Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

Concentration and integrity index of circulating cell-free DNA as a biomarker in pediatric patients with B-ALL

Jessica Fabiola Rodriguez-Ortiz[1,2], Anilú Margarita Saucedo-Sariñana[2,3], Mónica Alejandra Rosales-Reynoso[3], Janet Soto-Padilla[4], Rocío Ortíz-López[5], Ana Rebeca Jaloma-Cruz[1], César de Jesús Tovar-Jácome[2,3], Patricio Barros-Núñez[2*]

[1] División de Genética, Centro de Investigación Biomédica de Occidente, CMNO, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, México.
[2] Doctorado en Genética Humana, Centro Universitario de Ciencias de la Salud. Universidad de Guadalajara, Jalisco, México.
[3] División de Medicina Molecular, Centro de Investigación Biomédica de Occidente, CMNO, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, México.
[4] Servicio de Hematología. UMAE de Pediatría, CMNO, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, México.
[5] Escuela de Medicina y Ciencias de la Salud. Instituto Tecnológico de Monterrey. Monterrey, Nuevo León, México.

Article Info

Article history:

Received: April 04, 2025
Accepted: June 17, 2025
Published: August 31, 2025

Use your device to scan and read the article online

Abstract

The objective of this study was to evaluate the concentration and integrity index of circulating cell-free DNA (ccf-DNA) as biomarkers for the detection and monitoring of minimal residual disease (MRD) in pediatric patients with B-cell acute lymphoblastic leukemia (B-ALL). Comparison with a validated methodology for the quantification of monoclonal rearrangements of the IGH gene was made. Peripheral blood and bone marrow samples were collected from 10 pediatric patients with B-ALL at diagnosis, remission, and maintenance phases. Total ccf-DNA concentration was estimated using Qubit® fluorometry, and the integrity index was obtained through qPCR amplification of ALU247/ALU115 fragments. Monoclonal rearrangements of the IGH gene were quantified by multiplex PCR and detected by capillary electrophoresis. Results showed that at diagnosis, the mean ccf-DNA concentration was 5,607 ng/mL with an integrity index of 0.38; during remission induction, they were 968 ng/mL and 0.35; and during the maintenance phase, 748 ng/mL and 0.33, respectively. Differences between treatment phases were significant (p=0.02). The reference group had a mean ccf-DNA concentration of 247 ng/mL and an integrity index of 0.20, showing significant differences compared to the patient group (p=0.01). Monoclonality analysis showed significant differences between diagnosis and remission (p=0.022) and between diagnosis and maintenance (p=0.001). Linear regression analysis during treatment demonstrated a similar trend for ccf-DNA concentration and monoclonality. In conclusion, ccf-DNA concentration and integrity index could be useful biomarkers for monitoring MRD in patients with B-ALL, showing comparable efficacy to the detection of monoclonality in the IGH gene.

Keywords: Leukemia, Cell-free DNA, Minimal residual disease, B-ALL monitoring, IGH gene.

1. Introduction

B-cell acute lymphoblastic leukemia (B-ALL) is the most frequent malignant neoplasm in children. Its incidence in Latin America and Caribbean is 191 patients per million inhabitants per year in 2022; in Mexico, this incidence is 150 new cases each year in the last 18 years [1–4].

Despite the good initial response to chemotherapeutic treatment, a high percentage of patients tend to relapse into the disease; therefore, it is necessary to monitor the MRD (presence of 1 blast cell in one million of normal blood cells) to determine response to therapy [5]. Monitoring requires repeated biopsies, which are considered invasive, painful, and impractical methods; therefore, new alternatives and minimally invasive procedures have recently been proposed for the identification of biomarkers that can provide the necessary and adequate information to diagnose and monitor MRD in patients with leukemia [6, 7].

Among the recently recommended technologies, the detection of circulating cell-free DNA (ccf-DNA), also known as "liquid biopsy", provides a non-invasive and apparently more reliable molecular diagnosis than conventional biopsy. In this latter test, only part of the tumor is analyzed, whereas with ccf-DNA detection it is possible to obtain a cellular sample from both the primary tumor and metastases [8].

Liquid biopsy has several advantages: it is an easier, faster, and less invasive sampling that can be performed repeatedly to achieve follow-up and monitoring of MRD during and after treatment, as well as to predict the oc-

* Corresponding author.
E-mail address: pbarros_gdl@yahoo.com.mx (P. Barros-Núñez).
Doi: http://dx.doi.org/10.14715/cmb/2025.71.8.6

currence of acquired resistance to therapeutic agents [8]. The detection of ccf-DNA appears to be an accurate, sensitive, and non-invasive test for the recognition of malignant cells, but its efficacy in detecting different types of leukemia has yet to be verified and validated [9–11].

Furthermore, the development of emerging technologies such as digital PCR, nanopore sequencing, microfluids, and advanced sequencing techniques has significantly enhanced the detection and analysis of ccf-DNA in patients with B-ALL. These innovative methodologies have increased the sensitivity and specificity of ccf-DNA liquid biopsies, enabling more precise and minimally invasive MRD monitoring. Such progression can lead to earlier diagnoses and more effective treatments [9, 10, 12, 13]. This work aims to provide further insight into the use of ccf-DNA as a biomarker for the diagnosis and follow-up of MRD in patients with B-ALL.

2. Materials and methods

Samples from 10 patients with B-ALL were included (3 males and 7 females between 2 and 10 years of age), diagnosed by international criteria and by those established at the Pediatric Hospital of the Centro Médico Nacional de Occidente (CMNO) in Guadalajara, Mexico. Three peripheral blood and bone marrow samples were obtained from each patient: 1. at the time of diagnosis (before any treatment); 2. at the conclusion of the remission induction phase (1 month); and 3. during the maintenance phase (12th month). In all cases, blood and bone marrow samples were aliquots of the samples obtained by the Hematology Service for routine analysis of these patients. Clinical and laboratory information was collected from the medical records. The reference group included 10 healthy individuals of indistinct sex and ages ranging from 0 to 15 years, in whom the ccf-DNA concentration and the integrity index were established. The study was approved by the Ethics and Research Committees of the Mexican Institute of Social Security (R-2018-785-001). All participants parents signed informed consent, and an epidemiological questionnaire allowed us to collect sociodemographic data.

2.1 Sample collection and ccf-DNA isolation

5 mL of peripheral blood was collected in tubes with EDTA-K2 and processed within the first hour. Plasma separation was achieved by double centrifugation (1900 x g for 15 min and 1500 x g for 10 min) and stored at -80 °C until use. Isolation of ccf-DNA was achieved from 1 mL of plasma by the QIAamp circulating nucleic acid kit (QIAGEN Science, Inc., USA), according to the manufacturer's instructions.

About the concentration and integrity index, the total ccf-DNA concentration was estimated by Qubit® fluorometry using the dsDNA HS Assay Kit (Invitrogen, Carlsbad, CA) as stated in the manufacturer's instructions. The integrity index was obtained by q-PCR amplification of the ALU247/ALU115 fragments using the primers described by Umetani *et al*. [14] and the FastStart SYBR Green Master Kit (Roche, Mannheim, Germany), with precycling heat activation of DNA polymerase at 95 °C for 10 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 65 °C for 30 s, and extension at 72 °C for 30 s. The absolute equivalent amount of ccf-DNA in each sample was determined by a standard curve with serial dilutions of 1 ng/uL to 1 pg/uL (1000 ng/mL - 1 ng/mL) of genomic DNA.

In order to compare the values of both ccf-DNA concentration and integrity index with the results of a "gold standard" methodology, monoclonal rearrangements in the immunoglobulin heavy chain gene (VDJH-DJH) were quantified by multiplex PCR in bone marrow samples, following the protocol established by the BIOMED-2 Concerted Action BMH4-CT98-3936 and EuroClonality [15]. Subsequently, detection of the amplified fragments was performed by capillary electrophoresis on an Applied Biosystems™ ABI Prism 310 equipment and then analyzed using Peak Scanner© V 1.0 software (Thermo Fisher Scientific).

2.2. Statistical analysis

A Mann-Whitney U test was used to compare DNA concentration and integrity index between patient and control groups. For longitudinal analysis of concentration, integrity index, and monoclonal cells, the Friedman test was used. When the p-value was significant, the Bonferroni post hoc test was used for pairwise comparisons. ROC curves were performed to determine the diagnostic potential of ccf-DNA concentration or integrity index. All statistical analyses were performed in SPSS 25 and graphs in Graphpad Prism 8.0.1.

3. Results

The ccf-DNA concentration was determined by Qubit fluorometry in 30 blood samples obtained from 10 B-ALL patients with a mean age of 5 years, who were followed up during their treatment: at the time of diagnosis (sample 1); at the end of induction treatment (sample 2); and at the 12th month during maintenance treatment (sample 3). As a reference group, 10 healthy individuals with a mean age of 8 years were included. The results are shown in Table 1 and Figure 1.

The identification of ccf-DNA fragments released by apoptosis or necrosis was carried out by amplification of ALU 115 and 247 sequences by q-PCR. The amplicons obtained by ALU 115 identify total ccf-DNA (released by both cell death processes), whereas ALU 247 amplicons only represent the long fragments of ccf-DNA released by necrosis. The proportion of these fragments determines the

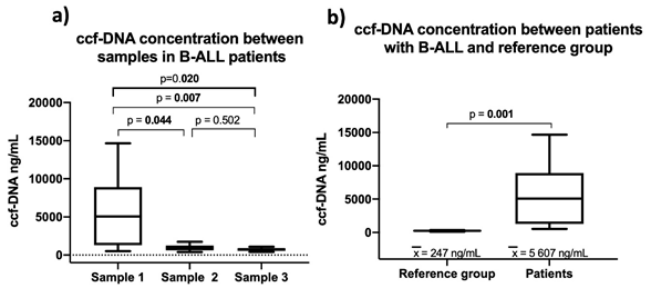

Fig. 1. Concentration of ccf-DNA in patients and reference group. **a:** Statistically significant differences (p=0.020) were observed between the mean concentrations of ccf-DNA samples from patients in the treatment phases (samples 1-3). Pairwise comparisons only showed significant differences between sample 1 vs. sample 2 (p=0.044), and sample 3 (p=0.022), but there was no statistical difference between samples 2 and 3 (p=0.50). **b:** ccf-DNA concentration between B-ALL patients at the diagnosis time and the reference group showed a significant difference (p=0.001).

Table 1. Concentration of ccf-DNA (ng/mL) and cellular monoclonality (FRU) in the reference group and B-ALL patients by stages of treatment.

Reference Group		Patients Group ccf-DNA in ng/mL / Monoclonality in RFU			
Individuals	ccf-DNA ng/mL		Diagnosis ng/mL / RFU	Induction ng/mL / RFU	Maintenance ng/mL / RFU
C1	304	P1	572 / 8,128	1,132 / 287	688 / 61
C2	208	P2	520 / 9,323	1,416 / 5,841	406 / 383
C3	194	P3	3,500 / 7,494	938 / 138	*13,460 / 6,750
C4	354	P4	8,900 / 7,876	1,190 / 1,057	790 / 69
C5	268	P5	1,540 / 7,134	617 / 195	750 / 75
C6	246	P6	2,620 / 6,714	596 / 100	1,090 / 111
C7	176	P7	8,200 / 7,823	708 / 111	754 / 64
C8	340	P8	6,620 / 7,292	398 / 3,805	976 / 314
C9	108	P9	8,940 / 7,868	1,738 / 1,471	870 / 137
C10	273	P10	14,660 / 7,410	956 / 4,265	408 / 164
Mean concentration	247		5,607 / 7,706	968 / 1,727	748 / 153

*Patient 3, in the maintenance therapy phase, presented a blast crisis coinciding with the sampling. This value was not included when calculating the mean ccf-DNA in the maintenance phase

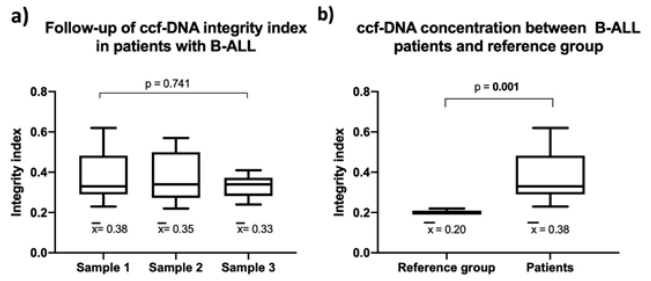

Fig. 2. ccf-DNA integrity index. **A:** No significant differences were found between the three samples obtained from patients at diagnosis and during their treatment. **B:** The ccf-DNA integrity index in the reference group versus B-ALL patients at diagnosis was statistically significant (p=0.0001).

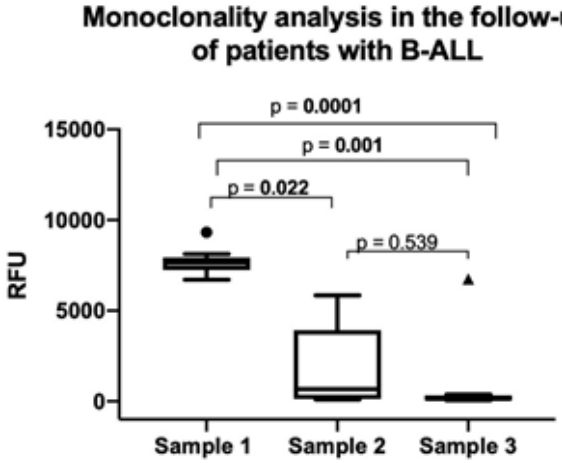

Fig. 3. Detection of monoclonal rearrangements during the treatment of the B-ALL patients.

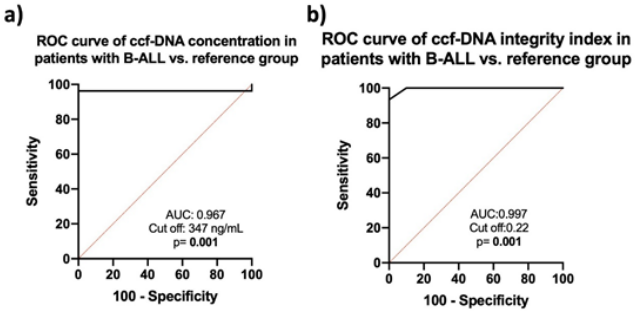

Fig. 4. ROC analysis. **A**: ccf-DNA concentration and B: ccf-DNA integrity index, calculated from the ALU 247/115 ratio. **B:** ccf-DNA integrity index, calculated from the ALU 247/115 ratio.

DNA integrity index in B-ALL patients at each treatment phase (Figure 2A) and versus the reference group (Figure 2B).

The monoclonal rearrangements present in the immunoglobulin heavy chains (IGH gene) were amplified by q-PCR using 27 consensus primers in five multiplex reactions, following the protocol of the BIOMED 2 group, for the diagnosis and follow-up of the 10 patients studied. Quantification of monoclonal rearrangements was established by Relative Fluorescence Units (RFU). Figure 3 shows the statistically significant differences (p=0.001) found in the number of monoclonal rearrangements of the IGH gene during the three treatment phases of the patients studied. Paired analysis by treatment phases shows significant differences between samples 1 and 2 (p=0.022) and between samples 1 and 3 (p=0.001). No differences were found between samples 2 and 3.

The ccf-DNA concentration showed a sensitivity and specificity of 100% with a cut-off from 376 ng/mL, while the integrity index has a sensitivity of 93.3% and specificity of 100% with a cut-off from 0.22 and an area under the curve of 0.997 (Figure 4).

Regression analysis of the DNA samples during the treatment of patients with B-ALL (Figure 5) showed a tendency to decrease the values of both the ccf-DNA concentration and the cell monoclonality. In the concentration of ccf-DNA, the linear regression model explains the 58% of the variance of the data, and the equation of the model is: Concentration=-2469.75 * (sample) + 7354.11 (Figure 5a); on the other hand, in the monoclonality analysis, the linear regression model explains the 79% of the variance of the data, and the equation of the model is: Monoclonal-

ity= -3441.28 * (sample) + 10059.24 (Figure 5b).

In the follow-up of patients with B-ALL, we found a tendency to a decrease in ccf-DNA concentration and monoclonality values. In ccf-DNA concentration, the linear regression model explains 58% of the variance of the data, and the model equation is: Concentration=-2469.75 * (sample) + 7354.11 (Figure 5a). On the other hand, in the monoclonal analysis, the linear regression model explains 79% of the variance of the data, and the model equation is: Monoclonality= -3441.28 * (sample) + 10059.24.

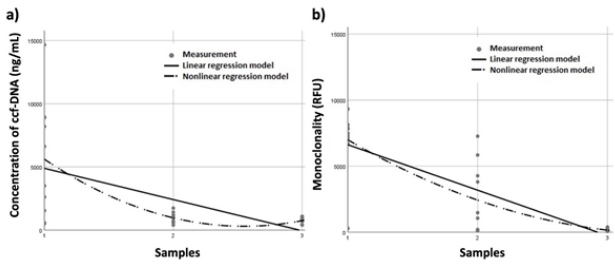

Fig. 5. Analysis of regression models. **A:** ccf-DNA concentration and **B:** monoclonality.

4. Discussion

Studies using ccf-DNA as a biomarker for diagnosis, prognosis, and follow-up of MRD in different types of cancer have multiplied in recent years; however, few studies have focused on the study of ccf-DNA in hematologic malignancies [9–11]. Moreover, the restricted studies carried out for this purpose do not compare the results of ccf-DNA with other procedures used in the diagnosis and follow-up of these patients. This work aims to provide more information on the use of ccf-DNA as a biomarker for the diagnosis and follow-up of MRD in patients with B-ALL in our population.

According to most reports, the concentration of ccf-DNA in patients undergoing a neoplastic process is higher than in other conditions. This is explained by the greater cell destruction within the tumor and the release of longer fragments (up to more than 10,000 bp) through the necrosis process [16–18]. In contrast, in healthy individuals, whose main source of ccf-DNA is apoptosis, smaller amounts of ccf-DNA (0-100 ng/mL) with shorter fragments (150-200 bp) are released [14, 19].

The results obtained here show that the concentration of ccf-DNA was significantly higher in patients with B-ALL (at the time of diagnosis) than in the reference group (Figure 1b); such a situation has already been reported in different studies and is explained by the fact that in neoplastic processes there is a higher release of non-apoptotic ccf-DNA [9, 14, 20–22]. Moreover, the concentration of ccf-DNA in these patients at the time of diagnosis was significantly higher compared to the ccf-DNA found during the treatment phases in samples 2 and 3 (p=0.020) (Figure 1a).

The concentration of ccf-DNA in all patients during their treatment behaves as expected, showing a progressive decrease in its concentrations throughout the treatment phases and a final concentration (sample 3) closer to that obtained in the reference group. Very interesting is the result observed in patient 3, in his third sample (maintenance therapy); such a result implies a very high sensitivity of the ccf-DNA quantification test, detecting the moment when this patient started a relapse. This result was subsequently confirmed by flow cytometry, monoclonal amplification, and clinical-biochemical parameters.

Analysis to determine the integrity index in patients and the reference group also showed a significant difference between the two groups, similar to the results found with ccf-DNA concentration. These results agree with the work published by Gao *et al*., in patients with acute myeloblastic leukemia (AML) that showed a mean integrity index of 0.51 versus 0.18 in controls [22]; however, we did not observe such a difference in patients between their three sampling times. It is unclear why the integrity index does not behave similarly to ccf-DNA concentration; that is, in these patients the proportion of non-apoptotic ccf-DNA does not decrease as treatment progresses. Perhaps ccf-DNA production caused by necrosis and apoptosis does not occur in ALL patients as it does in other diseases. To date, only Gao *et al*. have analyzed the integrity index in patients with leukemia [22].

Currently, only 4 studies have analyzed the use of ccf-DNA as a biomarker for the diagnosis and/or follow-up of leukemias. Schwarz *et al*. studied 21 ALL patients from a German population sample, reporting a mean concentration of 277 ng/mL at diagnosis, 248 ng/mL at day 3, and 62 ng/mL at day 4 of treatment, which was statistically like the 57 ng/mL observed in controls [20]. In 2010, Gao *et al.* analyzed the concentration and integrity index of cfDNA fragments in AML patients from a Chinese population sample, reporting a mean concentration of 8.8 ng/mL versus 3.42 ng/mL found in the control group [22]. In 2012, Jiang *et al*. analyzed the concentration of ccf-DNA fragments in AML patients in a Chinese population sample, reporting a mean concentration of 168 ng/mL that was significantly higher than those in three control groups [23]. Finally, Sonnenberg *et al*. analyzed 15 US patients with chronic lymphocytic leukemia (CLL) and obtained a mean concentration of ccf-DNA of 502 ng/mL compared to 50.3 ng/mL in healthy individuals [24].

Significant differences in the amount of ccf-DNA are observed in various populations. It is unknown which factors cause these variations; however, a likely explanation could be the methodology used in each study. Thus, in the studies conducted in the Chinese and German populations, the *ACTB* gene was amplified by q-PCR to determine the concentration of ccf-DNA. The differences between these two studies could also be influenced by the processing time; in this regard, Schwarz *et al*. suggest that no differences in ccf-DNA concentration are found when plasma separation is performed within the first 24 hours after blood collection [20]. On the contrary, Markus *et al.* established that after 4 hours after blood collection, the process of cell lysis releases a greater amount of ccf-DNA [25].

Papart-li *et al*., and Dessel *et al*., suggest that the observed variations in time are predominantly attributable to the type of collection tubes employed. Specifically, EDTA tubes exhibit an increase in ccf-DNA concentration after a three-hour post-sample collection, which is attributed to the lysis process, while Cell-Free DNA Blood Collection Tubes (BCT) demonstrate no significant cfDNA variation in concentration up to 96 hours post-sample collection [26, 27].

On the other hand, the study conducted in the US population used electrokinetic technology (dielectrophoresis) which is able to isolate ccf-DNA directly from the blood

sample, i.e., through a completely different process for obtaining ccf-DNA. The large methodological variability due to the lack of a standardized sample handling and processing technique would explain these differences among the various studies [28].

In addition, we evaluated the discriminatory power of ccf-DNA concentration and integrity index using a ROC curve (Figure 4), revealing that these two parameters can categorically distinguish healthy individuals from leukemia patients at diagnosis (p=0.001). The ccf-DNA concentration has a sensitivity and specificity of 100%, while the integrity index has a sensitivity of 93.3% and a specificity of 100%, coinciding with results observed in previously reported patients [20, 22, 24, 29].

Despite the wide variability of results in different populations (including the Mexican population of this study), a common pattern of very high levels of ccf-DNA in patients with B-ALL at their diagnosis and relapse is observed. Conversely, lower levels of ccf-DNA after the treatment and very low levels in reference groups confirm the validity of ccf-DNA as a biomarker for monitoring MRD in patients with B-ALL.

It is evident that q-PCR and flow cytometry are two reliable and reproducible methodologies capable of accurately defining MRD in samples from patients with ALL. Both approaches present high sensitivity, capable of detecting one malignant cell per 10,000 to 100,000 normal blood cells [12, 30]. Regarding the monoclonality results performed by q-PCR amplification in the present study, we found statistically significant differences (p=0.001) when IGH gene monoclonality was investigated in B-ALL patients at diagnosis and throughout the treatment phases (Figure 3). In pairwise analysis, significant differences were observed between samples 1 and 2 (p=0.022) and between samples 1 and 3 (p=0.001).

Regression analysis of DNA samples from B-ALL patients (Figure 5) showed a decreasing trend of both ccf-DNA concentration and cell monoclonality values during treatment. The linear regression model explained 58% of the variance of the data for ccf-DNA concentration and 79% of the variance for B-cell monoclonality. Notably, the number of monoclonal cells as determined by RFUs and obtained from bone marrow samples at diagnosis and during treatment is comparable, maintaining a similar trend to the concentration of ccf-DNA in peripheral blood samples. Thus, the quantification of monoclonal cells and ccf-DNA concentration are significantly increased at diagnosis in these patients, while they decrease significantly in samples 2 and 3. These results are expected when patients show a good response to treatment [9, 13, 21]. In congruence, statistical analysis found significant differences for both variables (ccf-DNA and monoclonal cells) in samples 1 vs. 2 and 3. Interestingly, in the third sample (maintenance treatment) of patient 3, a massive increase in both ccf-DNA and monoclonal cells was observed, showing higher values than in the first sample (at diagnosis). This result was caused by a blast crisis, an event that started after the third sample was obtained from this patient.

The ccf-DNA concentration and its integrity index could be useful biomarkers for the diagnosis and follow-up of patients with B-ALL. Further studies in different population groups should be performed to confirm these findings and to be able to establish ccf-DNA quantification as a safe liquid biopsy procedure to monitor minimal residual disease in these patients. The observation made in patient #3 during his maintenance phase exemplifies the sensitivity of ccf-DNA, detecting the precise moment when this patient initiated a relapse.

Conflict of interests

The authors stated that there are no conflicts of interest regarding the publication of this manuscript.

Consent for publications

The authors read and approved the final manuscript for publication.

Ethics approval and consent to participate

The study was approved by the Ethics and Research Committees of the Mexican Institute of Social Security (R-2018-785-001). All participants parents signed informed consent, and an epidemiological questionnaire allowed us to collect sociodemographic data.

Informed consent

All participants parents signed informed consent, and an epidemiological questionnaire allowed us to collect sociodemographic data.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

Patricio Barros-Núñez: conceptualization, supervision, funding acquisition, project administration supervision, original draft preparation, review and editing; Mónica Alejandra Rosales-Reynoso: conceptualization, data curation, original draft preparation, review and editing; Jessica Fabiola Rodriguez-Ortiz: Data curation, formal analysis, writing and editing, original draft preparation; Anilú Margarita Saucedo-Sariñana; data curation, formal analysis, review and editing; César de Jesús Tovar-Jácome; Data curation, review and editing; Janet Soto-Padilla: Investigation and methodology; Rocío Ortíz-López; Research and methodology; Ana Rebeca Jaloma-Cruz: original draft preparation, review and editing.

Funding

This study was supported by Grants from the "Fondo de Investigación en Salud" of the "Instituto Mexicano del Seguro Social" (FIS/IMSS/PROT/PRIO/18/072).

References

1. Smith AJ, Jiménez-Morales S, Mejía-Aranguré JM (2023) The genetic risk of acute lymphoblastic leukemia and its implications for children of Latin American origin. Front Oncol 13:. https://doi.org/10.3389/fonc.2023.1299355
2. Luna RR, Vargas AO, Velazco-Hidalgo L, et al (2024) El abrumador problema del cáncer infantil en México. Acta Pediatr Mex 45.https://doi.org/10.18233/apm.v45i5.2964
3. Paula Silva N, Colombet M, Moreno F, et al (2024) Incidence of childhood cancer in Latin America and the Caribbean: coverage, patterns, and time trends. Pan Am J Public Health 48:. https://doi.org/10.26633/RPSP.2024.11
4. Bray F, Laversanne M, Sung H, et al (2024) Global cancer sta-

tistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 74:229–263. https://doi.org/10.3322/caac.21834

5. Heuser M, Freeman SD, Ossenkoppele GJ, et al (2021) 2021 Update on MRD in acute myeloid leukemia: a consensus document from the European LeukemiaNet MRD Working Party. Blood 138:2753–2767. https://doi.org/10.1182/blood.2021013626
6. Colmenares R, Álvarez N, Barrio S, et al (2022) The Minimal Residual Disease Using Liquid Biopsies in Hematological Malignancies. Cancers (Basel) 14:. https://doi.org/10.3390/cancers14051310
7. Allam S, Nasr K, Khalid F, et al (2023) Liquid biopsies and minimal residual disease in myeloid malignancies. Front Oncol 13:. https://doi.org/10.3389/fonc.2023.1164017
8. Adhit KK, Wanjari A, Menon S, K S (2023) Liquid Biopsy: An Evolving Paradigm for Non-invasive Disease Diagnosis and Monitoring in Medicine. Cureus. https://doi.org/10.7759/cureus.50176
9. Lei S, Jia S, Takalkar S, et al (2023) Cancer Genomic Profiling and Minimal Residual Disease Monitoring By Cell-Free DNA Sequencing in Pediatric Leukemia. Blood 142:1604–1604. https://doi.org/10.1182/blood-2023-188158
10. Sorbini M, Carradori T, Togliatto GM, et al (2024) Technical Advances in Circulating Cell-Free DNA Detection and Analysis for Personalized Medicine in Patients' Care. Biomolecules 14:. https://doi.org/10.3390/biom14040498
11. Lim J, Kuss BJ, Talaulikar D (2021) Role of cell-free DNA in haematological malignancies. Pathology. https://doi.org/10.1016/j.pathol.2021.01.004
12. Bernardi S, Malagola M, Farina M, et al (2023) Digital PCR as a New Method for Minimal Residual Disease Monitoring and Treatment Free Remission Management in Chronic Myeloid Leukemia Patients: Is It Reliable? Hemato 4:1–11. https://doi.org/10.3390/hemato4010001
13. Sampathi S, Chernyavskaya Y, Haney MG, et al (2022) Nanopore sequencing of clonal IGH rearrangements in cell-free DNA as a biomarker for acute lymphoblastic leukemia. Front Oncol 12:. https://doi.org/10.3389/fonc.2022.958673
14. Umetani N, Kim J, Hiramatsu S, et al (2006) Increased Integrity of Free Circulating DNA in Sera of Patients with Colorectal or Periampullary Cancer: Direct Quantitative PCR for ALU Repeats. Clin Chem 52:1062–1069. https://doi.org/10.1373/clinchem.2006.068577
15. Langerak AW, Groenen PJTA, Brüggemann M, et al (2012) EuroClonality/BIOMED-2 guidelines for interpretation and reporting of Ig/TCR clonality testing in suspected lymphoproliferations. Leukemia 26:2159–2171. https://doi.org/10.1038/leu.2012.246
16. Kim J, Bae H, Ahn S, et al (2021) Cell-Free DNA as a Diagnostic and Prognostic Biomarker in Dogs With Tumors. Front Vet Sci 8:. https://doi.org/10.3389/fvets.2021.735682
17. Mettler E, Fottner C, Bakhshandeh N, et al (2022) Quantitative Analysis of Plasma Cell-Free DNA and Its DNA Integrity and Hypomethylation Status as Biomarkers for Tumor Burden and Disease Progression in Patients with Metastatic Neuroendocrine Neoplasias. Cancers (Basel) 14:. https://doi.org/10.3390/cancers14041025
18. Kowal-Wisniewska E, Jaskiewicz K, Bartochowska A, et al (2024) Towards effectiveness of cell free DNA based liquid biopsy in head and neck squamous cell carcinoma. Sci Rep 14:. https://doi.org/10.1038/s41598-024-52031-5
19. Bustamante P, Tsering T, Burnier JV (2022) Abstract 1694: Cytotoxic conditions alter cell free DNA concentration and fragment size in cancer cells. Cancer Res. https://doi.org/10.1158/1538-7445.am2022-1694
20. Schwarz AK, Stanulla M, Cario G, et al (2009) Quantification of free total plasma DNA and minimal residual disease detection in the plasma of children with acute lymphoblastic leukemia. Ann Hematol 88:897–905. https://doi.org/10.1007/s00277-009-0698-6ï
21. George NG, Rishi B, Singh A, et al (2023) Early prognosis prediction in acute myeloid and acute lymphoid leukemia patients using cell-free DNA concentration ratios. Front Mol Biosci 10:. https://doi.org/10.3389/fmolb.2023.1333943
22. Gao Y, He Y, Yang Z, et al (2010) Increased integrity of circulating cell-free DNA in plasma of patients with acute leukemia. Clin Chem Lab Med. https://doi.org/10.1515/CCLM.2010.311
23. Jiang Y, Pan S, Xia W, et al (2012) [Dynamic monitoring of plasma circulating DNA in patients with acute myeloid leukemia and its clinical significance]. J Exp Hematol 20 1:53–6. https://doi.org/10.1200/PO.22.00694
24. Sonnenberg A, Marciniak JY, Rassenti L, et al (2014) Rapid electrokinetic isolation of cancer-related circulating cell-free DNA directly from blood. Clin Chem 60:500–509. https://doi.org/10.1373/clinchem.2013.214874
25. Markus H, Contente-Cuomo T, Farooq M, et al (2018) Evaluation of pre-analytical factors affecting plasma DNA analysis. Sci Rep 8:. https://doi.org/10.1038/s41598-018-25810-0
26. Parpart-Li S, Bartlett B, Popoli M, et al (2017) The effect of preservative and temperature on the analysis of circulating tumor DNA. Clin Cancer Res 23:2471–2477. https://doi.org/10.1158/1078-0432.CCR-16-1691
27. Van Dessel LF, Beije N, Helmijr JCA, et al (2017) Application of circulating tumor DNA in prospective clinical oncology trials-standardization of preanalytical conditions. Mol Oncol 11:295–304. https://doi.org/10.1002/1878-0261.12037
28. Abdollahi A, Shokouhmand H (2022) Electrokinetic separation of cfDNA in insulator-based dielectrophoresis systems: a linear model of cfDNA and investigation of effective parameters. J Phys Condens Matter 34:. https://doi.org/10.1088/1361-648X/ac6476
29. Bonstingl L, Skofler C, Ulz C, et al (2024) Clinical Application of ISO and CEN/TS Standards for Liquid Biopsies - Information Everybody Wants but Nobody Wants to Pay For. Clin Chem 70:1140–1150. https://doi.org/10.1093/clinchem/hvae079
30. Zaher ER, Anwar MM, Kohail HMA, et al (2013) Cell-free DNA concentration and integrity as a screening tool for cancer. Indian J Cancer 50 3:175–83. https://doi.org/10.4103/0019-509X.118721
31. Saygin C, Cannova J, Stock W, Muffly L (2022) Measurable residual disease in acute lymphoblastic leukemia: methods and clinical context in adult patients. Haematologica 107:2783–2793. https://doi.org/10.3324/haematol.2022.280638