorders [11].

A key limitation is that routine infection markers are largely reactive and may have limited discriminatory value in iBCL. C-reactive protein (CRP) is a sensitive acute-phase reactant, but it is non-specific and increases in many inflammatory conditions [12]. Procalcitonin (PCT) is more specific for bacterial infection, but its performance is inconsistent in malignancy and treatment-related inflammation [13,14]. In febrile neutropenia, meta-analyses show only moderate diagnostic accuracy for PCT and CRP, highlighting the need for additional biomarkers in haematological patients [14].

This gap supports a mechanism-informed strategy: biomarkers that reflect immune injury, immune activation and innate inflammatory control may better capture

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infection-prone states in iBCL. FMS-like tyrosine kinase 3 ligand (Flt3L) regulates haematopoiesis and dendritic-cell development [15]. In lymphoma patients, circulating Flt3L has been investigated as a marker of chemotherapy-related myelosuppression and infectious complications, including neutropenic sepsis [16,17]. Soluble interleukin-2 receptor (sIL-2R) is released during T-cell activation and serves as a circulating marker of systemic immune activation in inflammatory disorders [18,19]. Growth arrest-specific 6 (Gas6) signals through TAM receptors to modulate innate immunity and has been associated with sepsis severity and outcomes [20, 21]. Recent studies have also investigated Gas6 in patients with lymphoma and post-chemotherapy infection, with higher Gas6 levels associated with poorer clinical outcomes [22].

However, the clinical value of integrating these immune-related markers for infection assessment in iBCL remains unclear. Here we characterized infection patterns in iBCL, identified clinical correlates of infection, and evaluated whether Flt3L, sIL-2R and Gas6, alone or in combination, could improve discrimination of infection in iBCL.

MATERIALS AND METHODS

Participants

This retrospective cohort included patients with iBCL treated in the Wuxi No.2 People's Hospital from January 2021 to June 2024 and identified from routine clinical records. Healthy controls were enrolled from the hospital health examination center during the same period and had no documented hematologic malignancy at the time of examination.

Sample size estimation

Sample size was estimated for logistic regression using a prespecified effect size. A two-sided α of 0.05 and 90% power ($\beta = 0.10$) were assumed, with an expected odds ratio of 2.0 for the exposure–infection association. Pilot data suggested an infection incidence of ~35% in the iBCL cohort. Under these assumptions, 95 infected and 152 non-infected patients were required. In addition, 70 healthy controls were included.

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Inclusion and exclusion criteria

iBCL infection group

Patients were included if they met the following criteria: (1) iBCL was confirmed by histopathology. The included iBCL subtypes were follicular lymphoma (FL), chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), and marginal zone lymphoma (MZL). Only classic FL (grade 1-3A) was included, while FL grade 3B and histological transformation cases were excluded due to their distinct biology and treatment strategies. MZL included extranodal MALT lymphoma, nodal marginal zone lymphoma, and splenic marginal zone lymphoma. (2) Infection was diagnosed based on microbiological evidence and/or imaging findings consistent with infection, together with compatible clinical manifestations (for example, fever or site-specific symptoms). (3) A serum sample was obtained on the day of infection diagnosis.

Patients were excluded if they met any of the following criteria: (1) A malignancy other than iBCL was present. (2) Immunosuppressive agents not related to iBCL therapy had been used within the preceding month.

iBCL non-infection group

Patients were included if they met the following criteria: (1) iBCL was confirmed by histopathology. (2) No infection episode was documented within the preceding 3 months. For cases without an identified pathogen, infection was clinically diagnosed when fever or site-specific symptoms, typical imaging findings, elevated inflammatory markers, response to empirical anti-infective therapy, and exclusion of non-infectious fever were all documented. These cases are reported as unidentified pathogen. (3) A serum sample was collected during the same study period.

Patients were excluded if they met the following criterion: (1) Key clinical data were missing.

Healthy control group

Controls were included if they met the following criteria: (1) no history of malignancy or infection was documented. (2) Age (±5 years) and sex were matched to the iBCL cohort. (3) Immune function was within the normal range.

Controls were excluded if they met the following criteria: (1) a family history of lymphoma was reported. (2) Infection or vaccination occurred within the preceding 3 months.

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Blood collection and serum processing

A total of 5 mL fasting venous blood was collected from each participant and centrifuged at 3,000 rpm for 10 min. Serum was separated, aliquoted, and stored at -80 °C to minimize freeze-thaw cycles. Samples were collected at infection diagnosis for the infection group and at study entry for the non-infection iBCL and healthy control groups.

Biomarker measurements

Serum Flt3L, sIL-2R and Gas6 were quantified by enzyme-linked immunosorbent assay (ELISA) using commercial kits according to the manufacturers' protocols. The kits and their performance characteristics were as follows: Flt3L (EH3074, Wuhan Fine Biotech, Wuhan, China; detection range 15.625–1000 pg/mL; intra-assay coefficient of variation [CV] ≤ 6.5%; inter-assay CV ≤ 6.0%), sIL-2R (ml038117, Shanghai Enzyme-linked Biotechnology, Shanghai, China; detection range 0.312–20 ng/mL; intra-assay CV ≤ 7.5%; inter-assay CV ≤ 8.0%), and Gas6 (EH0613, Wuhan Fine Biotech; detection range 62.5–4000 pg/mL; intra-assay CV ≤ 5.5%; inter-assay CV ≤ 5.5%). All standards and serum samples were tested in duplicate. Absorbance was measured at 450 nm, and serum concentrations were calculated from the corresponding standard curves according to the manufacturers' instructions. Laboratory personnel performing the assays were blinded to the clinical infection status of all samples. All procedures and quality-control steps were performed strictly according to the kit manufacturers' instructions.

Serum PCT was measured using a chemiluminescent immunoassay on a Beckman Coulter AU5800 automated biochemical analyzer (Beckman Coulter, USA) according to the manufacturer's instructions. The assay used a 35 μL sample volume, with a reported limit of quantitation of 0.02 ng/mL and a coefficient of variation of ≤8.0% at concentrations ≥0.150 ng/mL.

Ethical statement

The study was approved by Wuxi No.2 People's Hospital (#2025-HGYJ08), and informed written consent was waived since this was a retrospective analysis. The study was performed in strict accordance with the Declaration of Helsinki, Ethical Principles for Medical Research Involving Human Subjects.

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Statistical analysis

Continuous variables were presented as mean ± SD or median (interquartile range) and compared using two-sided t-tests or Mann-Whitney U tests, as appropriate. Categorical variables were reported as n (%) and compared using χ^2 or Fisher's exact tests. Candidate variables for multivariable logistic regression were selected based on clinical plausibility and a univariate threshold of $P < 0.05$. Bone-marrow involvement and Ann Arbor stage were considered measures of disease burden, while treatment regimen, prior rituximab, and chemotherapy within 3 months reflected treatment intensity. To minimize redundancy, Ann Arbor stage and chemotherapy within 3 months were retained as representative variables for disease burden and treatment intensity, respectively. All VIFs in the final model were <2.0. Complete-case analysis was performed; patients with missing key clinical data were excluded, while biomarker data were complete for all included subjects. Independent associations were assessed by multivariable logistic regression. The three biomarkers were entered as binary variables using their optimal cut-offs. As a sensitivity analysis, iBCL subtype (FL, CLL/SLL or MZL) was additionally included as a categorical covariate in the multivariable logistic regression model to assess whether the biomarker–infection associations were robust to subtype heterogeneity. Exploratory subtype-stratified analyses were also planned; however, because the sample size was estimated for the overall cohort rather than for subtype-specific analyses, the number of infection events within each subtype was small. Subtype-stratified estimates are therefore reported as exploratory only, with explicit acknowledgement of limited statistical power. Interaction between subtype and each biomarker was examined using likelihood ratio tests, but these analyses were underpowered and should be interpreted cautiously. These cut-offs were derived within the cohort by maximizing the Youden index (sensitivity + specificity − 1) on the ROC curve and were not prespecified. The combined model used the three biomarkers as continuous variables in a logistic regression model without clinical covariates. The model threshold was determined on the linear predictor scale by maximizing the Youden index; the resulting cut-off was −0.42, corresponding to a predicted probability of approximately 39.7%. Discrimination was evaluated by receiver operating characteristic (ROC) analysis, and correlations by Spearman's rank test. For comparisons involving multiple groups, Brown-Forsythe ANOVA with the Games-Howell post hoc test was used, as reported in Figure 1. Two-sided $P < 0.05$ was considered significant.

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RESULTS

Infection patterns in patients with iBCL

Given the immune dysfunction inherent to iBCL, we characterized infections by site, severity and acquisition setting among infected patients as shown in Table 1. Respiratory tract infection was most frequent (48/95, 50.5%). Skin and soft-tissue or other infections, including oral infection and sepsis, accounted for 28/95 (29.5%). Urinary tract infection accounted for 19/95 (20.0%). Most infections were mild and managed as outpatients (66/95, 69.5%). Severe infection requiring hospitalization or SOFA $\geq$ 6 occurred in 29/95 (30.5%). Community-acquired infection predominated (68/95, 71.6%), whereas 27/95 (28.4%) were hospital-acquired during chemotherapy or hospitalization. Overall, infections were mainly community-acquired and mild, most commonly involving the respiratory tract.

Pathogen distribution of infection in iBCL patients

To further define the infectious burden, we assessed the pathogen spectrum in infected patients (Table 2). Single bacterial infections were most frequent (60/95, 63.2%). Gram-negative bacteria were identified more often than Gram-positive bacteria (32/95, 33.7% versus 25/95, 26.3%), whereas anaerobes were uncommon (3/95, 3.2%). Single viral infections accounted for 14/95 (14.7%), with varicella–zoster virus as the leading virus (9/95, 9.5%), followed by influenza virus (5/95, 5.3%). Fungal infections, mainly Candida and Aspergillus, occurred in 7/95 (7.4%). Mixed infections (bacteria plus virus or fungus) were detected in 9/95 (9.5%). No pathogen was identified in 5/95 (5.3%), and these cases were diagnosed clinically according to the prespecified criteria described in the Methods (compatible symptoms, typical imaging findings, elevated inflammatory markers, response to empirical anti-infective therapy, and exclusion of non-infectious causes of fever). Overall, iBCL infections were mainly bacterial, with Gram-negative organisms predominating.

Univariate analysis of demographic and clinical features between iBCL non-infection and infection groups

To identify clinical features associated with infection, baseline characteristics were compared between non-infected and infected iBCL patients (Table 3). The infection group was older (median 66 vs 62 years; $P < 0.001$) and showed a higher prevalence of advanced Ann Arbor stage (III–IV; 61.1% vs 43.4%; $P = 0.009$) and bone marrow

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involvement (37.9% vs 23.0%; P = 0.014). Treatment exposure also differed, with less frequent watch-and-wait management (12.6% vs 40.8%) and more frequent immunochemotherapy (40.0% vs 21.1%; P < 0.001) among infected patients. Recent chemotherapy within 3 months and previous rituximab exposure were more common in the infection group (P = 0.003 and P = 0.021). Diabetes mellitus and hypogammaglobulinemia (IgG < 7 g/L) were also enriched (P = 0.007 and P < 0.001). Collectively, infection was associated with older age, greater disease burden, higher treatment intensity and impaired humoral immunity.

Multivariate Logistic regression analysis of factors associated with infection status in iBCL patients

To identify factors independently associated with infection, multivariable logistic regression was performed (Table 4). Age ≥65 years was associated with higher odds of infection (odds ratio [OR] 2.12, 95% confidence interval [CI] 1.25–3.58; P = 0.005). Advanced Ann Arbor stage (III–IV) was also associated with infection (OR 1.43, 95% CI 1.08–1.89; P = 0.012). Chemotherapy within 3 months remained significantly associated with infection status (OR 1.97, 95% CI 1.32–2.95; P < 0.001). Hypogammaglobulinemia showed a strong association (OR 2.26, 95% CI 1.39–3.67; P < 0.001), and diabetes mellitus also remained significant (OR 1.56, 95% CI 1.07–2.28; P = 0.021). Elevated Flt3L, sIL-2R and Gas6 were independently associated with infection (all P < 0.05). Overall, infection status in iBCL was associated with host factors, disease burden, recent therapy, impaired humoral immunity and elevated immune-related biomarkers.

Serum Flt3L, sIL-2R and Gas6 were elevated in infected iBCL patients

Given iBCL-associated immune dysregulation, serum biomarkers were compared among healthy controls, non-infected iBCL and infected iBCL groups. Median serum Flt3L increased progressively across groups (Fig. 1A), from 47.19 pg/mL in controls to 80.47 pg/mL in non-infected iBCL and 108.5 pg/mL in infected iBCL (P < 0.001). Median serum sIL-2R showed a similar stepwise increase (Fig. 1B), rising from 185.5 ng/mL in controls to 245.4 ng/mL in non-infected iBCL and 339.5 ng/mL in infected iBCL (P < 0.001). Median serum Gas6 also increased across groups (Fig. 1C), from 12.26 ng/mL in controls to 17.60 ng/mL in non-infected iBCL and 23.20 ng/mL in

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infected iBCL ($P < 0.001$). Overall, all three biomarkers were highest in infected iBCL, supporting their association with infection.

ROC analysis of serum Flt3L, sIL-2R and Gas6 in iBCL infection

To evaluate whether these biomarkers discriminated infection in iBCL, ROC analyses were performed (Fig. 2 and Table 5). Among individual biomarkers, Flt3L showed the best discrimination (area under the curve [AUC] 0.76, 95% CI 0.70–0.82), with 69.47% sensitivity (95% CI 59.61–77.83%) and 73.03% specificity (95% CI 65.47–79.45%) at the optimal cut-off (93 pg/mL). sIL-2R achieved an AUC of 0.73 (95% CI 0.66–0.79) with 61.05% sensitivity (95% CI 51.00–70.25%) and 76.97% specificity (95% CI 69.67–82.95%) at 318 ng/mL, whereas Gas6 yielded an AUC of 0.75 (95% CI 0.69–0.81), with 69.47% sensitivity (95% CI 59.61–77.83%) and 71.71% specificity (95% CI 64.08–78.27%) at 21 ng/mL.

To improve discrimination, a combined model integrating Flt3L, sIL-2R and Gas6 as continuous variables was built using multivariable logistic regression without clinical covariates. The model threshold was determined on the linear predictor scale by maximizing the Youden index. This model achieved an AUC of 0.89 (95% CI 0.85–0.94), with 80.00% sensitivity (95% CI 70.86–86.81%) and 84.87% specificity (95% CI 78.32–89.70%), and PPV/NPV of 76.77% (95% CI 67.54–83.99%) and 87.16% (95% CI 80.82–91.63%), respectively. These estimates reflect the apparent performance of the model in the present cohort and have not been externally validated. Overall, the three-marker model outperformed each individual biomarker, supporting its utility for infection assessment in iBCL.

Subgroup and sensitivity analyses

Because iBCL subtypes differ in disease biology and treatment exposure, we performed exploratory subtype-stratified and subtype-adjusted analyses. The numbers of infection events were 42 in the FL group, 35 in the CLL/SLL group and 18 in the MZL group. In a multivariable model that additionally adjusted for iBCL subtype, Flt3L (OR 1.62, 95% CI 1.09–2.40; P = 0.016), sIL-2R (OR 1.43, 95% CI 1.04–1.97; P = 0.028) and Gas6 (OR 1.54, 95% CI 1.07–2.21; P = 0.020) remained independently associated with infection status. Subtype-stratified estimates were directionally consistent but imprecise, with wide confidence intervals reflecting the small number of infection events within each subtype. Tests for interaction between subtype and biomarker were not statistically significant, but these analyses were

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underpowered. These exploratory analyses should therefore be interpreted as hypothesis-generating and do not establish subtype-specific validity.

Correlation analysis between serum Flt3L, sIL-2R, Gas6 levels and PCT in iBCL infection patients

Finally, to examine whether these biomarkers reflect inflammatory burden within infected patients, we assessed their relationship with PCT. Flt3L correlated positively with PCT ($r = 0.47$, $P < 0.001$; Fig. 3A). sIL-2R showed a similar association ($r = 0.51$, $P < 0.001$; Fig. 3B). Gas6 also correlated with PCT ($r = 0.44$, $P < 0.001$; Fig. 3C). Overall, higher Flt3L, sIL-2R and Gas6 levels were associated with higher PCT, consistent with these markers reflecting inflammatory burden during infection in iBCL.

DISCUSSION

In this retrospective, single-center study of patients with iBCL, we evaluated an immune-anchored serum panel comprising Flt3L, sIL-2R, and Gas6 for infection assessment. Infections were primarily community-acquired and respiratory, and were predominantly bacterial. Age ≥65 years, stage III–IV disease, chemotherapy within 3 months, hypogammaglobulinemia and diabetes were independently associated with infection, together with elevated Flt3L, sIL-2R and Gas6. A combined three-marker model showed stronger discrimination than any single biomarker. Collectively, these findings support the potential utility of this panel for infection assessment in iBCL.

In this hospital-based iBCL cohort, infections were largely community acquired, most frequently involved the respiratory tract, and were predominantly bacterial, with Gram-negative organisms detected more often than Gram-positive organisms. This distribution broadly aligns with previous reports, while also underscoring features relevant to iBCL. For example, a previous study of lymphoproliferative disorders, including patients treated with ibrutinib or idelalisib, reported an overall infection rate of 29.8%, with clinically and microbiologically defined infections comprising 49.3% and 50.7% of events, respectively [23]. However, that study did not distinguish community- from hospital-acquired infections or identify the respiratory tract as the predominant site. In a CLL cohort, infections occurred in 71.9% of patients and treatment exposure increased major infection risk, but pathogen distributions were not reported [24].

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In multivariable analysis, infection was independently associated with age ≥65 years, Ann Arbor stage III–IV, chemotherapy within 3 months, hypogammaglobulinemia and diabetes mellitus. These findings are consistent with previous work. For example, in CLL, severe lymphopenia, combination targeted therapy and previous rituximab exposure were independently associated with infection, highlighting susceptibility driven by immune compromise [11]. In multivariable analyses, Charlson comorbidity index and IgG deficiency were also strongly associated with severe infection, supporting a role for hypogammaglobulinemia [25]. In addition, immunoglobulin replacement therapy was associated with a lower infection risk, supporting immune deficiency as a key correlate of infection susceptibility [26]. Notably, our data show independent associations of advanced Ann Arbor stage and recent chemotherapy with infection in iBCL, aligning with reports that multiple treatment lines increase infection risk [27]. iBCL comprises biologically and clinically distinct subtypes, including FL, CLL/SLL and MZL, which differ in molecular features, immune dysfunction and treatment exposure [5]. This heterogeneity is particularly relevant for CLL/SLL, where cytogenetic and molecular features such as del(17p)/TP53 abnormalities and IGHV mutation status influence disease behaviour and treatment selection [6, 7]. FL and MZL also differ in histology, disease kinetics and treatment patterns. We fully acknowledge that pooling these subtypes may introduce heterogeneity related to disease biology and treatment modality, and this is a major limitation of the present study.

We analysed the subtypes together for three reasons, while recognising that this approach is exploratory. First, from a clinical perspective, patients with iBCL are generally evaluated through a common outpatient pathway when infection is suspected, and initial infection screening is not usually subtype-specific in routine practice. Second, although the upstream pathogenesis and treatment patterns differ across subtypes, they share core terminal pathways that increase infection susceptibility, namely humoral immune impairment and T-cell dysfunction. The three biomarkers evaluated here, Flt3L, sIL-2R and Gas6, were selected to reflect these shared immune abnormalities rather than subtype-specific biology. Third, the subtype distribution did not differ significantly between the infection and non-infection groups ($P = 0.205$), suggesting that subtype composition was balanced across groups.

To examine whether the core findings were robust to subtype heterogeneity, we performed a sensitivity analysis in which iBCL subtype (FL, CLL/SLL or MZL) was

included as a categorical covariate in the multivariable logistic regression model. After adjustment for subtype, Flt3L (OR 1.62, 95% CI 1.09–2.40; P = 0.016), sIL-2R (OR 1.43, 95% CI 1.04–1.97; P = 0.028) and Gas6 (OR 1.54, 95% CI 1.07–2.21; P = 0.020) remained independently associated with infection status, with effect estimates similar to those in the primary model. These findings suggest that the associations observed in this cohort were not materially confounded by subtype. However, this sensitivity analysis does not overcome the underlying heterogeneity in disease biology and treatment exposure, and the results should therefore be regarded as exploratory and hypothesis-generating rather than definitive for any individual iBCL subtype or treatment setting.

We found that elevated Flt3L, sIL-2R and Gas6 were independently associated with infection in iBCL, and that a combined model outperformed each marker alone in this cohort (AUC 0.89 vs 0.73–0.76), but this estimate reflects internal performance and requires independent validation. This association is biologically plausible, as Gas6–Axl signalling can dampen macrophage antibacterial responses and promote polarization toward an M2-like phenotype [28]. Circulating Flt3L has also been linked to chemotherapy-related myelosuppression and subsequent neutropenic sepsis in lymphoma patients [17]. Our findings extend this observation by showing that Flt3L was associated with infection status in a broader iBCL cohort, including patients with community-acquired infection. For Gas6, previous studies have linked its activity to inflammatory regulation and infectious conditions [29, 30]. More recently, Gas6 has been explored in lymphoma patients with post-chemotherapy infection [22]; however, its role in infection assessment among iBCL patients remains unclear. Unlike previous studies evaluating individual biomarkers in specific clinical settings, our study assessed the combined value of Flt3L, sIL-2R, and Gas6 for infection assessment in iBCL, providing a potential immune-based approach for patient stratification. Existing clinical scores, such as CLL-TIM, have not been evaluated in iBCL and do not incorporate Flt3L or sIL-2R [31]. Our model therefore provides a complementary, immune-anchored approach that was associated with infection-related immune dysregulation in this cohort, consistent with transcriptomic studies linking inflammatory signatures to infection events [32]. Because of the cross-sectional design, this association should be regarded as hypothesis-generating. Overall, these findings suggest that combining Flt3L, sIL-2R, and Gas6 may provide a complementary framework for infection assessment in iBCL.

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In iBCL, sIL-2R increased stepwise from healthy controls to non-infected and infected patients and correlated with PCT, consistent with greater immune activation in patients with infection [33-35]. iBCL and chemotherapy can impose chronic antigenic stimulation and regenerative stress, promoting T-cell activation and sIL-2R release, a marker linked to immune activation and exhaustion [35-37]. In this cross-sectional analysis, higher inflammatory burden, as reflected by higher PCT, was associated with higher sIL-2R [38,39]. Because biomarkers were measured at a single time point, these findings cannot establish temporal or causal relationships. Nonetheless, they support sIL-2R as a marker associated with immune vulnerability and infection-related immune dysregulation in iBCL, providing a plausible basis for its contribution to the combined model.

In iBCL, infections often arise in the community and are managed in outpatient settings, where symptoms can be non-specific and timely assessment is important. In our cohort, the Flt3L–sIL-2R–Gas6 model showed good discrimination. These findings suggest that the biomarker panel may have value as an adjunct to clinical assessment and triage when infection is suspected. However, the biomarkers were measured by ELISA, which may limit their use when rapid testing is required. Future studies should evaluate rapid detection platforms, such as chemiluminescence-based immunoassays and point-of-care testing, and assess the cost-effectiveness of combined biomarker testing. Prospective, adequately powered studies with prespecified eligibility criteria and standardized infection definitions are also needed to validate the panel and determine its clinical utility, including its ability to support patient stratification and clinical decision-making.

This study has several limitations. First, and most importantly, the cohort pooled three biologically and clinically distinct iBCL subtypes, namely FL, CLL/SLL and MZL, which differ in molecular features, immune dysfunction and treatment exposure. In addition, treatment modalities differed across patients and included watch-and-wait, targeted therapy, immunochemotherapy and prior rituximab. Pooling these subtypes and treatment groups may therefore introduce heterogeneity that cannot be fully addressed by statistical adjustment, and the results should not be directly extrapolated to any specific iBCL subtype or treatment setting. Subtype-stratified analyses were underpowered: the sample size was estimated for the overall cohort, and the numbers of infection events were only 18 in the MZL group and 35 in the CLL/SLL group. Subgroup analyses by subtype and by targeted-therapy exposure were therefore

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exploratory only and could not provide reliable subtype-specific estimates. CLL cytogenetic profiles (TP53/del(17p), IGHV status) were incompletely captured and could not be adjusted for. Future multicentre studies with larger sample sizes, prespecified subtype-stratified and treatment-stratified analyses, and homogeneous patient groups are required to validate the diagnostic performance of this biomarker panel. Second, the retrospective, single-center design may introduce selection bias and limits generalizability. Third, a subset of infections lacked microbiological confirmation and were defined clinically, raising the possibility of misclassification. Fourth, biomarkers were measured at a single time point, and the cut-offs were derived within this cohort. The reported AUC represents apparent performance and may be optimistic. In particular, the AUC of 0.89 for the combined model reflects performance in the present cohort and should be considered exploratory. Bootstrap internal validation and calibration assessment were not prespecified in this hypothesis-generating study and could not be completed in this analysis. Independent external validation, including calibration and decision-curve analysis, is required before clinical implementation. Fifth, formal comparison with conventional inflammatory markers (CRP and WBC) or validated scores such as CLL-TIM was not feasible. CRP and WBC were not uniformly measured, PCT was available only in infected patients, and CLL-TIM was developed specifically for CLL. Therefore, the incremental value of the panel over these tools remains to be established. Sixth, subgroup analyses by iBCL subtype and by targeted-therapy exposure were underpowered owing to small and imbalanced subgroup sizes and strong confounding by indication; CLL cytogenetic profiles (TP53/del(17p), IGHV status) were incompletely captured and could not be adjusted for. Seventh, the analyses addressed infection discrimination rather than prospective prediction, and clinical utility was not evaluated. Finally, without serial pre-infection and post-recovery samples, the panel cannot distinguish a chronic infection-prone state from incident active infection and should currently be regarded as a tool for infection assessment rather than a stand-alone diagnostic tool.

CONCLUSION

In conclusion, in this exploratory, retrospective, single-centre cohort of a pooled iBCL population, infection was associated with host vulnerability and immune dysregulation. Elevated Flt3L, sIL-2R and Gas6 were independently associated with

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infection, and their combined model outperformed each marker alone in this cohort. However, because the cohort pooled biologically and clinically distinct iBCL subtypes and treatment modalities, these findings should be regarded as hypothesis-generating and cannot be directly extrapolated to any specific subtype or treatment setting. The AUC of 0.89 reflects performance in the present cohort and requires independent validation in homogeneous, subtype-specific and treatment-specific cohorts before clinical use.

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TABLES AND FIGURES WITH LEGENDS

Table 1. Classification and distribution of infections in iBCL patients (*n* = 95)

Classification dimension	Specific type	Case number (*n*)	Constituent ratio (%)
Infection site	Respiratory tract infection	48	50.5
	Urinary system infection	19	20.0
	Skin and soft tissue/others (oral cavity, sepsis)	28	29.5
Infection severity	Mild infection (outpatient manageable)	66	69.5
	Severe infection (hospitalization required/SOFA ≥ 6)	29	30.5
Infection occurrence place	Community-acquired infection	68	71.6
	Hospital-acquired infection (during chemotherapy/hospitalization)	27	28.4

Abbreviations: iBCL, indolent B-cell lymphoma; SOFA, Sequential Organ Failure Assessment. Pasted markdown

Table 2. Pathogen distribution by infection site in iBCL patients (*n* = 95)

Pathogen type	Specific pathogen	Case number (*n*)	Constituent ratio (%)
Single bacterial infection	Gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae*)	32	33.7
	Gram-positive bacteria (*Staphylococcus aureus, Streptococcus*)	25	26.3
	Anaerobes (*Bacteroides fragilis*)	3	3.2
	Total bacteria	60	63.2

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Single viral infection	Varicella-zoster virus (VZV)	9	9.5
	Influenza virus	5	5.3
	Total viruses	14	14.7
Fungal infection	Candida, Aspergillus	7	7.4
Mixed infection (bacteria + virus/fungus)	-	9	9.5
Unidentified pathogen	Diagnosed by clinical symptoms + inflammatory indicators	5	5.3

Abbreviations**:** iBCL, indolent B-cell lymphoma; VZV, varicella–zoster virus. Pasted markdown

Table 3. Univariate analysis of demographic and clinical features in iBCL patients with and without infection

Features	**Non-Infection (*n* = 152)**	**Infection (*n* = 95)**	***p* value**
Age (years), median (range)	62 (45-80)	66 (50-83)	< 0.001
Gender, *n* (%)			
Male	80 (52.6%)	53 (55.8%)	0.694
Female	72 (47.4%)	42 (44.2%)	
iBCL subtype, *n* (%)			
FL	82 (53.9%)	42 (44.2%)	0.205
CLL/SLL	40 (26.3%)	35 (36.8%)	
MZL	30 (19.7%)	18 (18.9%)	
Ann Arbor stage, *n* (%)			
I-II	86 (56.6%)	37 (38.9%)	0.009

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III-IV	66 (43.4%)	58 (61.1%)	
Bone marrow infiltration, *n* (%)			
No	117 (77.0%)	59 (62.1%)	0.014
Yes	35 (23.0%)	36 (37.9%)	
Treatment regimen, *n* (%)			
Watch and wait	62 (40.8%)	12 (12.6%)	< 0.001
Targeted therapy	58 (38.2%)	45 (47.4%)	
Immunochemotherapy	32 (21.1%)	38 (40.0%)	
Recent chemotherapy history (within 3 months), *n* (%)			
No	126 (82.9%)	63 (66.3%)	0.003
Yes	26 (17.1%)	32 (33.7%)	
Rituximab use history			
No	107 (70.4%)	53 (55.8%)	0.021
Yes	45 (29.6%)	42 (44.2%)	
Diabetes mellitus, *n* (%)			
No	123 (80.9%)	62 (65.3%)	0.007
Yes	29 (19.1%)	33 (34.7%)	
Hypertension, *n* (%)			
No	101 (66.4%)	58 (61.1%)	0.415
Yes	51 (33.6%)	37 (38.9%)	
Hypogammaglobulinemia (IgG <7 g/L), *n* (%)			
No	117 (77.0%)	51 (53.7%)	< 0.001
Yes	35 (23.0%)	44 (46.3%)	

Continuous data (e.g., age) were analyzed using the Mann-Whitney U test (due to non-normal distribution), and categorical data were analyzed using the $\chi 2$ test. **Abbreviations**: CLL/SLL, chronic lymphocytic leukemia/small lymphocytic

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lymphoma; FL, follicular lymphoma; iBCL, indolent B-cell lymphoma; IgG, immunoglobulin G; MZL, marginal zone lymphoma.

Table 4. Multivariate logistic regression analysis of factors associated with infection status in iBCL patients

Variable	OR value	95% CI	*p* value
Age ≥ 65 years	2.12	1.25–3.58	0.005
Ann Arbor Stage III-IV	1.43	1.08–1.89	0.012
Chemotherapy within 3 months	1.97	1.32–2.95	< 0.001
Hypogammaglobulinemia	2.26	1.39–3.67	< 0.001
Diabetes mellitus	1.56	1.07–2.28	0.021
Flt3L ≥ 92.75 pg/mL	1.68	1.14–2.47	0.008
sIL-2R ≥ 317.66 ng/mL	1.47	1.08–2.01	0.015
Gas6 ≥ 20.62 ng/mL	1.59	1.12–2.25	0.009

Candidate variables were selected based on a priori clinical plausibility and a univariate threshold of $p < 0.05$, using complete-case analysis (patients with missing key clinical data were excluded, while biomarker data were complete for all included subjects). Bone-marrow involvement and Ann Arbor stage both indicate disease burden, whereas treatment regimen, prior rituximab, and chemotherapy within 3 months all indicate treatment intensity. In a model that simultaneously forced all candidate variables, the VIF within these correlated clusters rose to 2.32 for the disease-burden pair and up to 2.58 for the treatment-intensity variables. To avoid this redundancy, only one representative per cluster was retained: Ann Arbor stage for disease burden and chemotherapy within 3 months for treatment intensity. After this adjustment, all VIFs in the final model were below 2.0. The three biomarkers were entered as binary variables defined by their optimal cut-offs, which were derived within this cohort by maximizing the Youden index (sensitivity + specificity - 1) on the ROC curve to provide clinically actionable thresholds. For this risk-factor model, Flt3L, sIL-2R, and Gas6 were dichotomized at their cohort-derived optimal cut-offs (Flt3L ≥ 92.75 pg/mL; sIL-2R ≥ 317.66 ng/mL; Gas6 ≥ 20.62 ng/mL), whereas

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continuous biomarker values were used in the combined diagnostic model shown in Table 5. **Abbreviations:** CI, confidence interval; Flt3L, FMS-like tyrosine kinase 3 ligand; Gas6, growth arrest-specific 6; iBCL, indolent B-cell lymphoma; OR, odds ratio; ROC, receiver operating characteristic; sIL-2R, soluble interleukin-2 receptor; VIF, variance inflation factor.

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Table 5. ROC analysis of Flt3L, sIL-2R, and Gas6 for discriminating infection status in iBCL patients

Indicator	AUC (95% CI)	Cut-off	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictivity (95% CI)	Negative predictivity (95% CI)
Flt3L	0.76 (0.70-0.82)	92.75 pg/mL	69.47% (59.61% to 77.83%)	73.03% (65.47% to 79.45%)	61.68% (52.22% to 70.34%)	79.30% (71.83% to 85.17%)
sIL-2R	0.73 (0.66-0.79)	317.66 ng/mL	61.05% (51.00% to 70.25%)	76.97% (69.67% to 82.95%)	62.36% (52.21% to 71.54%)	76.00% (68.65% to 82.04%)
Gas6	0.75 (0.69-0.81)	20.62 ng/mL	69.47% (59.61% to 77.83%)	71.71% (64.08% to 78.27%)	60.55% (51.17% to 69.22%)	78.99% (71.45% to 84.95%)
Flt3L+sIL-2R+Gas6	0.89 (0.85-0.94)	-0.42	80% (70.86% to 86.81%)	84.87% (78.32% to 89.70%)	76.77% (67.54% to 83.99%)	87.16% (80.82% to 91.63%)

For individual biomarkers, cutoff values were derived by maximizing the Youden index (sensitivity + specificity − 1) on the ROC curve. For the combined Flt3L+sIL-2R+Gas6 model, all three biomarkers were entered as continuous variables, and the linear predictor of the logistic regression was computed as: 0.042 * *Flt3L + 0.008* * sIL2r + 0.192 * Gas6 – 10.563. The optimal cutoff for this combined model was −0.42 on the linear predictor scale, also determined by maximizing the Youden index. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated against the actual infection status at this threshold. **Abbreviations:** AUC, area under the receiver operating characteristic curve; CI, confidence interval; Flt3L, FMS-like tyrosine kinase 3 ligand; Gas6, growth arrest-specific 6; iBCL, indolent B-cell lymphoma; ROC, receiver operating characteristic; sIL-2R, soluble interleukin-2 receptor.

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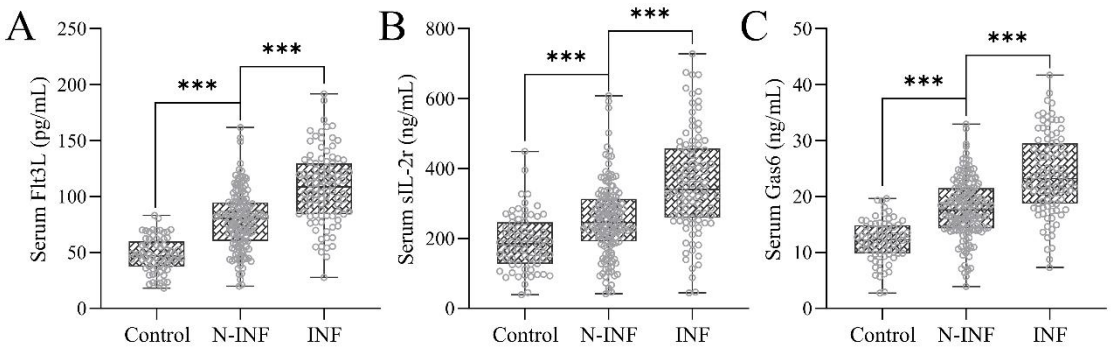


Figure 1. Serum Flt3L, sIL-2R, and Gas6 levels across healthy controls and patients with iBCL stratified by infection status. Box-and-whisker plots compare serum biomarker concentrations among healthy controls (n = 70), patients with iBCL without infection (N-INF; n = 152), and patients with iBCL and infection (INF; n = 95). **(A)** Serum Flt3L levels increased progressively from controls to the N-INF and INF groups. **(B)** Serum sIL-2R levels showed a similar stepwise increase across the three groups. **(C)** Serum Gas6 levels were also highest in the INF group. Boxes represent the interquartile range, horizontal lines indicate the median, whiskers indicate the observed range, and circles represent individual participants. Group differences were assessed using Brown–Forsythe analysis of variance followed by the Games–Howell multiple-comparisons test. Brackets indicate pairwise comparisons; ***p < 0.001. **Abbreviations**: Flt3L, FMS-like tyrosine kinase 3 ligand; Gas6, growth arrest-specific 6; iBCL, indolent B-cell lymphoma; INF, infection; N-INF, non-infection; sIL-2R, soluble interleukin-2 receptor.

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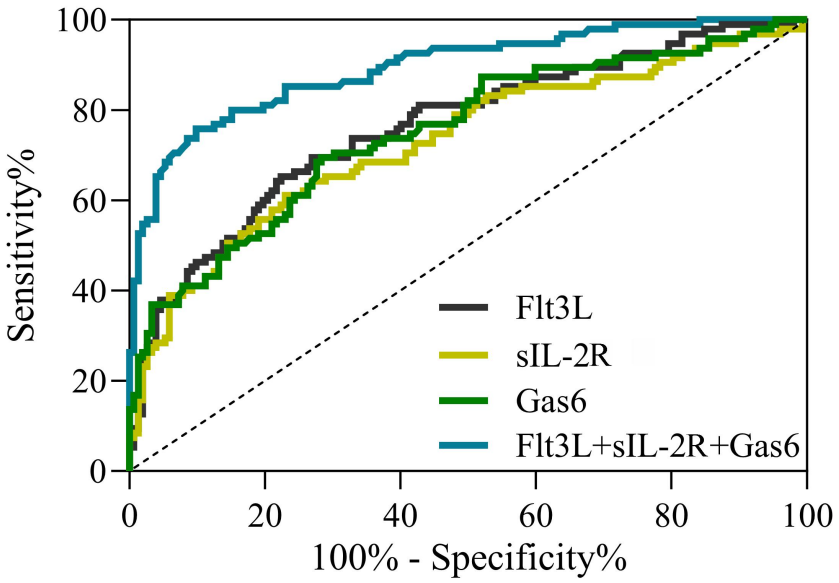


Figure 2. Discriminatory performance of serum Flt3L, sIL-2R, Gas6, and their combined model for infection status in patients with iBCL. Receiver operating characteristic curves compare the ability of serum Flt3L, sIL-2R, and Gas6, individually and in combination, to distinguish patients with infection (n = 95) from those without infection (n = 152). Among the individual biomarkers, Flt3L achieved an area under the curve of 0.76 (95% confidence interval [CI] 0.70–0.82), Gas6 achieved 0.75 (95% CI 0.69–0.81), and sIL-2R achieved 0.73 (95% CI 0.66–0.79). The combined model, constructed by entering all three biomarkers as continuous variables into a logistic regression model without clinical covariates, showed the highest apparent discrimination, with an area under the curve of 0.89 (95% CI 0.85–0.94), 80.0% sensitivity, and 84.9% specificity at the cohort-derived optimal threshold. The dashed diagonal line represents chance-level discrimination. The performance of the combined model was estimated in the same cohort in which it was developed and requires independent external validation. **Abbreviations**: AUC, area under the receiver operating characteristic curve; CI, confidence interval; Flt3L, FMS-like tyrosine kinase 3 ligand; Gas6, growth arrest-specific 6; iBCL, indolent B-cell lymphoma; ROC, receiver operating characteristic; sIL-2R, soluble interleukin-2 receptor.

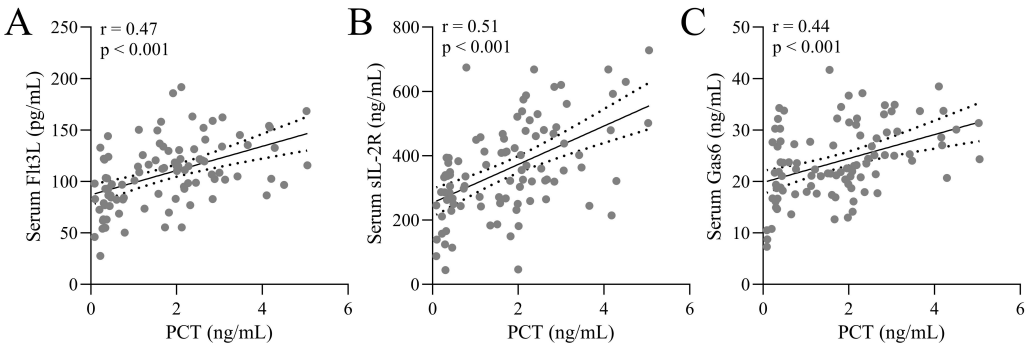


Figure 3. Correlations of serum Flt3L, sIL-2R, and Gas6 levels with procalcitonin in patients with iBCL and infection. Scatter plots show the associations of serum PCT levels with **(A)** Flt3L, **(B)** sIL-2R, and **(C)** Gas6 levels in patients with iBCL and infection (n = 95). Spearman's rank correlation analysis showed significant positive correlations between PCT and Flt3L (r = 0.47), sIL-2R (r = 0.51), and Gas6 (r = 0.44); all $p < 0.001$. Each point represents an individual patient. Solid lines represent least-squares linear fits, and dashed lines indicate the corresponding 95% confidence bands; these lines are included for visualization of the observed trends. **Abbreviations**: Flt3L, FMS-like tyrosine kinase 3 ligand; Gas6, growth arrest-specific 6; iBCL, indolent B-cell lymphoma; PCT, procalcitonin; sIL-2R, soluble interleukin-2 receptor.

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