Leukemia Research 86 (2019) 106220

Contents lists available at ScienceDirect

Leukemia Research

journal homepage: www.elsevier.com/locate/leukres

Letter to the Editor

Buccal cell telomere length is not a useful marker for comorbidities in chronic lymphocytic leukemia

Chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL) are common biologically similar diseases, with a rising incidence as the population ages [1]. Most patients will require therapy at some point and while new therapies, such as ibrutinib and venetoclax, hold promise for most patients, chemoimmunotherapy remains the initial treatment of choice [2]. The type of chemoimmunotherapy depends on the patient's age, renal function, performance status and comorbidities, with the burden of comorbidities being commonly measured using the Cumulative Illness Rating Scale (CIRS) score [3–5]. As the median age at diagnosis in the clinic is 68 years, comorbidities are common, influencing tolerance to therapy and cause of death [6,7]. However, CIRS measurements are quite subjective and an objective measurement of comorbidites would be useful to ensure consistency. In the present study, we have determined whether telomere length (TL) in buccal cells (BCs) would be a useful and reproducible surrogate marker for comorbidities in CLL/SLL.

Telomeres are multiple nucleotide repeats of TTAGGG at the ends of chromosomes, maintaining DNA integrity and controlling cellular senescence [8]. TL in peripheral blood leukocytes or lymphocytes is shortened by oxidative stress from comorbidities, environmental factors and aging, with short telomeres predicting a reduced life span [8]. TL in CLL has been extensively evaluated in the leukemic cells, with CLL cell TL being shorter than autologous polymorphonuclear leukocytes, and shorter than normal B cells [9]. Moreover, telomere shortening is most prominent in *IGHV* unmutated cells and those with del 11q or del 17p; short TL correlates with short time to treatment, progression-free survival and overall survival [9–12]. In addition, TL in CLL cells shorten over time [13]. In contrast to normal cells, shortening of telomeres in CLL may reflect more rapid cell proliferation, abnormal DNA repair or dysfunctional shelterins [9,14].

While TL may vary between different normal tissues, the degree of shortening is similar with a good correlation between TLs in buccal cells (BCs), leukocytes and fibroblasts in patients with congenital disorders of telomeres [15]. Thus, in the present study we measured TL in BCs of CLL patients, to minimize contamination with leukemic cells. We studied 165 patients (143 CLL (131 Rai stages 0/I/II, 12 Rai stage III/IV) and 22 SLL) diagnosed between January 1, 2007 and December 31, 2011, who were referred to the CLL clinic at CancerCare Manitoba, University of Manitoba. One hundred and forty two patients had matching leukemic cell and BC samples while the remainder had either a leukemic cell (11) or BC sample (12) only. Eleven age-matched non-CLL controls were also evaluated. Median follow-up was 6.85 years (range, 0.05–9.98 years), median age at diagnosis was 65 years with male to female ratio of 1.8. Seventy eight percent had a CIRS score ≤ 6 and 22% a score > 6. Overall, 56% of patients required treatment at a median of 27.3 months (range, 0–109.7 months), and 24% died during the follow-up period.

For the measurement of TL, B cells were isolated from the peripheral blood of newly diagnosed CLL patients and healthy age- and sex-matched controls, as previously described [14]. BCs were obtained by scraping the buccal mucosa onto Whatman FTA micro cards and genomic DNA was isolated from 4 (3 mm) diameter single-hole punches. The QIAamp DNA investigator's kit (Qiagen) was used for DNA extraction, as per the manufacturer's instructions. TL measured by multiplex quantitative PCR (qPCR) and expressed as a ratio of telomere length against beta globulin gene [16].

As predicted, the median TL in CLL cells was considerably shorter in *IGHV* unmutated samples compared to mutated cells. Moreover, median TL was shorter in CLL cells (0.53) than in normal B cells (0.98; $p < 0.0001$) [9] and much shorter than in autologous BC (2.06; $p < 0.0001$). However, there was no correlation between TLs in BCs and leukemic cells, demonstrating that different factors were influencing telomere attrition in normal and malignant tissue (Wilcoxon two-sample tests and Pearson correlations; $r = 0.07184$, $p = 0.396$). Moreover, while BC telomeres shortened with patient age ($p = 0.0114$) this did not occur with the CLL cells ($p = 0.6599$) (Fig. 1**A**) [15].

The CIRS score correlated closely with age ($p < 0.0001$) and, as previously reported, also correlated inversely with survival [17]. Using a cut-off of > 6 to define fit and ≤ 6 for unfit [18], survival of patients with a high score was significantly poorer than those with a low score ($p = 0.0227$) and multivariate analysis demonstrated that this was independent of age ($p = 0.0082$). Surprisingly, despite the fact that both BC TL and CIRS correlated with age there was only a trend towards significance when comparing BC TL with CIRS score ($p = 0.6595$; multivariable logistic regression and proportional hazard ratio regression analysis) (Fig. 1B, $p = 0.0666$). In contrast to the CIRS score, BC TL did not correlate with survival.

In contrast, by multivariate survival analysis (with death as a censor), short CLL TL was associated with a shorter time to treatment ($p < 0.0001$) [19]. However, there was no correlation between CLL cell TL and time to treatment for those receiving therapy ($p = 0.4086$, $r = 0.09306$). Short CLL TL was also associated with reduced treatment free survival (death or treatment as endpoints) and overall survival (Fig. 2) [20]. However, BC TL was included in the multivariate analysis but did not significantly improve the predictive value over CLL TL alone. An interesting observation was that CLL TL decreased with increasing CIRS score (Fig. 1B, $p = 0.0223$), suggesting an effect of comorbidities on CLL cell biology.

In summary, this study demonstrates that BC TL measurements are not useful as surrogate marker for CIRS. In contrast to CLL TLs, BC telomeres do not correlate with patient survival or improve prognostic significance when combined with leukemic cell TL. However, we confirm the importance of CIRS score as a measure of overall survival, an effect that is independent of patient age.

https://doi.org/10.1016/j.leukres.2019.106220
Received 4 July 2019
Available online 28 August 2019

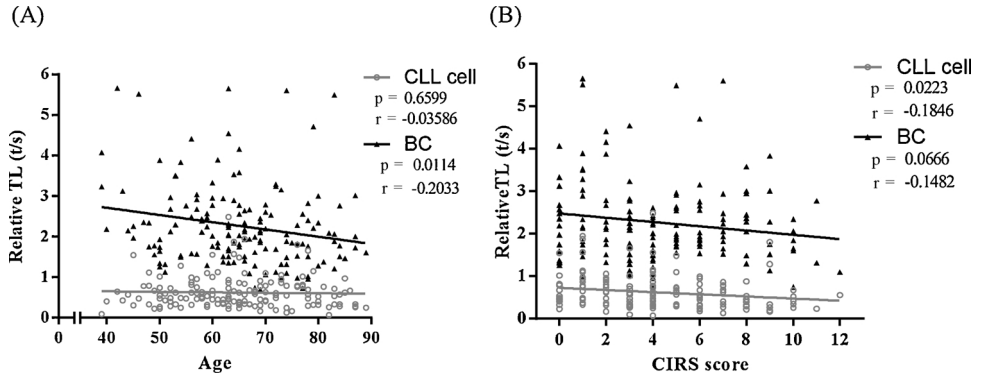

Fig. 1. Telomere shortening was only affected by increasing age in BC, not in CLL cells and shorter TLs in CLL cells, not BCs, were associated with increased CIRS score. (A) BC and CLL cell TLs from CLL patients as a function of age. (B) TLs in BCs and CLL cells in relationship to increasing CIRS scores.

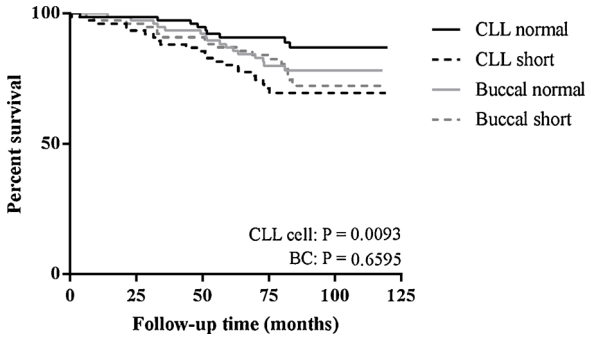

Fig. 2. Shorter TLs in CLL cells, not BCs, were associated with decreased patient survival. Kaplan Meier survival curve comparing short and normal TLs from CLL and BCs using median TL as cut-off. This showed the survival differences within the CLL TL group but not BC TL group.

Acknowledgement

This study was funded by the Research Manitoba, grant number 1291.

References

[1] M.D. Seftel, A.A. Demers, V. Banerji, S.B. Gibson, C. Morales, G. Musto, M.W. Pitz, J.B. Johnston, High incidence of chronic lymphocytic leukemia (CLL) diagnosed by immunophenotyping: a population-based Canadian cohort, Leuk. Res. 33 (2009) 1463–1468.

[2] M. Hallek, T.D. Shanafelt, B. Eichhorst, Chronic lymphocytic leukaemia, Lancet 391 (2018) 1524–1537, https://doi.org/10.1016/S0140-6736(18)30422-7.

[3] B. Eichhorst, A.-M. Fink, J. Bahll, R. Busch, G. Kovacs, C. Maurer, E. Lange, First-line chemoimmunotherapy with bendamustine and rituximab versus fludarabine, cyclophosphamide, and rituximab in patients with advanced chronic lymphocytic leukaemia (CLL10): an international, open-label, randomised, phase 3, non-inferiority trial, Lancet Oncol. 17 (2016) 928–942.

[4] F. Salvi, M.D. Miller, A. Grilli, R. Giorgi, A.L. Towers, V. Morichi, L. Spazzafumo, L. Mancinelli, E. Espinosa, A. Rappelli, P. Dessì-Fulgheri, A manual of guidelines to score the modified Cumulative Illness Rating Scale and its validation in acute hospitalized elderly patients, J. Am. Geriatr. Soc. 56 (2008) 1926–1931.

[5] V. Goede, K. Fischer, R. Busch, A. Engelke, B. Eichhorst, C.M. Wendtner, T. Chagorova, J. de la Serna, M.-S. Dilhuydy, T. Illmer, S. Opat, C.J. Owen, O. Samoylova, K.-A. Kreuzer, S. Stilgenbauer, H. Döhner, A.W. Langerak, M. Ritgen, M. Kneba, E. Asikanius, K. Humphrey, M. Wenger, M. Hallek, Obinutuzumab plus chlorambucil in patients with CLL and coexisting conditions, N. Engl. J. Med. 370 (2014) 1101–1110.

[6] S. Beiggi, V. Banerji, A. Deneka, J. Griffith, S.B. Gibson, J.B. Johnston, Comparison of outcome of patients with CLL who are referred or nonreferred to a specialized CLL clinic: a Canadian population-based study, Cancer Med. (2016).

[7] P. Strati, S.A. Parikh, K.G. Chaffee, N.E. Kay, T.G. Call, J. Sara, J.R. Cerhan, S.L. Slager, T.D. Shanafelt, Relationship between co-morbidities at diagnosis, survival and ultimate cause of death in patients with chronic lymphocytic leukaemia (CLL): a prospective cohort study, Br. J. Haematol. 178 (2019) 394–402.

[8] E.H. Blackburn, E.S. Epel, J. Lin, Human telomere biology: a contributory and interactive factor in aging, disease risks, and protection, Science 350 (2015) 1193–1198.

[9] R.N. Damle, F.M. Batliwalla, F. Ghiotto, A. Valetto, E. Albesiano, C. Sison, S.L. Allen, J. Kolitz, V.P. Vinciguerra, P. Kudalkar, T. Wasil, K.R. Rai, M. Ferrarini, P.K. Gregersen, N. Chiorazzi, Telomere length and telomerase activity delineate distinctive replicative features of the B-CLL subgroups defined by immunoglobulin V gene mutations, Blood 103 (2004) 375–382.

[10] T.T. Lin, K. Norris, N.H. Heppel, G. Pratt, J.M. Allan, D.J. Allsup, J. Bailey, L. Cawkwell, R. Hills, J.W. Grimstead, R.E. Jones, B. Britt-Compton, C. Fegan, D.M. Baird, C. Pepper, Telomere dysfunction accurately predicts clinical outcome in chronic lymphocytic leukaemia, even in patients with early stage disease, Br. J. Haematol. 167 (2014) 214–223.

[11] P. Hillmen, K. Norris, C.D. Fegan, D.M. Baird, C. Pepper, A. Rawstron, R. Hills, Telomere length predicts for outcome to FCR chemotherapy in CLL, Leukemia (2019).

[12] D. Steinbrecher, B.M.C. Jebaraj, C. Schneider, J. Edelmann, F. Cymbalista, V. Leblond, A. Delmer, S. Ibach, E. Tausch, A. Scheffold, J. Bloehdorn, M. Hallek, P. Dreger, H. Döhner, S. Stilgenbauer, Telomere length in poor-risk chronic lymphocytic leukemia: associations with disease characteristics and outcome, Leuk. Lymphoma 59 (2018) 1614–1623.

[13] P. Ghione, E. Genuardi, D. Rossi, D. Drandi, B. Mantoan, D. Barbero, E. Bernocco, L. Monitillo, M. Cerri, M. Ruggeri, P. Omede, C. Deambrogi, L. De Paoli, R. Passera, M. Coscia, F. Cavallo, M. Massaia, M. Boccadoro, G. Gaidano, M. Ladetto, S. Ferrero, Progressive telomere shortening is part of the natural history of chronic lymphocytic leukaemia and impacts clinical outcome: evidences from long term follow-up, Br. J. Haematol. 181 (2018) 693–695.

[14] G. Ishdorj, S.E.F. Kost, S. Beiggi, Y. Zang, S.B. Gibson, J.B. Johnston, A novel spliced variant of the TIN2 shelterin is present in chronic lymphocytic leukemia, Leuk. Res. 59 (2017) 66–74.

[15] S.M. Gadalla, R. Cawthon, N. Giri, B.P. Alter, S.A. Savage, Telomere length in blood, buccal cells, and fibroblasts from patients with inherited bone marrow failure syndromes, Aging (Albany NY) 2 (2010) 867–874.

[16] R.M. Cawthon, Telomere length measurement by a novel monochrome multiplex quantitative PCR method, Nucleic Acids Res. 37 (2009).

[17] G.M. Rigolin, M. Cavallari, F.M. Quaglia, L. Formigaro, E. Lista, A. Urso, E. Guardalben, C. Liberatore, D. Faraci, E. Saccenti, C. Bassi, L. Lupini, M.A. Bardi, E. Volta, E. Tammiso, A. Melandri, M. Negrini, F. Cavazzini, A. Cuneo, S. Ematologia, S. Mediche, A. Ospedaliero-universitaria, A.S. Anna, D. Morfologia, In CLL, comborbidities and the complex karyotype are associated with an inferior outcome independently of CLL-IPI, Blood 129 (2017) 3495–3499.

[18] B. Eichhorst, V. Goede, M. Hallek, Treatment of elderly patients with chronic lymphocytic leukemia, Leukemia Lymphoma. 50 (n.d.) 171–178.

[19] D. Rossi, A. Bruscaggin, V. Spina, S. Rasi, H. Khiabanian, M. Messina, M. Fangazio, T. Vaisitti, S. Monti, S. Chiaretti, A. Guarini, I. Del Giudice, M. Cerri, S. Cresta, C. Deambrogi, E. Gargiulo, V. Gattei, F. Forconi, F. Bertoni, S. Deaglio, R. Rabadan, L. Pasqualucci, R. Foà, R. Dalla-Favera, G. Gaidano, Mutations of the SF3B1 splicing factor in chronic lymphocytic leukemia: association with progression and fludarabine-refractoriness, Blood 118 (2011) 6904–6908.

[20] J.C. Strefford, L. Kadalayil, J. Forster, M.J.J. Rose-Zerilli, A. Parker, T.T. Lin, N. Heppel, K. Norris, A. Gardiner, Z. Davies, D. Gonzalez de Castro, M. Else, A.J. Steele, H. Parker, T. Stankovic, C. Pepper, C. Fegan, D. Baird, A. Collins, D. Catovsky, D.G. Oscier, Telomere length predicts progression and overall survival in chronic lymphocytic leukemia: data from the UK LRF CLL4 trial, Leukemia (2015) 1–3.

Lin Yang*
Section of Hematology/Medical Oncology, Department of Internal Medicine, University of Manitoba, Winnipeg, MB, Canada
E-mail address: lyang@cancercare.mb.ca.

Sara E.F. Kost, Sara Beiggi, Yunli Zhang, Robert Schmidt
Research Institute of Oncology and Hematology (formerly, Manitoba Institute of Cell Biology), University of Manitoba, Winnipeg, MB, Canada

Zoann Nugent
Department of Epidemiology and Cancer Registry, Cancer Care Manitoba, Winnipeg, MB, Canada

Aaron Marshall
Department of Immunology, University of Manitoba, Winnipeg, MB, Canada

Versha Banerji[a,b,c]
[a] *Section of Hematology/Medical Oncology, Department of Internal Medicine, University of Manitoba, Winnipeg, MB, Canada*
[b] *Research Institute of Oncology and Hematology, University of Manitoba, Winnipeg, MB, Canada*
[c] *Department of Biochemistry and Medical Genetics, University of Manitoba, Winnipeg, MB, Canada*

Spencer B. Gibson[a,b]
[a] *Research Institute of Oncology and Hematology, University of Manitoba, Winnipeg, MB, Canada*
[b] *Department of Biochemistry and Medical Genetics, University of Manitoba, Winnipeg, MB, Canada*

James B. Johnston[a,b]
[a] *Section of Hematology/Medical Oncology, Department of Internal Medicine, University of Manitoba, Winnipeg, MB, Canada*
[b] *Research Institute of Oncology and Hematology, University of Manitoba, Winnipeg, MB, Canada*

* Corresponding author at: CancerCare Manitoba, 2055a-675 McDermot Avenue, Winnipeg, MB, R3E 0V9, Canada.