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SHORT REPORT

Haematological Malignancy – Clinical

Prognostic impact of pretreatment cell-free DNA concentration in newly diagnosed peripheral T-cell lymphomas

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Summary
Peripheral T-cell lymphomas (PTCLs) have a poor prognosis and, to date, there are no reliable predictive biomarkers of response. In this work we explored the prognostic impact of cell-free DNA (cfDNA) concentration in 75 newly diagnosed patients enrolled in a prospective multicenter study. Pre-treatment cfDNA was strongly associated with clinical risk factors and was identified as a superior predictor for shorter progression-free survival in multivariable analysis, outweighing canonical risk parameters. Furthermore, we identified a cfDNA value above which survival worsens. In conclusion, pre-treatment cfDNA concentration represents an easily usable predictive biomarker that is highly associated with survival of PTCL patients.

KEYWORDS
DNA prognosis, haematological malignancies, prognostic factors, T-cell lymphoma

INTRODUCTION

PTCLs represent 10%–15% of all non-Hodgkin lymphoma (NHL). Except for patients affected by anaplastic large-cell lymphoma (ALCL) harbouring anaplastic lymphoma kinase (*ALK*) gene rearrangement, the prognosis of PTCLs is poor.[1] Although some clinical features associated with worse prognosis have been recognized (including lactate

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dehydrogenase [LDH] value above upper normal limit, poor performance status, a high International Prognostic Index [IPI] or Prognostic Index for T-cell lymphoma [PIT] score),[2] there are no reliable predictive biomarkers that allow for a risk-adapted treatment strategy. In recent years, cfDNA has become an increasingly important marker in oncology because of its correlation with tumour burden and the possibility of searching for tumour-associated mutations.[3] In cancer patients, cfDNA is composed of both circulating tumour DNA (ctDNA)[4] and normal DNA fragments, and cfDNA concentration ([cfDNA]) is often higher than in healthy controls.[5] Some studies have shown a correlation between elevated [cfDNA] and high-risk clinical parameters in NHL patients,[6,7] leading to unfavourable outcome.[8] However, the impact of [cfDNA] in PTCLs is poorly investigated and only in retrospective studies where PTCLs were grouped with other histologies.[6,9] Thus, the aim of this study was to assess whether pretreatment [cfDNA] in plasma was associated with clinical risk factors and prognosis in patients enrolled in the PTCL13 clinical trial (NCT02223208), whose plasma samples were prospectively collected. This was a multicentre phase Ib-II study that included newly diagnosed patients affected by peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS), anaplastic lymphoma kinase-negative anaplastic large-cell lymphoma (ALK-ALCL) and nodular T-follicular helper lymphoma (nTFHL), whose results have been recently published.[10] Patients received 6 cycles of chemotherapy combined with romidepsin, followed by consolidation with haematopoietic stem cell transplantation, in those who achieved a response to induction and were eligible to the procedure.

METHODS

This study received Ethics Committee approval from all the involved centres. After informed consent acquisition, peripheral blood samples were collected at baseline, then plasma was obtained by gradient separation as previously described[11] and subsequently stored at −80°C. For cfDNA extraction, the Maxwell® RSC LV ccfDNA Kit, Custom (Promega) was used, following the manufacturer's instruction. Qubit® 2.0 Fluorometer (Invitrogen) was used for quantification. Size profile analysis was performed immediately after cfDNA extraction using the 4200 TapeStation® capillary electrophoresis system (Agilent) with a High Sensitivity D5000 ScreenTape. Samples with evidence of significant contamination of high molecular weight genetic material (>1000 base pairs) were excluded. After extraction, libraries were prepared using KAPA HyperCap Workflow (version 3.0, Roche) for stable sample storage and future sequencing. Statistical analyses were performed using GraphPad Prism (version 9.3.1) and, for univariable and multivariable analyses, using R (version 4.1.2). Detailed inclusion and exclusion criteria, extended protocol and outcome are described in the published PTCL13 study.[10]

RESULTS AND DISCUSSION

Of the 89 enrolled patients, 85 had available baseline plasma but 10 had evidence of high molecular weight DNA contamination and were therefore excluded. Overall, baseline [cfDNA] was assessable in 75 patients, whose characteristics and outcome are listed in Tables S1–S5 and overlap with those of the population enrolled in the PTCL13 study.[10]

Cell-free DNA concentration, expressed in ng of cfDNA/mL of plasma (ng/mL), had a median value of 19.1 ng/mL (range: 2.27–1082) and it was associated with clinical risk factors (Figure 1A–E). There was a strong association between high [cfDNA] and a high IPI (median cfDNA: IPI 0–2 = 12.27, IPI 3–5 = 63.86 ng/mL, $p < 0.0001$) and PIT scores (median cfDNA: PIT 0–1 = 14.20, PIT 2–4 = 31.22 ng/mL, $p = 0.0006$), increased LDH levels (median cfDNA: normal LDH = 10.1, increased LDH = 40 ng/mL $p < 0.0001$) and poor performance status (median cfDNA: Eastern Cooperative Oncology Group performance status of 0 = 12.27, performance status ≥1 = 37 ng/mL, $p = 0.0003$). A moderate association was found with the presence of extra-nodal disease ($p = 0.04$), while no association was detected between age, Ann Arbor stage, bone marrow involvement and [cfDNA] (Figure 1F–H). In nTFHLs, [cfDNA] was lower than in ALK-ALCLs (median cfDNA 11.1 and 42.78 ng/mL, respectively, $p = 0.016$, Figure 1I), but was similar to PTCL-NOS (median cfDNA 15.71 ng/mL), despite the fact that nTFHLs had a better progression-free survival (PFS) only when compared to PTCL-NOS ($p = 0.0335$) but not when compared to ALK-ALCLs (Figure S1A). A higher baseline [cfDNA] was found in patients who progressed compared with those who achieved at least a partial response at the end of the six chemotherapy and romidepsin courses (median cfDNA: 27.6 vs. 14.76 ng/mL, respectively, $p = 0.0303$, Figure S1B) and in patients who died compared to survivors (Figure S1C).

Importantly, elevated pretreatment [cfDNA] had a negative impact on PFS (Univariable Cox linear model, $p = 0.0001$, Figure S2A) and overall survival (OS, univariable Cox nonlinear model, $p = 0.0105$, Figure S2B). Multivariable analysis confirmed a significant influence of [cfDNA] on PFS [multivariable Cox model: HR 1.34, 95% confidence interval (CI) 1.14–1.58, $p = 0.0004$; and HR 1.28, 95% CI 1.11–1.48, $p = 0.0006$, Figure 2A] greater than that of IPI and PIT scores, LDH, performance status and extra-nodal disease.

Patients with a very high [cfDNA] (>100 ng/mL, $N = 9$) were characterized by poor clinical features (poor performance status, high IPI, increased LDH), all but one had a PFS event and six of them (66%) died. Considering progression events occurring by the interim evaluation performed after the third cycle, which was observed in seven patients, an extremely high baseline [cfDNA] was found in three of these patients (cfDNA range 118.8–1082.4 ng/mL). However, the other four early progressor had a lower [cfDNA]. For this reason, to identify a specific pretreatment [cfDNA] cut-off associated with a worse prognosis, an analysis by receiver operating characteristic (ROC) curve was performed in relation to 1-year OS (Figure S3). In doing so, a cut-off of

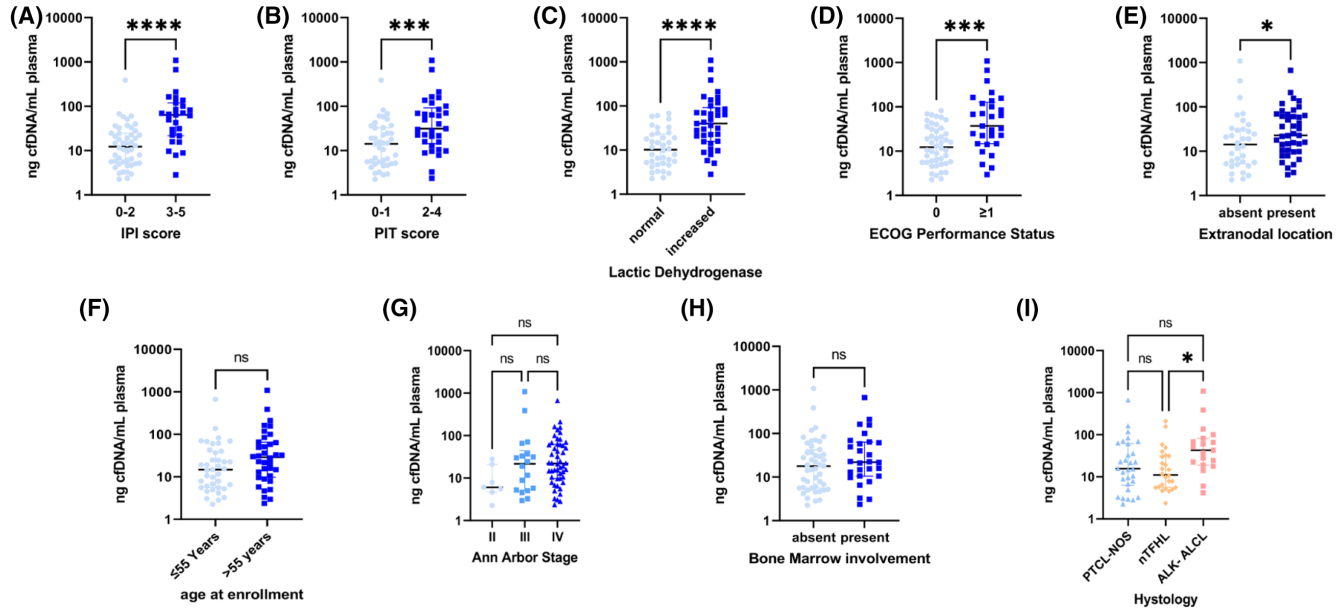

FIGURE 1 Correlation between pretreatment cfDNA concentration and clinical parameters. This figure shows the correlation of cfDNA concentration with clinical risk factors such as International Prognostic Index (IPI 0–2 vs. 3–5, A) and Prognostic Index for T-cell lymphoma (PIT 0–1 vs. 2–4, B) scores, lactic dehydorgenase levels (LDH normal vs. increased, C), Eastern Cooperative Oncology Group (ECOG) performance status (0 = normal vs. ≥ 1, D), extra-nodal disease (absent vs. present, E), age (lower versus higher than the median value, which was 55 years, F), Ann Arbor stage (II vs. III vs. IV, G) and bone marrow involvement (absent versus present, H). The graph I shows the cfDNA concentration according to histology, where PTCL-NOS is shown in light blue, nTFHLs in orange and ALK-ALCLs in salmon. Dichotomous variables were analysed with the Mann–Whitney test, while variables with more than two group (G, I) with the Kruskal-Wallis test by ranks. The concentration of cfDNA is shown in logarithmic scale to better appreciate the distribution of concentrations, especially for interquartile values. ALK-ALCL, anaplastic large-cell lymphoma without *ALK* gene rearrangement; cfDNA, cell-free DNA; ECOG, Eastern Cooperative Oncology Group; IPI, International Prognostic Index; LDH, lactic dehydrogenase; not otherwise specified; nTFHL, nodular T-follicular helper lymphoma; PIT, Prognostic Index for T-cell lymphoma; PTCL-NOS, peripheral T-cell lymphoma.

42.78 ng/mL was identified using Youden's J statistics, above which PFS and OS were significantly reduced (log-rank test: $p = 0.0177$ and <0.0001 respectively, Figure 2B,C). Among the 21 patients with a [cfDNA] > 42.78 ng/mL, 8 were PTCL-NOS, 4 nTFHLs and 9 ALK-ALCLs, with nTFHLs being significantly less represented than ALK-ALCLs ($p = 0.043$). Considering the response evaluation after the six chemotherapy and romidepsin courses (Table S2), available in 69 patients, a [cfDNA] > 42.78 ng/mL was slightly associated with a lower probability of complete remission (47% vs. 71.2% in those with lower [cfDNA], $p = 0.085$, Figure S4A) and with more progression events by the end of induction therapy (35.3% vs. 19.2%, Figure S4B), despite the fact that the response rates showed no statistically significant differences in the two groups.

On the other hand, multivariable analysis confirmed a significant effect on PFS and OS when [cfDNA] above or below 42.78 ng/mL was studied together with IPI, LDH and ECOG (PFS: HR 2.76, 95% CI 1.24–6.16, $p = 0.0128$; OS: HR 9.45, 95% CI 3.10–28.81, $p = 0.0001$, Figure S5A,C). When tested together with PIT, ECOG and extra-nodal disease, both [cfDNA] and PIT score were associated with OS (HR 9.30, 95% CI 3.22–26.87, $p < 0.0001$ and HR 0.34, 95% CI 0.12–0.94, $p = 0.037$ respectively, Figure S5D), while no significant associations with PFS were found (cfDNA ≥ vs. <42.78 ng/mL, HR 1.83, 95% CI 0.86–3.91, $p = 0.1178$, Figure S5B).

In conclusion, our data demonstrate that pretreatment [cfDNA] has a strong prognostic impact in PTCLs and represents a superior predictor of survival, compared to canonical parameters. Although the association with adverse risk factor and outcome of cfDNA has already been described in heterogeneous NHL case series,[6,8] in this study the analysis focused on homogeneously treated PTCLs. Its influence on survival in the context of a prospective study identifies cfDNA as a strong and easily assessable biomarker that could become part of those to be measured in newly diagnosed PTCLs.

While the multivariable analysis performed considering [cfDNA] as a continuous variable demonstrated a strong influence on survival, the ones performed considering the identified cut-off was significant only when corrected for one group of factors but not for the others. However, considering [cfDNA] as a continuous variable represents a better approach, and the correlation with survival could be strengthened as participants' follow-up is updated. In addition, in this study, OS curves differed more than PFS curves. This difference could reside in the approach taken to calculate the cut-off, that specifically considered the OS, as well as in a better prognosis of patients with low baseline [cfDNA] even when treated with subsequent treatment lines, although this latter hypothesis will need to be investigated in future studies. Notably, patients with extremely high [cfDNA] were characterized by poor clinical features and a bad prognosis,

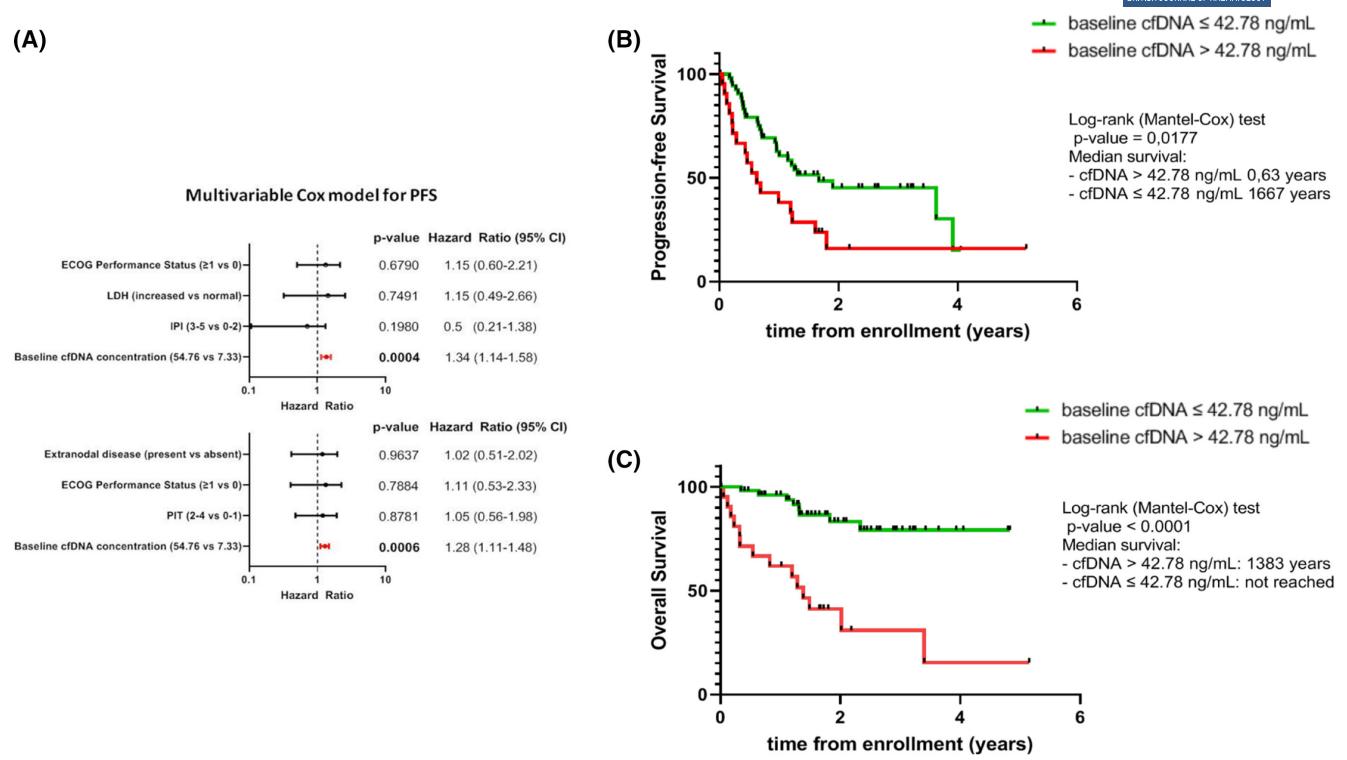

FIGURE 2 Association between cell-free DNA concentration and survival outcome. (A) Multivariable Cox models for progression-free survival where baseline cfDNA concentration is analysed as a continuous variable, corrected by two sets of covariates (in order to correct for a wide variety of covariates while respecting the ratio of the number of events observed and the number of covariates included). Hazard ratios reported compare the third to the first quartile of the continuous variable. (B, C) Progression-free survival (B) and overall survival (C), with respect to baseline cfDNA concentration being higher (red) or lower (green) than the identified cut-off of 42.78 ng cfDNA/mL of plasma. ALK-ALCL, anaplastic lymphoma kinase-negative anaplastic large-cell lymphoma; cfDNA, cell-free DNA; ECOG, Eastern Cooperative Oncology Group; IPI, International Prognostic Index; LDH, lactic dehydrogenase; nTFHL, nodal T-follicular helper lymphoma; PFS, progression-free survival; PIT, Prognostic Index for T-cell lymphoma; PTCL-NOS, peripheral T-cell lymphoma, not otherwise specified.

indicating that [cfDNA] could be indeed used as a biomarker to guide closer monitoring and a more intensive treatment.

Despite the fact that sequencing of cfDNA is crucial to identify molecular alterations that could guide therapeutic interventions, its applicability in routine clinical practice is limited because of high costs and long turnaround time. In contrast, [cfDNA] is an inexpensive biomarker that can quickly provide prognostic information in the real-world scenario. In our opinion, the inclusion of this parameter in clinical trials design should be evaluated with the aim of tailoring treatment intensity according to baseline [cfDNA].

AUTHOR CONTRIBUTIONS

E. Fardella and G. Zanirato performed data analysis and conducted the experiments; M. Magni processed the samples and contributed to the experiments; E. Fardella and A. Chiappella collected the clinical data; E. Fardella interpreted data and wrote the manuscript; S. Ljevar contributed to the statistical analysis (univariable and multivariable, and ROC curve); L. Orsucci, A. Re, S. V. Usai, V. Stefoni, C. Castellino, F. G. Rossi, A. Pinto, A. Dodero and A. Chiappella provided the samples and revised the work: G. Zanirato, M. Magni, N. Caldarelli, C. Carniti and P. Corradini revised the work; C. Carniti and P. Corradini conceived the study, supervised the project and co-ordinated the experiments.

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CONFLICT OF INTEREST STATEMENT

Paolo Corradini. Advisory boards: AbbVie, ADC Therapeutics, Amgen, BeiGene, Celgene, Daiichi Sankyo, Eli Lilly, Gilead/Kite, GSK, Incyte, Janssen, Kyowa Kirin, Nerviano Medical Science, Novartis, Roche, Sanofi, SOBI, Takeda. -Honoraria for lectures: AbbVie, Amgen, Celgene, Gilead/Kite, Incyte, Janssen, Novartis, Roche, Sanofi, SOBI, Takeda. Annalisa Chiappella. Advisory board: Gilead-Sciences, Roche, Ideogen, Takeda; Educational activities/

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DATA AVAILABILITY STATEMENT

For data sharing and any further information please contact paolo.corradini@unimi.it. Permission was granted by the authors for the reproduction of material from other sources.

ETHICS STATEMENT

The study was registered in the European Union Drug Regulating Authorities Clinical Trials Database (EudraCT) under EudraCT Number 2013-005179-41. It was registered in ClinicalTrials.gov under the clinical trial registration number NCT02223208. The study received approval from the ethics committee at each participating centre. The study was approved by the ethics committee of the leading centre on 27 May 2014, internal number 87/14.

PATIENTS' CONSENT

A written informed consent was obtained from every patient enrolled in the study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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