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ORIGINAL ARTICLE

Clonal evolution as detected by interphase fluorescence *in situ* hybridization is associated with worse overall survival in a population-based analysis of patients with chronic lymphocytic leukemia in British Columbia, Canada

Steven J. Huang [a,b], Krystal Bergin [a,1,2], Adam C. Smith [c,d,2,3], Alina S. Gerrie [a,e], Helene Bruyere [b], Chinmay B. Dalal [a], Daniele K. Sugioka [d], Monica Hrynchak [f], Khaled M. Ramadan [g], Aly Karsan [c], Tanya L. Gillan [b,4], Cynthia L. Toze [a,e,*]

[a] *Division of Hematology, Vancouver General Hospital, University of British Columbia, Vancouver, BC, Canada;* [b] *Pathology and Laboratory Medicine, Vancouver General Hospital, University of British Columbia, Vancouver, BC, Canada;* [c] *Cancer Genetics Laboratory, Pathology and Laboratory Medicine, British Columbia Cancer Agency, University of British Columbia, Vancouver, BC, Canada;* [d] *The Pelé Research Institute, Hospital Pequeno Prinicipe, Brazil;* [e] *Leukemia/BMT Program of BC, Vancouver General Hospital and British Columbia Cancer Agency, University of British Columbia, Vancouver, BC, Canada;* [f] *Cytogenetics Laboratory, Royal Columbian Hospital, New Westminster, BC, Canada;* [g] *Division of Hematology, St. Paul's Hospital, University of British Columbia, Vancouver, BC, Canada*

This study evaluates prognostic markers as predictors of clonal evolution (CE) and assesses the impact of CE on overall survival (OS) in a population-based cohort of 159 consecutive eligible patients with chronic lymphocytic leukemia (CLL) obtained from the British Columbia Provincial CLL Database. CE was detected by interphase fluorescence *in situ* hybridization (FISH) in 34/159 patients (21%) with 65% of CE patients acquiring deletion 17p or 11q. CD38 positive status (≥30%) on flow cytometry predicted 2.7 times increased risk of high-risk CE (acquisition of deletion 17p or 11q) on multivariate analysis. Prior CLL therapy was not a significant predictor of CE. CE was associated with 4.1 times greater risk of death when analyzed as a time-dependent variable for OS after adjusting for age, lymphocyte count, and FISH timing. High-risk CE was associated with worse OS while acquisition of low/intermediate-risk abnormalities (trisomy 12, deletion 13q, and IGH translocation) had no difference in OS. Our study demonstrates the negative impact of CE detected by FISH on OS in this population-based cohort. These data provide support for repeating FISH testing during CLL follow-up as patients with high-risk CE have reduced survival and may require closer observation.

Keywords Chronic lymphocytic leukemia, clonal evolution, fluorescence *in situ* hybridization, overall survival, population, prognosis

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* Corresponding author.
E-mail address: ctoze@bccancer.bc.ca

[1] Present address: Department of Haematology at The Alfred, Melbourne, Australia.

[2] ACS and KB contributed equally to the manuscript.

[3] Present address: Laboratory Medicine Program, University Health Network, University of Toronto, Toronto, Ontario, Canada.

[4] Present address: Medical Genetics Laboratory, Department of Laboratory Medicine, Eastern Health, St. John's, NL, Canada.

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Introduction

Chronic lymphocytic leukemia (CLL) is a heterogeneous disease with clinical course varying from indolent to aggressive with early death (1). The detection of four cytogenetic abnormalities at diagnosis by interphase fluorescence *in situ* hybridization (FISH) can predict a patient's prognosis as poor (deletion 17p or deletion 11q), intermediate (trisomy 12 or normal FISH) or favorable (deletion 13q) (2,3) giving additional prognostic information to the traditional Rai stage (4). Clonal evolution (CE), the acquisition of new cytogenetic abnormalities during the disease course, may occur. Previous studies evaluating CE in CLL by FISH have shown an association between CE and worse overall survival (OS), mostly in single-center studies and most had fewer than 100 patients (5–12). The largest such study included 202 patients but used FISH and mutation analysis (11). Clonal evolution in CLL has also been investigated by newer methods including microarray (13–16) and gene sequencing (11,17–21). Newer tests such as gene sequencing may not be commonly available in most centers. Predictive factors for risk of clonal evolution have been evaluated with mixed results obtained for ZAP70 expression, immunoglobulin heavy chain (IGHV) mutation status and baseline FISH abnormalities, and no significance seen for CD38 expression (9–11,22–24).

The British Columbia Provincial CLL Database (BCPCLL Database) contains clinical, laboratory and outcome data for all patients with CLL who have had FISH testing for recurrent cytogenetic abnormalities performed in British Columbia, Canada since inception of the test in 2004 (25). Protocols and policies for CLL patient management are set centrally by the British Columbia Cancer Agency and care is delivered in a similar and standard manner in both academic and community settings in a distributed fashion. This setting therefore provides an opportunity to study clonal evolution in CLL by FISH on a province-wide basis in a cohort of CLL patients that has received similar testing and clinical management protocols.

We report here a population-based study confirming the negative relationship between overall survival and CE and assessing various prognostic factors as predictors of CE. This is the first study to report on the association of CE studied by FISH and OS on a population level.

Patients and methods

Patients

The BCPCLL Database was queried for patients with CLL for whom sequential FISH data with a minimum of three months between follow-up FISH and initial FISH was available between March 2004 and December 2014. The study population was limited to patients diagnosed after March 2004 in order to eliminate the potential bias of selecting possibly better risk patients who survived to have a FISH test if patients diagnosed before FISH testing was implemented were included. Patients were diagnosed between 2004 and 2013; follow-up was available until November 2015. A diagnosis of CLL was made according to National Cancer Institute CLL Working Group 1996 criteria (26). Patients positive for translocation (11;14), which is typically diagnostic of mantle cell lymphoma, were excluded from the study. Patients were seen at the British Columbia Cancer Agency, Vancouver General Hospital, Royal Columbian Hospital, St. Paul's Hospital or by a community physician. Research ethics board approval was obtained from the University of British Columbia, Vancouver Coastal Health Research Institute, the Fraser Health Authority and British Columbia Cancer Agency.

Fluorescence *in situ* hybridization

FISH was performed on interphase nuclei from either peripheral blood cells or bone marrow samples as previously described with probes for the MYB, ATM, 12 centromere, 13q14.3, IGH and TP53 loci (25). Cutoff values for a positive FISH result was 11% (abnormal results > 10%) for all probes except for detection of biallelic deletion 13q and IGH translocation which used a 1% cutoff.

Routine FISH testing is generally performed for patients with CLL in British Columbia prior to starting treatment or when the lymphocyte count is above 20×10^9/L. Follow-up FISH testing is not routinely performed at defined intervals but is requested by the treating physician under the following conditions: (1) when there is a major change in the patient's CLL clinically and/or there is disease progression, (2) treatment is considered, (3) prior to allogeneic hematopoietic stem cell transplant (HSCT), or (4) post-transplant for monitoring disease status and clearance of FISH abnormality.

Statistics

The primary study end point was overall survival calculated as time from first FISH to time of death from any cause or last follow up (censoring). Survival was determined using the Kaplan–Meier method and Cox regression analysis. The log-rank test was used to compare covariates on Kaplan–Meier analysis. Clonal evolution was analyzed as a time-dependent covariate in order to minimize immortal-time bias (27). Patient characteristics were compared using the chi-square test or Fisher's exact test for categorical variables and the Mann–Whitney test for continuous variables. Baseline characteristics, common prognostic factors for CLL and treatment status (treated vs. untreated) were tested using logistic regression to determine predictors for development of high-risk clonal evolution including: sex, age at diagnosis, Rai stage at diagnosis, CD38 expression (defined as positive if ≥30%), lymphocyte count at diagnosis and baseline cytogenetic abnormalities, after adjusting for timing of FISH testing. Factors with a p-value < 0.15 on univariate analysis were entered into multivariate analysis. To address two limitations of the study: (1) FISH was performed long after diagnosis for some patients, so some patients may have experienced clonal evolution prior to the first FISH study, and (2) several patients had a first FISH study after treatment was initiated therefore some of the abnormalities may be treatment-related, a subgroup analysis was performed on patients who had initial FISH within 2 years of diagnosis and had no treatment greater than 2 months before initial FISH. All statistical tests were two-sided. A p-value of <0.05 was considered statistically significant. Relative risk (RR) was calculated with 95% confidence intervals (CI). Data were analyzed using Statistical Software Package for the Social