Circulating MicroRNAs as Biomarkers in Diffuse Large B-cell Lymphoma: A Pilot Prospective Longitudinal Clinical Study

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ABSTRACT:

OBJECTIVES: Diffuse large B-cell lymphoma (DLBCL) is highly heterogeneous in terms of phenotype and treatment response in patients. These characteristics make the prognosis difficult to establish and hinder the use of new personalized treatments in clinical practice. In this context, there is currently a need to define new biomarkers enabling a better definition of DLBCL subtypes, prognosis evaluation, and an overview of the resistance to chemotherapeutics. The aim of this study was to evaluate the use of microRNAs found in plasma from patients with DLBCL as biomarkers of tumor evolution in these patients.

METHOD: For this purpose, a plasma biobank was created with samples from patients with DLBCL. The evolution of the level of selected microRNAs during treatment has been studied. A total of 19 patients with DLBCL were included in this pilot mono-centered study and a total of 68 samples were analyzed.

RESULTS: The first step of this study was the selection of the microRNAs to be quantified in all the samples of the biobank and that could potentially be used as biomarkers. To this end, quantification of 377 microRNAs was performed on the plasma samples of 2 selected patients with DLBCL and 1 healthy donor with no history of cancer. Among the 377 microRNAs evaluated, 7 were selected and analyzed in the entire biobank.

CONCLUSIONS: This study highlighted 5 circulating microRNAs whose plasma levels would be worth further investigating for the characterization of DLBCL evolution in patients. MiR-21 and miR-197 had a significant higher plasmatic level in patients with tumors unresponsive to treatment. With a higher plasma level in patients with complete remission, miR-19b, miR-20a, and miR-451 could enable to differentiate, at the remission review, patients with residual tumor, from patients with complete remission.

KEYWORDS: Diffuse large B-cell lymphoma, circulating microRNAs, biomarkers, plasma

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Introduction

Diffuse large B-cell lymphoma (DLBCL) is an aggressive B-cell non-Hodgkin lymphoma (NHL) accounting for 30% to 35% of NHL.[1] The survival is relatively high for patients with limited disease with a 5-year progression-free survival (PFS) around 80% to 85%. Patients with advanced and/or symptomatic disease have a worse prognosis with a 5-year PFS around 50%.[2]

Considering its major clinical and biological heterogeneity, DLBCL comprises many subtypes described by the World Health Organization (WHO).[3] This classification is principally based on morphological differences. However, the gene expression profiling technology enabled the discovery of different molecular subtypes in the DLBCL not otherwise specified (NOS) category: activated B-cell like (ABC) and germinal center B-cell like (GCB) subtypes,[4] associated with different prognoses with the same common treatment (R-CHOP).[5]

Despite this advance, the molecular classification is not yet taken into account for the treatment choice of DLBCL in clinical practice. Following the European Society for Medical Oncology (ESMO) clinical practice guidelines, the treatment choice is based on patient age and his general state together with the age-adjusted International Prognostic Index (aaIPI) based on the stage, lactate dehydrogenase level, and performance status.[6]

The most commonly used treatment against DLBCL is the R-CHOP chemotherapy, involving the administration of cyclophosphamide, hydroxydaunorubicin, vincristine, and prednisolone (CHOP), together with a monoclonal antibody directed against CD20: the rituximab (R).[6]

Although a significantly improved survival was attributed to the addition of rituximab to the patient treatment,[7] an important number of patients remains refractory to this first-line chemotherapy or experiences relapse, with a resulting poor outcome.[2] Therefore, the discovery of new predictors enabling an early identification of patients with inherent or acquired refractory disease (RD) during treatment would be valuable to help in prognosis evaluation and in the implementation of new personalized treatment in clinical practice.

MicroRNAs are small single-stranded noncoding RNAs of about 19 to 25 nucleotides in length that are able to negatively regulate gene expression by a translational repression of messenger RNAs.[8] These gene regulatory molecules have a major impact on cell phenotype.[9] Considering the major roles of microRNAs in tumorigenesis and their already studied use as biomarkers in tumor cells,[10] the discovery of circulating forms of microRNAs in 2008 triggered numerous studies investigating the potential of circulating microRNAs as biomarkers in cancer.[11,12] With the asset of being easily accessible by blood sampling, these extracellular microRNAs would be able to give clues about the tumor state.[13] Despite the great number of publications assessing their levels in various cancers, the measurement of circulating microRNAs has not yet entered the clinical practice. Discordant studies and very few overlaps between studies hinder their reproducible evaluation. These interstudy variations are due at least in part to the lack of standardization of the pre-analytical and analytical settings.[12]

It is now well known that microRNA deregulation is implicated in DLBCL. Four microRNAs (miR-17-92 cluster, miR-21, miR-155, and miR-34a) particularly draw attention in DLBCL tumors.[14] In the context of circulating microRNAs, it is expected that the levels of circulating microRNAs would reflect the deregulation found inside cancer cells. The study of circulating microRNAs in DLBCL began with the work of Lawrie and colleagues in 2008, becoming simultaneously the pioneers of circulating microRNA research.[15] Since then, other studies analyzed circulating microRNAs as biomarkers in DLBCL. They were all performed on serum and revealed some interesting profiles for several microRNAs including miR-21, miR-155, and miR-34a, mentioned earlier as deregulated in DLBCL cells.[15–18] MiR-21 particularly draws attention and was linked with patient prognosis in 3 independent studies.[15,17–19] All these studies were performed on samples obtained before treatment. However, a recent study investigated the continuous evolution of specific microRNA level during patient follow-up after the first-line treatment and revealed some interesting evolution profiles related to the disease state progression.[19]

The aim of this pilot study was to evaluate the use of microRNAs found in plasma from patients with DLBCL as biomarkers of tumor evolution in these patients. For this purpose, a plasma biobank was created with samples from patients with DLBCL at different times of their treatment. The evolution of the level of selected microRNAs during treatment has been studied.

Methods

DLBCL patient characteristics

A total of 19 patients with DLBCL and 1 healthy donor were prospectively included in this single-center study between March 2014 and June 2017. Written informed consents were obtained from each participant following the requirements of the local research ethics committee which approved the study (ethical agreement number B039201419613). Their diagnosis and classification were performed following the WHO recommendations.[3] The DLBCL NOS patients were classified as GCB or ABC subtypes by immunohistochemistry according to Hans' algorithm.[20]

Plasma sampling

Blood was collected into 0.109 M sodium citrate (9:1 v/v) tubes (Venosafe; Terumo, Leuven, Belgium) at patient catheter. Samples were obtained at the administration of the first chemotherapeutic cure (C0) (just before administration), at the administration of the second and the fourth cycles of chemotherapy (C2 and C4), and at the remission review (Cf). In the case of an autograft, a final sample was taken at the postgraft review. These times were chosen considering the evaluation of the response to treatment performed by ^{18}fluorodeoxyglucose positron emission tomography (FDG-PET)/computed tomography (CT) during the fourth chemotherapeutic cure. The patient response was evaluated using the Deauville score[21] and according to the Lugano classification.[22] Platelet-free plasma was obtained following strict pre-analytical setting already published by our research group.[23] Plasma samples were frozen in liquid nitrogen and stored at −80°C.

MicroRNA extraction from plasma

Total RNA were isolated from 200 µL of plasma with the miRNeasy Serum/Plasma Kit from QIAGEN Benelux B.V. (Venlo, Netherlands) following the manufacturer protocol. For technical normalization, 5.6×10^8 copies of a nonhuman synthetic microRNA (miRNeasy Serum/Plasma Spike-In Control, *C. elegans* miR-39 miRNA mimic) were added to plasma as a spike-in control during the extraction step, before the chloroform addition, as advised by the manufacturer. The final total RNA fraction was eluted in 15 µL of RNase-free water.

MicroRNA profiling by microarray

The first step of this study was the selection of the microRNAs that will be quantified in all the samples of the biobank and that could potentially be used as biomarkers. To this end, 2 patients with DLBCL and 1 healthy donor with no history of cancer

were selected for a microRNA profiling. These patients were selected based on their highly different response to treatment.

A profiling of 377 microRNAs was performed by TaqMan Low-Density Array (Applied Biosystems, Life Technologies Europe BV, Merelbeke, Belgium) on 26 µL of total RNA fraction extracted from patient samples. This volume of extracted RNA was concentrated to dryness in a vacuum concentrator (Hetovac VR-1, Heto Lab) and used for the reverse transcription step with the Taqman MicroRNA Reverse Transcription Kit and the Megaplex RT Primers from Applied Biosystems, Human Pool A v2.1. The quantification of 377 microRNAs was finally performed in a TaqMan Array, Human MicroRNA A Card v2.0 using the ViiA 7 system (Applied Biosystems, Life Technologies Europe BV, Merelbeke, Belgium). MicroRNAs with a Cq higher than 32 were considered as nonexpressed. The relative expression of microRNAs was calculated by the $2^{-\Delta\Delta Cq}$ method using the U6-snRNA level, selected by the NormFinder software, as an endogenous control for normalization.

Scoring for biomarker evaluation

To evaluate the potential as biomarkers of the microRNAs profiled by microarray, we determined some criteria to use in a scoring system. Following this scoring detailed as additional file (see Additional file 1), 1 point was given to a microRNA each time it meets the criteria enabling it to be defined as a potential diagnostic, prognostic and/or remission biomarker, biomarker of a disease progression, biomarker of an inherent resistance to treatment, and/or biomarker of an acquired resistance to treatment. Due to the lack of replicates that would enable statistical testing, a differential expression was defined as a more than 2-fold change.

MicroRNA quantification by quantitative reverse transaiption–polymerase chain reaction

About 5 µL of total RNA extracted from plasma was reverse-transcribed with the miScript II RT Kit (QIAGEN). The resulting complementary DNA sample was diluted in 200 µL of water and 2.5 µL was used per quantitative polymerase chain reaction (qPCR). This last step was performed with the miScript SYBR Green PCR Kit and the specific miScript Primer Assays (QIAGEN) for the microRNAs to quantify (see Additional file 2). Each qPCR reaction was performed in technical duplicates. The relative expression of microRNAs was expressed using the ΔCq method using the spike-in microRNA mimic added during the RNA extraction step for normalization.

Sample classification depending on patient prognosis

Plasma samples were classified based on patient prognosis. Concretely, a bad prognosis was defined as a RD after the first-line treatment or a relapse diagnosed after the first line and a good prognosis as a complete remission (CR) after the first-line treatment that was maintained during the study.

Sample classification depending on the tumor responsiveness to treatment

Plasma samples were classified based on the chemotherapeutic treatment and the tumor responsiveness to treatment. Concretely, samples were separated first depending on their sampling on a patient under chemotherapeutic treatment (C2 or C4) or not (C0 or Cf), considering that this parameter could affect circulating microRNAs. The tumor state was then taken into account for further classification. All the C0 samples were grouped into a single category considering their progressing tumor status. C2 and C4 samples were classified depending on the tumor response to treatment. This classification was based on the FDG-PET/CT scan results evaluated during treatment. On one hand, when this examination revealed a partial metabolic response or a CR, the samples collected before this scan were attributed to a responsive tumor (RT). On the other hand, when the Deauville score did not change or increased between 2 FDG-PET/CT scans, the samples collected during the period between these 2 examinations were attributed to an unresponsive tumor (URT). Finally, the final samples (Cf) from patients with CR were separated from those obtained on patients with a residual tumor (ResT).

Statistical analysis

Comparison between different conditions was performed using unpaired *t* test on GraphPad Prism software (version 5.04).

Results

Characteristics of included patients

A total of 19 patients with DLBCL were included in the study (Table 1). The median age was 74 years. Most of the patients had a stage IV and were diagnosed with a DLBCL NOS. Patients were predominantly treated with R-CHOP or R-CHOP-like treatments of 6 or 8 cycles. We were not able to collect all the samples for every patients due to technical issues or because patients were sometimes treated in another hospital during their chemotherapeutic treatment. Patients 14 and 19 had an autologous stem cell transplantation. For patient 15, only the sample collected at C0 was used because this patient developed an additional adenocarcinoma during the treatment.

Candidate microRNA selection

The first step of this study was the selection of the candidate microRNAs to be quantified in all the samples of the biobank. To this end, 2 patients with DLBCL and 1 healthy donor with no history of cancer were selected for microRNA profiling. These patients were selected based on their highly different response to

Table 1. Summary of clinical characteristics of the 19 patients with DLBCL and the healthy donor.

PATIENTS	AGE AT C0, Y	GENDER	WHO CLASSIFICATION	STAGE	AAIPI	TREATMENT	RESPONSE AFTER C4	RESPONSE AFTER CF	SAMPLES				RELAPSE AFTER FIRST LINE
									C0	C2	C4	CF	
1	75	M	DLBCL, NOS ABC	III	2	R-CHOP 21 (8 cycles)	Partial response	Complete remission	IT	RT	RT	CR	No
2	65	M	DLBCL intermediated with Burkitt lymphoma	IV	3	R-CEOP 21 (8 cycles)	Partial response	Refractory disease	IT	RT	RT	ResT	NA
3	70	F	PMBL	II	1	R-CHOP 21 (8 cycles)	Partial response	Refractory disease	IT	RT	RT	ResT	NA
4	62	M	DLBCL, NOS ABC	IV	2	R-MPVA	Partial response	Complete remission	IT	RT	RT	CR	Yes
5	81	M	DLBCL, NOS ABC	II	1	R-CHOP 21 (6 cycles)	*ND*	Complete remission	IT	RT	—	—	No
6	84	F	DLBCL, NOS	I	0	R-CHOP 21 (6 cycles)	Complete remission	Progressive disease	IT	RT	RT	—	NA
7	40	F	PMBL	II	1	R-ACVBP	Partial response	Complete remission	IT	RT	RT	CR	No
8	79	F	DLBCL, NOS GCB	IV	1	R-CHOP 21 (8 cycles)	Partial response	Complete remission	IT	RT	RT	CR	No
9	77	M	DLBCL, NOS EBV+	IV	1	R-CHOP 21 (8 cycles)	Partial response	Complete remission	IT	RT	RT	CR	No
10	62	M	DLBCL, NOS ABC	I	0	R-CHOP 21 (4 cycles)	Complete remission (after C2)	Complete remission	IT	RT	RT	CR	No
11	23	F	DLBCL, NOS GCB	IV	2	GA101-ACVBP	Partial response	Complete remission	IT	RT	RT	CR	No
12	72	M	DLBCL, NOS ABC	IV	1	R-CHOP 21 (8 cycles)	Partial response	Complete remission	—	RT	RT	CR	No
13	79	F	PCLBCL, leg type	IV	2	R-CHOP 21 (6 cycles)	Progressive disease (after C2)	Refractory disease	IT	URT	URT	ResT	NA
14	39	F	DLBCL, NOS ABC	II	1	R-ICE (3 cycles) + BEAM and ASCT	Complete remission (after C3)	Complete remission (after ASCT)	IT	RT	RT	CR	Yes
15	76	M	DLBCL, NOS GCB	II	0	R-CHOP 21 (4 cycles)	Partial response	Adenocarcinoma	IT	—	—	—	—
16	82	F	DLBCL, NOS ABC	IV	1	R-CHOP 21 (6 cycles)	Complete remission	Complete remission	IT	RT	RT	CR	No
17	77	M	DLBCL, NOS GCB	IV	1	R-CHOP 21 (8 cycles)	Partial response	Complete remission	IT	RT	RT	CR	No
18	74	M	DLBCL, NOS GCB	II	1	R-CHOP 21 (6 cycles)	*ND*	Complete remission	IT	RT	RT	—	No
19	45	M	DLBCL, NOS ABC	IV	1	R-ACVBP (4 cycles) + 2 methotrexate	Partial response (after C2) and refractory disease (after C4)	Complete remission (after ASCT)	IT	RT	URT	—	No
20	72	F	Healthy and no past of cancer	NA	NA	Pradaxa 150 mg, Rytmonorm 225 mg, Bisoprolol 5 mg	NA	NA					

Abbreviations: ABC, activated B-cell like; ASCT, autologous stem cells transplantation; BEAM, carmustine, etoposide, cytarabine, melphalan; CR, complete remission; DLBCL, NOS, diffuse large B-cell lymphoma, not otherwise specified; EBV, Epstein-Barr virus; GCB, germinal center B-cell like; ICE, ifosfamide, carboplatin, etoposide; IT, initial tumor; MPVA, methotrexate, procarbazine, vincristine, ara-C-cytarabine; PCLBCL, primary cutaneous DLBCL; PMBL, primary mediastinal large B-cell lymphoma; ResT, residual tumor; RT, responsive tumor; URT, unresponsive tumor.

Table 2. Selected microRNAs for the entire biobank screening.

SELECTED MICRORNAS	CRITERIA
MiR-197	5 points
MiR-20a	5 points
MiR-451	5 points
MiR-122	4 points
MiR-19b	4 points
Let-7e	Prognostic at C0, C2, and C4
MiR-21	Bibliographic selection

treatment. One of them (patient 8) obtained a CR after an R-CHOP treatment (CR patient), whereas the other (patient 3) presented a RD to the same treatment (RD patient) (Table 1). These patients and the healthy donor were women with close ages (79, 70, and 72 years of age at C0).

Among the 377 microRNAs quantified into the plasma of the 3 selected donors, 81 microRNAs were detected. These microRNAs are listed as additional file (see Additional file 3) and raw data and processed results have been deposited in the ArrayExpress database at EMBL-EBI (www.ebi.ac.uk/arrayexpress) under accession number E-MTAB-6063. Using the scoring system, we set up to evaluate the potential as biomarkers of the quantified microRNAs, and we selected 4 microRNAs with the highest biomarker scores of 5 or 4 points: miR-197, miR-20a, miR-451, miR-122, and miR-19b (Table 2). MiR-19a also presented a biomarker score of 4 points. However, its expression strictly followed the same tendency as miR-19b. For this reason, and because miR-19b had a higher expression level compared to miR-19a, we decided to analyze only miR-19b. Let-7e was also selected for its potential prognostic value at C0, C2, and C4. Considering its citation in other circulating microRNA studies on DLBCL mentioned in this introduction, miR-21 was also selected for further testing. This microRNA obtained a score of 2 with our biomarker scoring system.

Expression of the candidate microRNAs in the entire biobank

These candidate microRNAs were quantified in all the samples constituting the plasma biobank. The analysis of the level of these microRNAs comparing age groups, sex, WHO classification, stage, aaIPI, and prognosis groups revealed no significant result (see Additional files 4 to 9). No correlation was observed between the microRNA level and the blood count of patients (see Additional file 10).

However, the samples were also grouped based on the treatment and the tumor state as described in the materials and methods part. Among the 7 microRNAs tested (Table 2), 5 gave interesting results with significant differences between the patient groups (Figure 1). MiR-122 and let-7e did not show particular trends (see Additional file 11). MiR-21 and miR-197 showed interesting levels during treatment (C2 and C4), being significantly more expressed in plasma from patients with tumors unresponsive to treatment (URT) compared with RT. MiR-19b, miR-20a, and miR-451 are differentially expressed at Cf between patients with residual tumor (ResT) and patients with CR, with higher plasma levels in patients with CR.

Discussion

Several research works already tried to use circulating microRNAs as biomarkers in DLBCL with sometimes contradictory results.[17,18] In addition, they principally focused on samples collected before treatment.[15–18] In this longitudinal study, we decided to analyze circulating microRNAs during the treatment course of patients and to evaluate their potential link with disease evolution. Considering the impact of pre-analytical settings on circulating microRNA evaluation,[12] we also followed a strict procedure for plasma sampling and storage. Unlike the other studies on circulating microRNAs in DLBCL, we chose to use platelet-free plasma as a source of circulating microRNAs and not serum. Indeed, platelets are known to release a high number of extracellular vesicles (EVs) containing microRNAs during coagulation.[24] For this reason, the use of plasma enables the analysis of microRNAs originally circulating in blood and not released afterward during serum formation.

The first step of this study was the selection of the circulating microRNAs with the highest potential as biomarkers in DLBCL. The choice of 2 patients with DLBCL for this selection was based not only on their major differences in tumor response to treatment but also on their close age (near 75 years) and their same sex (women) to minimize interindividual variability. The microRNA screening on these patients and the evaluation of their potential as biomarkers with our scoring system aimed at identifying microRNAs with high variability between patients and during treatment with a potential as biomarkers. By the comparison with the levels in a healthy donor, we also selected microRNAs with plasmatic expression potentially linked with the pathological cancer state. In this context, the miR-197, miR-20a, miR-451, miR-122, and miR-19b were selected for their high score. The miR-197 has already been highlighted for its role as oncogene or anti-oncogene depending on the cancer type and would target various tumorigenic or tumor-suppressive genes.[25] This microRNA would also be underexpressed in the plasma of patients with DLBCL compared with healthy donors.[26] The miR-19b and miR-20a belong to the miR-17-92 cluster, often overexpressed in cancer and that would have an oncogenic potential in B-cell lymphoma.[27] In DLBCL, this cluster frequently undergoes genetic amplification in GCB subtype.[28] The miR-451 has been previously highlighted for its dysregulation in DLBCL compared with healthy lymphocytes[29] and would be downregulated in GCB subtype compared with the ABC.[30,31]

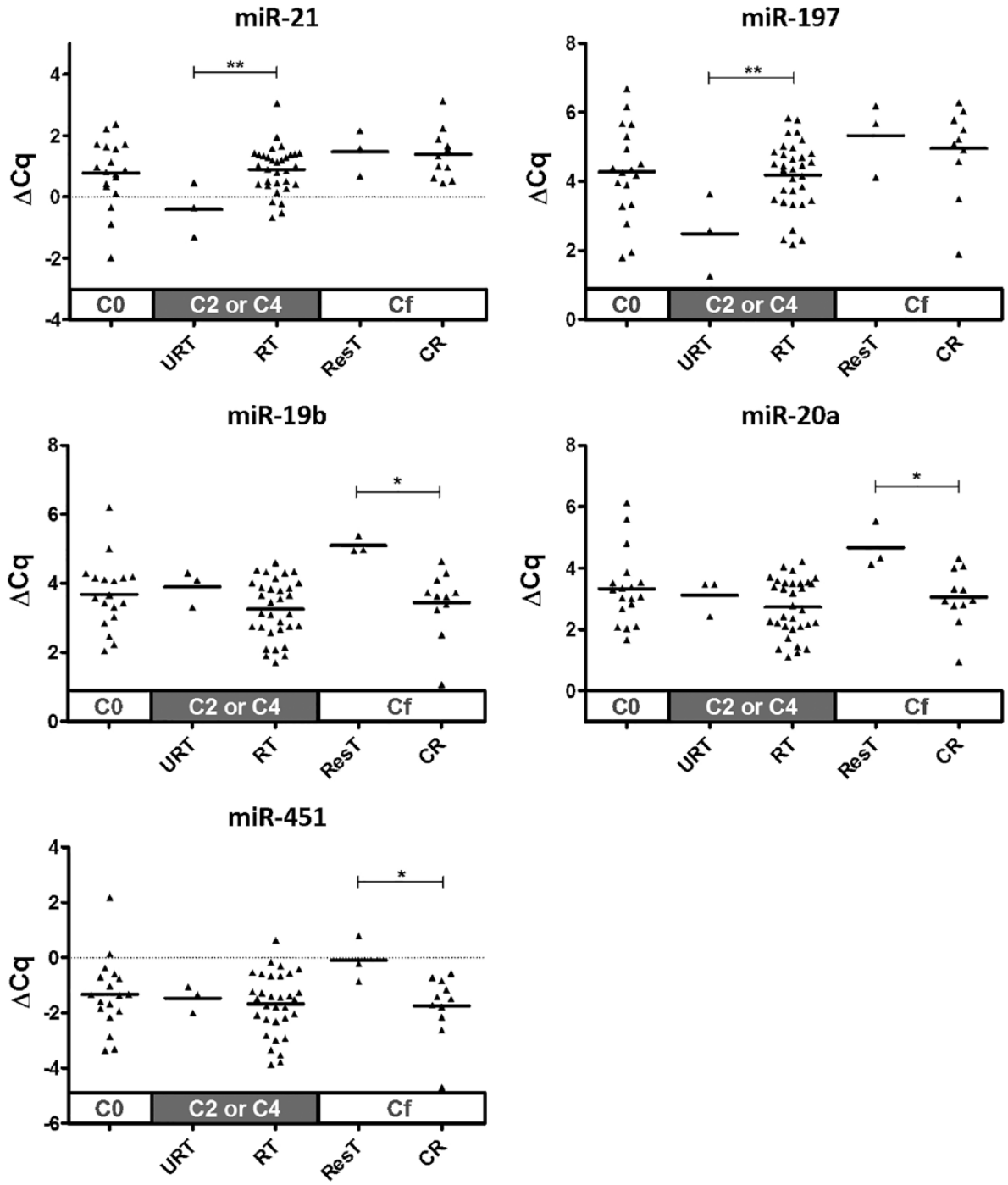

Figure 1. MiR-21, miR-197, miR-19b, miR-20a and miR-451 expression among the different groups of DLBCL patients. The selected microRNAs were quantified in all the samples constituting the plasma biobank. Samples were obtained at the administration of the first chemotherapeutic cure (C0), at the administration of the second and the fourth cycles of chemotherapy (C2 and C4) and at the remission review (Cf). The samples were grouped following the criteria described in the materials and methods, depending on the treatment and the tumor state. The relative expression of the microRNAs was evaluated by RT-qPCR and expressed using the ΔCq method using a spike-in microRNA mimic for normalization. CR indicates complete remission; ResT, residual tumor; RT, responsive tumor; URT, unresponsive tumor. Data with significant difference between groups are indicated with *($P < .05$) or with **($P < .01$).

The miR-122 would be underexpressed in DLBCL plasma compared with healthy one.[26] Its roles as oncogene or anti-oncogene would depend on the cancer type.[32,33] The let-7e was also chosen for its prognostic value at C0, C2, and C4. This microRNA has been highlighted as tumor suppressor in many cancers[34] and would be downregulated in DLBCL tissue compared with normal tissue.[35] Due to the low number of donors, the results of this first screening need to be analyzed carefully and needed to be validated in a higher number of patients. For its numerous citations in the literature, we also selected miR-21 with a biomarker score of 2.[14,15,17–19] MiR-21 would have an oncogenic role in DLBCL by the targeting of

PTEN[36] and Bcl-2,[37] increasing cell growth and inhibiting apoptosis.

The level of these microRNAs in plasma was finally evaluated in all the samples constituting the plasma biobank of 19 patients with DLBCL. A potential link between patient clinical characteristics (age, sex, WHO classification, stage, and aaIPI) and these circulating microRNA levels at C0 was also analyzed and revealed no significant result.

By considering all the samples collected during treatment course, no link was found between microRNA plasma levels and the prognosis. However, by grouping samples depending on the tumor state and the treatment, some interesting circulating microRNA profiles were highlighted. MiR-21 and miR-197 presented similar expression profiles with a significant higher plasmatic level in patients with tumors unresponsive to treatment. These results highlight a potential clinical use of these circulating microRNAs to monitor the tumor response to treatment. With a higher plasma level in patients with CR, miR-19b, miR-20a, and miR-451 enable to differentiate, at the remission review, patients with residual tumor, from patients with CR.

The origin of these plasma-circulating microRNAs is still unclear. It is well known that a part of these microRNAs are encapsulated in EVs and can be generated by almost all cell types.[38,39] Tumor cells are described to release a major amount of EVs but other cells such as blood cells are known to produce circulating microRNAs.[40] In this study, no correlation was observed between blood count and microRNA levels, suggesting that the observed variations in microRNA levels would not only be attributed to blood count differences.

The major asset of this clinical study is the follow-up of the microRNA plasma level during the course of treatment. Indeed, no trend based on prognosis or molecular subtypes has been observed before treatment in this study. These results need, however, to be confirmed in a larger cohort, but a careful attention should be paid to the pre-analytical settings especially if we consider a pluricentric study. A higher number of patients and samples would enable a more accurate view of the variation of these microRNAs during treatment and tumor evolution. A combination of several circulating microRNAs as biomarkers can also be considered. The measurement of circulating microRNAs also needs to be compared, in terms of sensitivity, specificity, cost, time consumption, and invasiveness, with the current clinical technique of the FDG-PET/CT, used for the evaluation of the tumor extension and the response to treatment.

Conclusions

In conclusion, we highlighted 5 circulating microRNAs whose plasma levels would be worth further investigating for the characterization of DLBCL evolution in patients. The results of this pilot study need to be confirmed on a larger cohort of patients but demonstrates nonetheless the importance of carrying out longitudinal studies to discover new biomarkers.

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Author Contributions

BC, WA, DJ-M, CC, and GC participated in the design of the research. The experiments were performed by BC and GF and all the authors helped for the data analysis and interpretation. This paper was written by BC and reviewed by all the authors for final approval of the version to be published.

Availability of Data and Materials

The data sets generated and analyzed during the current study are available in the ArrayExpress—EMBL-EBI repository under accession number E-MTAB-6063, https://www.ebi.ac.uk/arrayexpress/.

Consent for Publication

Written informed consents for publication were obtained from each participant.

Ethical Approval and Consent to Participate

Written informed consents were obtained from each participant following the requirements of the local research ethics committee (CHU UCL Namur, site Mont-Godinne) which approved the study (ethical agreement number B039201419613).

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