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Immune-biomarker signature predicts early molecular response in Iraqi chronic myeloid leukemia patients receiving front-line tyrosine-kinase inhibitors

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Chronic myeloid leukemia (CML) is characterized by ABL1::BCR-driven leukemogenesis. Although tyrosine-kinase inhibitors (TKIs) have transformed prognosis, predicting early molecular response (EMR) and treatment-free remission (TFR) remains clinically challenging. Immune mediators such as interleukin-2 (IL)-2, IL-7, and perforin may serve as biomarkers reflecting host-leukemia interactions. This study aimed to evaluate the diagnostic and prognostic potential of IL-2, IL-7, and perforin as predictive biomarkers in Iraqi patients with CML. A total of 215 participants were enrolled, including healthy controls (n = 55), newly diagnosed CML patients (n = 20), treatment-free remission patients (TFR) (n = 20), and patients receiving imatinib (n = 30), nilotinib (n = 30), or bosutinib (n = 30). Serum IL-2, IL-7, and perforin levels were quantified by ELISA, and molecular responses were monitored using qRT-PCR standardized to the International Scale. ROC analysis determined optimal biomarker cut-offs and predictive performance for EMR and TFR. The results indicated that IL-2 and IL-7 were significantly elevated in newly diagnosed CML patients (312.4 ± 133.2 and 158.9 ± 108.4 pg/mL) compared to controls (104.6 ± 50.7 and 53.8 ± 8.1 pg/mL, $P < 0.0001$). Perforin was markedly reduced (1.6 ± 0.9 and 8.7 ± 5.2 ng/mL). ROC analysis revealed IL-2 (AUC = 0.980), IL-7 (AUC = 1.000), and perforin (inverse AUC < 0.001) as highly discriminative for diagnosis, while perforin (AUC = 0.999, cut-off 3.76 ng/mL) emerged as the strongest predictor of TFR. An immune-biomarker panel integrating high IL-2/IL-7 and depleted perforin at diagnosis effectively predicts EMR and sustained TFR. Incorporating immune profiling into CML management may refine early risk stratification and enable personalized TKI selection.

Keywords: perforin; interleukin-7; interleukin-2; tyrosine-kinase inhibitors; chronic myeloid leukemia.

Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm driven by ABL1::BCR activity of tyrosine-kinase; first-line tyrosine-kinase inhibitors (TKIs) like the bosutinib, nilotinib, and imatinib changing clinical focus from long-term treatment-free remission (TFR) and temporary regulation of illness optimization of early molecular responses (EMRs). Contemporary guiding principles highlight EMR – International Scale of ABL1::BCR, which is ≤10% at 3 months – as a key revolutionary that stratifies risk and guides adaptation of therapy and tracking intensity. Failure to reach EMR portends inferior survival and deeper rates of responses, while accomplishing EMR is strongly associated with consequent deep and major molecular responses. These guiding principles are organized in current updates of the European LeukemiaNet (ELN), which proposed molecular calculation at least every three months till constant main molecular responses are detected (Cross et al., 2023; Saugues et al., 2024; Apperley et al., 2025). Translational and modeling information on a leukemia–immune dynamic that can tip patients in the direction of molecular recurrence or durable TFR, highlighting the value of immunity biomarkers that reproduce lymphocyte homeostasis and cytotoxic capacity (Hsieh et al., 2021; Huuhtanen et al., 2024).

Among candidate biomarkers, perforin, interleukin-2 (IL-2) and interleukin-7 (IL-7), display complementary, different axes of antileukemic immune responses. IL-7 is a key homeostatic cytokine for T-cell survival and repertoire maintenance; recent oncology and immunotherapy literature highlights IL-7/STAT5 signaling in restoring T-cell numbers and function under lymphopenic or chronically stimulated states – conditions relevant to CML diagnosis and to post-TKI immune surveillance. IL-2 orchestrates effector expansion and activation, with soluble and cellular readouts of the IL-2 axis associating with illness activity in leukemias. Perforin, the pore-forming effector molecule of NK cells and cytotoxic T lymphocytes, is indispensable for direct killing of leukemic cells; its expression and functionality have been linked to non-relapse phenotypes and effective innate/adaptive cytotoxic responses in myeloid leukemias and CML. Together, these markers capture homeostatic (IL-7), effector programming (IL-2), and terminal cytotoxic machinery (perforin) (Decroos et al., 2024a; Fu et al., 2024; Janowski et al., 2024a).

Clinically, EMR varies across first-line TKIs, and differences in off-target and immunomodulatory profiles may shape immune readouts. Contemporary updates and cohort analyses report high EMR rates with modern dosing/schedules and reinforce the centrality of early kinetics for long-term outcomes, including TFR attempts. Thus far, the field still lacks pragmatic signatures of immunity that predict EMR through particular front-line TKIs in routine practice, particularly in under-studied populations. Defining such signatures could refine early risk stratification beyond levels of transcript alone and inform selection of TKIs, tailoring of doses and readiness of TFR (Jabbour & Kantarjian, 2024; Apperley et al., 2025). There is also a geographic determinism. Information from settings in the Middle East, including Iraq, supposes measurable changes in cytokine milieus and innate immune responses in CML and indicates that care pathways, illness biology and local demographics may impact phenotypes of immunity and clinical responses. Characterizing biomarkers of immunity through context-relevant clinical cohorts – newly detected CML patients on particular front-line TKIs, including bosutinib, nilotinib and imatinib, and patients in TFR – can consequently create practicable, locally effective predictive tools contributing to worldwide immunobiology of CML (Faraj et al., 2023).

This work aims to assess the predictive value of signatures of immune-biomarker including perforin, interleukin-7 (IL-7) and interleukin-2 (IL-2), in prediction of early molecular responses (EMRs) in chronic myeloid leukemia (CML) patients in Iraq receiving front-line TKIs. Particularly, it seeks to compare and quantify the perforin, IL-7 and IL-2 levels through 6 classes – patients receiving bosutinib, pa-

tients receiving nilotinib, patients receiving imatinib, patients in treatment-free remission (TFR), newly diagnosed CML patients, and healthy controls – and to detect their associations with achievement of EMR (at 3 months; BCR:ABL1^IS ≤10%). Also, the work aims to find whether a combined profile of biomarker–immunity can be considered as an early, dependable predictor of molecular responses beyond traditional molecular and clinical limitations, thus facilitating decisions to be made on individualized therapeutic approaches and optimization of outcomes of long-term treatment in management of CML.

Materials and methods

Study design and cohorts. This observational cross-sectional study was conducted at the National Center of Hematology, Mustansiriyah University (Baghdad, Iraq), a tertiary referral institution accredited by the Iraqi Ministry of Higher Education and Scientific Research. Written informed consent was gained from all contributors before collection of samples.

Study population and group classification. A total of 215 individuals were enrolled and stratified into 6 classes: bosutinib-treated patients (n = 30), nilotinib-treated patients (n = 30), imatinib-treated patients (n = 30), treatment-free remission (TFR) patients (n = 20), newly diagnosed CML patients (n = 20) and healthy controls (n = 55). According to European criteria of LeukemiaNet (Apperley et al., 2025), all patients with CML were diagnosed according to molecular, cytogenetic, hematologic, and clinical findings. Patients were excluded if they had ongoing immunomodulatory therapy, secondary malignancies, autoimmune illness, or concurrent infections.

Sample collection. Peripheral venous blood samples (10 mL) were obtained under aseptic conditions. Samples were transported within 30 minutes at 22 ± 2 °C to the central processing laboratory. Serum samples were collected in plain tubes, allowed to clot, and centrifuged at 3000 rpm for 10 minutes. The supernatant was aliquoted and stored at –80 °C until cytokine and perforin analysis. Whole blood samples, collected in EDTA tubes, were processed within 2 hours for complete blood count (CBC) and molecular assays.

Quantification of immune biomarkers. Serum concentrations of interleukin-2 (IL-2), interleukin-7 (IL-7), and perforin were quantified using commercially available sandwich ELISA kits (FineTest®, China; IL-2: EH0189, IL-7: EH0203, Perforin: EH1487). The detection limits were 0.5, 5.0, and 0.1 ng/mL, respectively. All assays were performed in duplicate following the manufacturer's instructions, and optical density was measured at 450 nm using a microplate reader.

Molecular monitoring. For determination of transcript levels of ABL1::BCR and their standardization according to the International Scale (IS), quantitative real-time PCR (qRT-PCR) was utilized. Major molecular response (MR^3) was defined as ≤ 0.1% IS and EMR was defined as ABL1::BCR ≤ 10% IS at 3 months, according to guidelines of ELN 2025.

Biochemical and hematological analysis. Routine biochemical assays – including hepatic (ALT, AST, ALP, total bilirubin), renal (serum creatinine, blood urea), pancreatic (amylase, lipase), and electrolyte (Na^+, K^+, Cl^-) parameters – were performed using an automated clinical chemistry analyzer (Beckman Coulter AU5800, USA) at baseline and at 3, 6, and 9 months. Hematological parameters (WBC, hemoglobin, platelets) were analyzed using an automated hematology analyzer (Sysmex XN-1000, Japan).

Statistical analysis. SPSS version 29.0 (IBM Corp.) was utilized for analyzing of data. Replaying on distributions normality was evaluated via the Shapiro–Wilk test, continuous variables were expressed as median (IQR) or mean ± SD. Intergroup comparisons were shown utilizing the Kruskal–Wallis test followed by correction of Dunn–Bonferroni post hoc. Relations between molecular response and biomarkers were studied utilizing Spearman's correlation and generalized estimating equations (GEE) for estimation of odds ratios. Receiver operating characteristic (ROC) curves were used to determine diagnostic accuracy, optimal cut-off points, sensitivity, and specificity. A false discovery rate (FDR)-adjusted $P < 0.05$ was considered statistically significant.

Results

In Table 1, the concentrations of IL-2, IL-7, and perforin are shown in six groups: control (n = 55), CML (n = 20), TFR (n = 20), imatinib (n = 30), nilotinib (n = 30), and bosutinib (n = 30). For IL-2, the mean ± SD in the control group was 104.6 ± 50.7 pg/mL, while it was markedly higher in newly diagnosed CML patients (312.4 ± 133.2 pg/mL). In the TFR group, IL-2 dropped to 108.4 ± 42.9 pg/mL, while in imatinib, nilotinib, and bosutinib groups, the means were 173.9 ± 58.7, 183.0 ± 61.3, and 165.9 ± 83.4 pg/mL, respectively ($P < 0.0001$). IL-7 showed a similar trend: 53.8 ± 8.1 pg/mL in controls, 158.9 ± 108.4 pg/mL in CML, 58.3 ± 13.2 pg/mL in TFR, 68.5 ± 18.9 pg/mL in imatinib, 68.9 ± 55.8 pg/mL in nilotinib, and 71.4 ± 10.3 pg/mL in bosutinib ($P < 0.0001$). Conversely, perforin levels were significantly lower in CML (1.6 ± 0.9 ng/mL) compared to controls (8.7 ± 5.2 ng/mL) and higher in treated groups: TFR (6.1 ± 3.7 ng/mL), imatinib (4.6 ± 2.4 ng/mL), nilotinib (4.1 ± 3.0 ng/mL), and bosutinib (5.4 ± 2.9 ng/mL), confirming significant restoration of cytotoxic potential after therapy ($P < 0.0001$).

Table 1

Biomarkers parameters across six cohorts' one-way ANOVA F- and P-value Tukey post-hoc letters (mean ± SD, groups sharing the same letter are NOT significantly different, $P \geq 0.05$)

Parameters	Control (n = 55)	CML (n = 20)	TFR (n = 20)	Imatinib (n = 30)	Nilotinib (n = 30)	Bosutinib (n = 30)	P- value
IL-2, pg./mL	104.6 ± 50.7^{a}	312.4 ± 133.2^{c}	108.4 ± 42.9^{a}	173.9 ± 58.7^{ab}	183.0 ± 61.3^{b}	165.9 ± 83.4^{ab}	< 0.0001
IL-7, pg./mL	53.8 ± 8.1^{a}	158.9 ± 108.4^{b}	58.3 ± 13.2^{a}	68.5 ± 18.9^{a}	68.9 ± 55.8^{a}	71.4 ± 10.3^{a}	< 0.0001
Perforin, ng/mL	8.7 ± 5.2^{b}	1.6 ± 0.9^{a}	6.1 ± 3.7^{b}	4.6 ± 2.4^{b}	4.1 ± 3.0^{b}	5.4 ± 2.9^{b}	< 0.0001

Table 2 summarizes biochemical and hematological parameters in 215 participants (control n = 55). Alanine aminotransferase (ALT) was significantly elevated in the bosutinib group at 41 (31–55) U/L compared to the control 30 (26–36) U/L, CML 34 (26–40), TFR 34 (26–41), imatinib 30 (26–36), and nilotinib 30 (28–37) ($P = 0.020$). Aspartate aminotransferase (AST) showed no significant difference across cohorts: control 25 (20–31), CML 28 (23–33), TFR 27 (23–33), imatinib 27 (19–31), nilotinib 29 (20–33), and bosutinib 29 (20–33) ($P = 0.22$). Total serum bilirubin (TSB) ranged from 0.73 (0.58–0.88) mg/dL in controls to 0.87 (0.58–1.0) in CML and 0.90 (0.58–1.2) in bosutinib ($P = 0.08$). Blood urea varied between 33 (28–37) mg/dL in controls and 37 (28–45) in bosutinib ($P = 0.15$). Serum creatinine ranged from 0.74 (0.70–0.90) mg/dL in nilotinib to 1.0 (0.75–1.2) in bosutinib ($P = 0.19$). Sodium, potassium, and chloride values were stable across groups: Na 135–140 mmol/L ($P = 0.52$), K 3.7–4.2 mmol/L ($P = 0.68$), Cl 95–105 mmol/L ($P = 0.24$). Amylase showed significance ($P = 0.026$) with highest values in CML 97 (85–110) U/L and bosutinib 100 (82–120) U/L compared to control 87 (78–95) U/L. Lipase varied from 87 (77–101) U/L in nilotinib to 107 (91–120) in CML ($P = 0.10$). WBC was markedly elevated in CML 137 (110–200)×10^3/μL versus control 7.6 (6.5–9.1) ($P < 0.001$). Hemoglobin decreased significantly in CML 10.8 (9.9–11.9) g/dL versus control 13.0 (12.3–14.5) ($P < 0.001$), while platelets were highest in CML 385 (309–550)×10^3/μL compared to control 270 (228–327) ($P = 0.009$).

For newly diagnosed CML patients, the ROC analysis of IL-2, IL-7, and perforin demonstrated strong discriminative capability. IL-2 showed an AUC of 0.9800 with a P-value of 0.001 and 95% confidence interval (CI) from 0.9460 to 1.000. IL-7 achieved a perfect AUC of 1.000 ($P = 0.001$, CI 1.000–1.000), indicating complete separation between illness and control groups. Perforin had an AUC of 0.0000 ($P = 0.001$, CI 0.0000–0.0000), signifying an inverse diagnostic relation due to its depletion in active illness.

Table 2
Biochemical and hematological parameters across six cohorts (Kruskal-Wallis; data are median (inter-quartile range); only parameters with P < 0.05 are considered statistically significant after Benjamini-Hochberg correction; post-hoc pairwise comparisons performed by Dunn-Bonferroni test; control group n = 55; total n = 215)

Parameter, unit	Control (n = 55)	CML (n = 20)	TFR (n = 20)	Imatinib (n = 30)	Nilotinib (n = 30)	Bosutinib (n = 30)	P-value
ALT	30 (26–36)	34 (26–40)	34 (26–41)	30 (26–36)	30 (28–37)	41 (31–55)	0.020
AST	25 (20–31)	28 (23–33)	27 (23–33)	27 (19–31)	29 (20–33)	29 (20–33)	0.22
TSB	0.73 (0.58–0.88)	0.87 (0.58–1.0)	0.83 (0.58–0.98)	0.81 (0.66–0.95)	0.87 (0.66–1.0)	0.90 (0.58–1.2)	0.08
B. Urea	33 (28–37)	34 (25–42)	34 (28–41)	32 (28–39)	26 (19–32)	37 (28–45)	0.15
S. Creatinine, mg/dL	0.79 (0.70–0.88)	0.77 (0.70–1.0)	0.82(0.75–1.0)	0.92 (0.75–1.1)	0.74 (0.70–0.90)	1.0 (0.75–1.2)	0.19
Na, mmol/L	138 (135–140)	138 (135–140)	138 (135–140)	137 (135–139)	138 (136–140)	137 (135–140)	0.52
K, mmol/L	4.0 (3.8–4.2)	4.1 (3.8–4.2)	3.9 (3.7–4.2)	3.9 (3.7–4.2)	4.0 (3.8–4.2)	4.0 (3.8–4.2)	0.68
Cl, mmol/L	100 (98–104)	101 (98–104)	98 (95–104)	98 (95–102)	102 (99–105)	99 (95–104)	0.24
Amylase, U/L	87 (78–95)	97 (85–110)	94 (83–110)	93 (82–101)	89 (85–103)	100 (82–120)	0.026
Lipase, U/L	93 (85–100)	107 (91–120)	101 (83–120)	94 (82–120)	87 (77–101)	101 (82–120)	0.10
WBC, $10^3/\mu L$	7.6 (6.5–9.1)	137 (110–200) [2]	7.2 (5.9–8.9)	7.4 (5.4–9.9)	8.3 (5.4–9.9)	7.8 (6.5–9.1)	< 0.001
Hb, g/dL	13.0 (12.3–14.5)	10.8 (9.9–11.9)	11.2 (10.2–12.9)	11.7 (10.7–12.9)	12.6 (11.3–13.4)	12.0 (10.9–13.0)	< 0.001
PLT, $10^3/\mu L$	270 (228–327)	385 (309–550)	285 (206–345)	218 (181–270)	228 (156–312)	238 (188–285)	0.009

Table 3
Area under the curve analysis of IL-2, IL-7, and perforin for new diagnosis CML

Test result variable	Area	P-value	Asymptotic 95% Confidence Interval	
			lower bound	upper bound
IL-2, pg./mL	0.9800	0.0010	0.9460	1.0000
IL-7, pg./mL	1.0000	0.0010	1.0000	1.0000
Perforin, ng./mL	0.0000	0.0010	0.0000	0.0000

Note: under the nonparametric assumption; null hypothesis: true area = 0.5.

At the optimal diagnostic thresholds, IL-2 revealed a cut-off of 146.05 pg/mL with 100% sensitivity and 95% specificity, while IL-7 showed an optimal cut-off of 32.8 pg/mL achieving both 100% sensitivity and 100% specificity. Perforin had no applicable (NA) cut-off or values of specificity/sensitivity due to its expression being contrarywise associated to status of illness, displaying levels of near-zero detection in patients with CML.

In the remission phase, ROC performance was reversed. IL-2 recorded a minimal AUC of 0.045 (P = 0.0001, CI 0.000–0.127), while IL-7 had an even lower AUC of 0.008 (P = 0.0001, CI 0.000–0.026), indicating they were not predictive markers in remission. In contrast, perforin displayed a near-perfect AUC of 0.999 (P = 0.0001, CI 0.994–1.000), identifying it as the most reliable biomarker for treatment-free remission in CML. This shift highlights the reactivation of cytotoxic immune mechanisms once the illness is controlled.

For treatment-free remission, IL-2 and IL-7 did not yield valid cut-off or sensitivity/specificity data (NA), reinforcing their limited predictive role at this stage. However, perforin achieved a decisive cut-off value of 3.76 ng/mL, exhibiting 100% sensitivity and 95% specificity, confirming its potential as a robust biomarker to distinguish patients capable of maintaining remission after discontinuation of therapy.

Discussion

The IL-2 was markedly elevated at CML diagnosis (312.4 ± 133.2 pg/mL) versus controls (104.6 ± 50.7 pg/mL), then partially normalized with therapy (imatinib 173.9 ± 58.7; nilotinib 183.0 ± 61.3; bosutinib 165.9 ± 83.4 pg/mL) and returned to near-control levels in TFR (108.4 ± 42.9 pg/mL; P < 0.0001 across groups). This pattern supports the view that untreated CML is accompanied by a pro-inflammatory/immune-activation milieu which contracts as the leukemic burden falls under TKI pressure. Recent prospective immune-profiling in newly diagnosed CML showed broad cytokine fluctuations during the first treatment year that tracked molecular responses, consistent with our observed IL-2 fall on TKIs and re-equilibration in TFR. Moreover, sustained TFR appears to coincide with an immune set-point compatible with surveillance rather than overt activation, aligning with larger TFR reviews that emphasize immune normalization once BCR:ABL1 is durably suppressed (Hughes et al., 2025; Janowski et al., 2024b).

Table 4
ROC analysis: cut-off, sensitivity, and specificity for IL-2, IL-7 and perforin

Test result variable	Cut off	Sensitivity	Specificity
IL-2, pg./mL	146.1	100	95
IL-7, pg./mL	32.8	100	100
Perforin, ng./mL	NA	NA	NA

Table 5
Area under the curve analysis of IL-2, IL-7, and perforin for treatment-free remission in CML

Test result variable	Area	P-value[b]	Asymptotic 95% confidence Interval	
			lower bound	upper bound
IL-2, pg./mL	0.0450	0.00010	0.0	0.1270
IL-7, pg./mL	0.0080	0.00010	0.0	0.0260
Perforin, ng./mL	0.9990	0.00010	0.9940	1.0000

Note: the test result variable: perforin has at least one tie between the positive actual state group and the negative actual state group; statistics may be biased; under the nonparametric assumption; null hypothesis: true area = 0.05.

Table 6
ROC analysis: cut-off, sensitivity, and specificity for IL-2, IL-7 and perforin

Test Result Variable(s)	Cut off	Sensitivity	Specificity
IL-2, pg./mL	NA	–	–
IL-7, pg./mL	NA	–	–
Perforin, ng./mL	3.76	100	95

IL-7 rose at diagnosis (158.9 ± 108.4 pg/mL vs 53.8 ± 8.1 pg/mL in controls), then decreased with treatment (imatinib 68.5 ± 18.9; nilotinib 68.9 ± 55.8; bosutinib 71.4 ± 10.3 pg/mL) and approximated control in TFR (58.3 ± 13.2 pg/mL; P < 0.0001). Although clinical datasets on circulating IL-7 in CML are limited, experimental and translational work implicates IL-7 signaling in microenvironment-mediated TKI resistance via IL-7/JAK1/STAT5; therefore, the higher IL-7 at diagnosis plausibly reflects stromal-immune crosstalk that wanes as illness control is achieved. The near-physiologic IL-7 in TFR is compatible with a reduced reliance on IL-7–driven survival cues once leukemic pressure is removed (Birru et al., 2024). Perforin was suppressed at diagnosis (1.6 ± 0.9 vs. 8.7 ± 5.2 ng/mL in controls) and rose after therapy (TFR 6.1 ± 3.7; imatinib 4.6 ± 2.4; nilotinib 4.1 ± 3.0; bosutinib 5.4 ± 2.9 ng/mL; P < 0.0001), indicating restoration of cytotoxic potential. This mirrors contemporary evidence that TKIs, beyond reducing illness, reshape innate immunity – particularly NK cells – toward improved effector function; reviews since 2022 describe TKI-linked enhancement of NK phenotype/function, which mechanistically involves perforin/granzyme-mediated cytotoxicity. Clinically, higher NK cell counts and functionality are associated with successful TKI cessation, providing a biological rationale for the higher perforin level we observed in the treated groups and especially in patients maintaining TFR (Allison et al., 2022; Bruzzese et al., 2025).

The convergence of IL-2 and IL-7 toward control values, coupled with rising perforin, level is consistent with an "immune-rebalanced" state favorable for TFR. Large contemporary syntheses report that patients who stop treatment with TKIs successfully often display immune features compatible with ongoing surveillance (e.g., higher NK numbers/competence), while emerging biomarker studies suggest that cytokine signatures (e.g., IL-6/MCP-1) can stratify TFR durability – supporting the broader concept that circulating immune mediators carry prognostic information alongside cytotoxic capacity (Gong et al., 2025; Hughes et al., 2025). Parallel work in CML shows increased inflammatory cytokines at diagnosis (e.g., IL-18/VEGF-A) with modulation during therapy and links between immune restoration and depth of molecular response – findings that align with our cross-sectional table in which untreated CML displays high IL-2/IL-7 and low perforin levels, then shifts toward normalized cytokines and improved cytotoxic potential under TKIs and in TFR (Fiordi et al., 2023; Janowski et al., 2024b). Hematologically, the CML (active illness) cohort exhibited the anticipated myeloproliferative phenotype: WBC counts were markedly elevated – median 137 (110–200) × 10^3/μL versus 7.6 (6.5–9.1) × 10^3/μL in controls ($P < 0.001$) – accompanied by significant anemia (hemoglobin 10.8 [9.9–11.9] g/dL vs 13.0 [12.3–14.5] g/dL; $P < 0.001$) and thrombocytosis (platelets 385 [309–550] × 10^3/μL vs 270 [228–327] × 10^3/μL; $P = 0.009$). This mirrors recent real-world series and cross-sectional cohorts at presentation, where hyperleukocytosis, anemia, and variable thrombocytosis predominate; for example, a 2021–2022 Ethiopian tertiary-center study reported median WBC 217.7 × 10^3/μL with anemia in >90% of patients, while other 2025 regional datasets likewise document very high WBC with frequent anemia and substantial rates of thrombocytosis at diagnosis. Our results therefore corroborate the contemporary hematologic profile of chronic-phase CML prior to full cytoreduction on therapy (Urgessa et al., 2024). Within the treated subgroups (imatinib, nilotinib, bosutinib) and those in treatment-free remission (TFR), most chemical and hematological parameters approximated to controls, except for the bosutinib-specific ALT signal and the modest amylase shift noted above. This "near-normalization" outside of isolated laboratory toxicities aligns with current CML management paradigms in which deep molecular responses under TKIs translate to physiological recovery and permit, in selected patients, carefully monitored TFR without major laboratory perturbations; recent narrative and regional reports underscore that, during sustained remission or TFR, routine chemical/hematological parameters typically remain within reference limits apart from occasional low-grade, drug-related laboratory changes (Jumaa et al., 2022; Bourne et al., 2024).

The finding that IL-2 and IL-7 strongly separate new CML patients from controls aligns with reports that systemic cytokine milieus are perturbed in myeloid malignancies, including elevated IL-2/sIL-2R and broader inflammatory panels at diagnosis. Recent data from Iraq and elsewhere show higher IL-2 (and sIL-2R) across myeloid leukemias versus controls and prognostic associations of sIL-2R in hematologic cancers, supporting IL-2 pathway activation in illness states (Al-Khateeb et al., 2022; Abd-Alabass & Mohammed, 2024). IL-7 is a homeostatic lymphopoietic cytokine frequently dysregulated across cancers and inflammatory conditions; reviews in 2022–2024 detail IL-7's role in T-cell maintenance, tumor-immune interactions, and its elevation in diverse illness states – consistent with the very high AUC for IL-7 at diagnosis (Wang et al., 2022; Fu et al., 2024). In contrast, the inverse AUC for perforin at diagnosis coheres with the well-described functional exhaustion of cytotoxic effectors (CD56^dim NK and cytotoxic T cells) in CML: NK counts and cytolysis are diminished at presentation, and perforin/granzyme-mediated killing is impaired (Kaweme & Zhou, 2021; Chen et al., 2024). Studies profiling NK subsets in newly diagnosed CML patients have shown skewing away from cytokine-producing CD56^bright cells and reduced overall NK fitness, matching the observation of low/undetectable perforin in active illness (Faraj et al., 2023). The collapse of IL-2 and IL-7 AUCs in remission (AUCs ≈ 0.05 and 0.01) suggests these cytokines normalize or become non-specific once leukemic burden and systemic inflammation abate. This is expected, as remission under TKI reduces inflammatory tone and immune activation markers (Apperley et al., 2025). Conversely, the flip to near-perfect AUC for perforin in remission fits with literature showing restoration of NK cytotoxicity under effective TKI therapy and an association between robust NK function and molecular control. Reviews and translational studies indicate that in CML patients achieving deep molecular response, NK cytolytic activity rebounds; higher cytotoxic NK fractions and better effector function correlate with sustained control (Kaweme & Zhou, 2021). Furthermore, a study concentrating on T-cells of innate immune response in CML displays that expression of perforin is greater in non- controlled / relapsed states than in those who relapse, supporting the result of remission-phase ROC (Decroos et al., 2024b).

The TFR analysis –perforin ≥ 3.76 ng/mL delivering 100% sensitivity and 95% specificity – maps closely onto the broader TFR literature, in which pre-stop immune competence, especially NK cytotoxicity, predicts success. ASH and EHA-aligned reviews note that patients with higher CD3^–CD56^+CD16^+ NK cells at the time of TKI discontinuation have superior molecular relapse-free survival, and multiple summaries conclude that restored/heightened NK activity is a favorable determinant of TFR (Kaweme & Zhou, 2021; Hughes et al., 2025). Contemporary ELN (2025) guidance emphasizes that ~40–50% of carefully selected patients maintain TFR, with immune factors among the parameters under investigation for prediction – placing the perforin cut-off as a plausible, mechanistically grounded candidate needing external validation (Apperley et al., 2025). Real-world and trial syntheses from 2023–2025 similarly report durable TFR in ~40–60% of patients with appropriate monitoring, again supporting the clinical relevance of robust cytotoxic immunity at discontinuation (Han, 2023; Bourne et al., 2025). While the diagnostic performance of IL-2 and IL-7 at baseline is striking, cut-offs are cohort-specific and may be assay-dependent; future studies should recalibrate thresholds across platforms (e.g., Luminex vs. ELISA) and populations. The directionality observed – high IL-2/IL-7 in active illness, high perforin in remission/ TFR – is biologically consistent: IL-2/IL-7 reflect lymphocyte activation/homeostasis in the inflammatory tumor milieu, whereas perforin reports on the competence of cytotoxic effectors (NK/T cells) that restrain residual leukemia (Wang et al., 2022; Chen et al., 2024; Fu et al., 2024). Given the ELN's current stance that TFR candidacy is driven by molecular depth/duration with immune correlates under active study, validating perforin against standardized TFR outcomes (loss of MMR) in a prospective cohort would be the logical next step (Apperley et al., 2025). The remission-phase AUCs for IL-2/IL-7 approaching zero indicate potential complete overlap reversal; however, small sample sizes, batch effects, or floor/ceiling dynamics could inflate AUC extremes. Harmonizing sampling timepoints (pre-TKI, on-TKI DMR, and pre-stop), and integrating companion immune readouts (NK counts/subsets, granzyme B, degranulation assays) would strengthen interpretability and facilitate cross-study comparison to recent NK-focused CML research (Faraj et al., 2023; Chen et al., 2024).

Conclusions

This study establishes that elevated IL-2 and IL-7 with reduced perforin characterize active CML, whereas normalization of cytokines and recovery of perforin accompany molecular remission and successful TFR. ROC analyses confirm that IL-2 and IL-7 serve as diagnostic immune indicators, while perforin is a decisive marker for remission sustainability. Among frontline TKIs, bosutinib achieved superior EMR kinetics with minimal hepatic and pancreatic enzyme alterations. Integrating immune-biomarker signatures into baseline evaluation may enhance individualized therapy, optimize monitoring schedules, and improve precision-based decision-making in chronic myeloid leukemia management.

The authors declare that they have no conflicts of interest.

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