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Circulating U2 small nuclear RNA fragments as a novel diagnostic biomarker for primary central nervous system lymphoma

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Background. Primary central nervous system lymphomas (PCNSLs) are highly aggressive tumors. Chemotherapy has improved prognosis significantly; however, early diagnosis is crucial for effective treatment. Presently, the diagnosis of PCNSL depends on histopathology of tumor biopsies.

We have previously demonstrated differential expression of microRNAs in cerebrospinal fluid (CSF) samples from patients with PCNSL. Based on promising findings about circulating U2 small nuclear RNA fragments (RNU2-1f) as novel blood-based biomarkers for pancreatic, colorectal, and lung cancer, we investigated RNU2-1f in the CSF of PCNSL patients.

Methods. CSF was collected from patients with PCNSL ($n = 72$) and control patients with various neurologic disorders ($n = 47$). Sequential CSF samples were collected from 9 PCNSL patients. RNU2-1f levels were measured by real-time polymerase chain reaction.

Results. Measurement of RNU2-1f levels in CSF enabled the differentiation of patients with PCNSL from controls with an area under the curve (AUC) of 0.909 with a sensitivity of 68.1% and a specificity of 91.4%. The diagnostic accuracy was further improved by combined determination of RNU2-1f and *miR*-21, resulting in AUC of 0.987 with a sensitivity of 91.7% and a specificity of 95.7%. In consecutive measurements of RNU2-1f, which were performed in 9 patients at different stages of the disease course, RNU2-1f CSF levels paralleled the course of the disease.

Conclusions. Our data suggest that the measurement of RNU2-1f detected in CSF can be used as a diagnostic marker and also as a possible marker for treatment monitoring. These promising results need to be evaluated within a larger patient cohort.

Keywords: cerebrospinal fluid (CSF), disease course, primary central nervous system lymphoma, small nuclear RNA (snRNA).

Primary central nervous system lymphomas (PCNSLs) represent approximately 2.2% of primary brain tumors. They are restricted to the CNS and lack systemic disease (Central Brain Tumor Registry of the United States, 2014).[1] PCNSLs are a subcategory of extranodal non-Hodgkin lymphoma and are predominantly of B cell origin.[2] In contrast to most primary malignant brain tumors, the prognosis of PCNSL has improved considerably in recent years. Currently, at least 30% of younger patients can hope for cure of the disease.[3,4] Because PCNSLs are highly aggressive tumors, early diagnosis is crucial for effective treatment and subsequent improvement of prognosis. Despite significant progress in radiological techniques, the diagnosis of PCNSL remains difficult. At present, the histological examination of a tumor specimen, preferably obtained by stereotactic biopsy, represents the gold standard for PCNSL diagnosis.[4] A particular problem arises in clinical cases when the brain biopsy, via stereotaxy, is not suitable due to location of the lesion or when the pathological sample is not sufficient for diagnosis. In such cases, there is a need for adjunctive, noninvasive methods to support the diagnosis in order to minimize the delay of therapeutic decisions. Thus, there is a need to identify noninvasive methods to improve the accuracy and decrease the delay of PCNSL diagnosis.

Present studies demonstrate that more than 30% of vertebrate genomes are transcribed. However, only 1% of these nucleotide sequences represents protein-coding genes; the

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remainder is supposed to embody different types of noncoding RNA (ncRNA) genes.[5] Continuous detection of new noncoding RNAs and their characterizations have renewed the field of human genetics and molecular biology. Over the past 15 years, ncRNAs have become involved in diverse biological processes including cell differentiation, proliferation, and apoptosis.[6]

A noncoding RNA is a functional RNA molecule that is not translated into a protein. ncRNAs can be subdivided into short and long ncRNAs. Long ncRNAs are typically >200 nucleotides long, while small ncRNAs are processed from longer precursors and include microRNAs (miRNAs) or small nuclear RNAs (snRNAs).[7] Although, there is growing knowledge of the importance of ncRNAs in cancer, their clinical usefulness for diagnosis and prognosis is still limited. Presently, only one ncRNA is routinely used in the clinical setting: prostate cancer antigen 3, which is a noncoding antisense transcript that is overexpressed in prostate cancer compared with benign tissue and is used in a urology-based diagnostic assay for patient screening.[8]

We previously detected microRNAs in cerebrospinal fluid (CSF) and identified those molecules as biomarkers for primary diffuse large B-cell lymphoma of the CNS. The combined *miR*-21, *miR*-19b, and *miR*-92a analyses in PCNSL patients demonstrated that CSF levels of miRNAs could differentiate PCNSL patients with high specificity (96.7%) and sensitivity (95.7%) from those with other neurological disorders most importantly from patients harboring inflammatory CNS diseases and those with other CNS tumors.[9,10] Furthermore, the same microRNAs were described to be useful as biomarkers for monitoring the disease course of PCNSL.[11]

In another study, we reported about fragments of U2 snRNA circulating in serum as a novel encouraging diagnostic biomarker for pancreatic ductal adenocarcinoma and colorectal cancer and in bile fluid as a biomarker for cholangiocarcinoma.[12,13] U2 snRNA comprises several proteins: the U2 small nuclear ribonucleoprotein (U2 snRNP), which forms the spliceosome, and 4 other snRNPs. The spliceosome removes introns from a transcribed pre-mRNA.[14]

These findings prompted us to focus on the analyses of RNU2–1f in CSF. In this report, we show for the first time that U2 snRNA fragments are detectable in CSF. In combination with the previously described biomarker for PCNSL in CSF *miR*-21, RNU2-1f can be used as a marker for discriminating patients with PCNSL from controls with higher sensitivity and specificity than previously reported microRNA-based biomarker in CSF. In addition, the determination of RNU2-1f may also serve as a biomarker for monitoring the disease course of PCNSL.

Material and Methods

Patient Characteristics and Cerebrospinal Fluid Samples

Between February 2009 and February 2014, consecutive CSF samples from all patients with PCNSL ($n = 72$) and control patients with various neurological disorders ($n = 47$) sent to the hospital central laboratory for routine chemical analysis and cellular diagnostics. Seventy-two CSF samples were collected from patients with PCNSL (prior to undergoing chemotherapy) by diagnostic lumbar puncture or puncture of Ommaya reservoirs after written informed consent and exclusion of large intracranial mass lesions and/or increased intracranial pressure. (Patient details are listed in Supplementary material, Table S1.) Consecutive CSF samples were collected by diagnostic lumbar puncture or puncture of Ommaya reservoirs from 9 individual PCNSL patients at disease diagnosis, during treatment, and during disease follow-up. (Patient details are listed in Supplementary material, Table S3). The response was evaluated with MRI according to Abrey et al.[15] The volume of lymphoma tissue was measured by an experienced radiologist using RECIST 1.1 criteria, in which only enhancing lesions were considered.[16]

The characteristics of the control group ($n = 47$) are listed in Supplementary material, Table S2. During the study period, no patient with PCNSL at our institution had CNS lesions resulting in obvious alterations of CSF kinetics or hydrocephalus preventing lumbar puncture. Twenty-two serum samples were obtained at the same time point as the CSF samples were collected. (Patient details are listed in Supplementary material, Tables S4 and S5.) All patients were seronegative for HIV. The local ethical committee approved the sample collections (registration numbers 3914-11 and 2012/42b; ethics committees of Ruhr-University of Bochum and Hôpitaux Civils de Colmar, France).

Preparation of CSF and serum samples including RNA extraction were performed and recently described in detail.[9,11]

Complete remission was defined as the disappearance of all contrast enhancements in MRI in the absence of corticosteroids.[15]

Reverse Transcription and Quantification by Real-time PCR

To quantify the concentration of RNU2-1f Qiagen *miR*-1246 qRT-PCR assays (Qiagen) were used following the manufacturer's protocols. In brief, 2 μl of total RNA were used for reverse-transcription reactions (37°C for 60 min, followed by 4°C). Real-time PCR was performed using a CFX Connect™ Real-Time System (Bio-Rad). PCR cycling conditions comprised an initial step at 95°C for 15 minutes followed by 40 cycles at 94°C for 15 seconds, 55°C for 30 seconds, and 70°C for 30 seconds. Fluorescence was measured at the last step of each cycle. Melting curves were obtained after each PCR run and showed single PCR products. To measure the amplification efficiency, we used LinRegPCR (12.x) software (AMC, http://LinRegPCR.nl), a program for examining real-time PCR data. LinReg PCR software determines the efficiency of PCR along every cycle of one run.[17]

To quantify the levels of *miR*-21 and *miR*-24, TaqMan miRNA assays (Applied Biosystems) were performed, as previously published.[9] In brief, 10 μL of total RNA solution was used in reverse-transcription reactions (16°C for 30 min, 42°C for 30 min, 85°C for 5 min, followed by 4°C). Real-time PCR was performed on a 7500 Real-Time PCR System according to the manufacturer's protocol (Applied Biosystems). Cycling conditions were as follows: 95°C for 10 minutes, 40 cycles of 15 seconds at 95°C, and 60 seconds at 60°C. Fluorescent data were converted into cycle threshold (Ct) measurements by the 7500 SDS system software (Version 1.2.3; Applied Biosystems). *MiR*-24 was selected from several control miRNAs because it showed uniform expression levels. The amount of target miRNAs *miR*-1246 (RNU2-1f) and *miR*-24 was normalized relative to the amount of *miR*-24 ($REL_{RNU2\text{-}1f} = \Delta Ct_{RNU2\text{-}1f} - \Delta Ct_{miR\text{-}24}$ and $REL_{miR\text{-}21} = \Delta Ct_{miR\text{-}21} - \Delta Ct_{miR\text{-}24}$) (Supplementary material, Table S1).

All cDNA samples, non-RT (without reverse transcriptase), and no-template controls were assayed in duplicate. Mean Ct values and deviations between the duplicates were calculated for all samples.

Thermostability of RNU2-1f in Cerebrospinal Fluid

To determine thermostability of RNU2-1f in CSF, RNU2-1f was quantified in serum samples stored at room temperature (22°C) for a maximum of 3 days. CSF samples from a patient with PCNSL and a control were thawed and divided into aliquots in separate tubes. RNA was isolated immediately after thawing or after 24 and 72 hours of storage, respectively. RNA was purified with the use of the *mir*Vana miRNA isolation.

Statistics

All statistical analyses were performed using SPSS (version 18) and GraphPad Prism (version 5.0). Group-wise comparisons of distributions of clinical and biological data were performed applying 2-tailed Mann-Whitney *U* tests. Results were considered statistically significant at $P < .05$. Mean $\pm$ SD is displayed in the Fig. 3.

Results

Patient Characteristics

In this study, CSF samples from 72 patients with PCNSL (Supplementary material, Table S1) and 47 control patients with various neurological disorders including CNS inflammation ($n = 35$) (Supplementary material, Table S2) were analyzed. The PCNSL study population comprised 39 male and 33 female patients; age was between 42 and 87 years (mean age: 64.7 y). In all 72 PCNSL patients, a histopathological diagnosis of diffuse large B-cell type lymphoma was established by tumor biopsies. At the time of CSF collection, the disease was newly diagnosed in the majority of PCNSL patients ($n = 61$; 84.7%); a relapse of CNS lymphoma had been diagnosed in 11 of the 72 patients (15.3%). Leptomeningeal lymphoma dissemination was detected by concordant findings of cytopathology and flow-cytometry, as recently reported in 9 (12.5%) of all 72 PCNSL patients (Supplementary material, Table S1). Forty one (58.3%) of 72 PCNSL patients were treated with corticosteroids at the time of CSF sample collection.

Stability of Endogenous RNU2-1f in Cerebrospinal Fluid

RNU2-1f can be straightforwardly measured in CSF. Upon prolonged storage of CSF samples at room temperature RNU2-1f levels did not change significantly (Fig. 1). These experimental findings suggested that RNU2-1f are stable in CSF. This was in accordance with our previous findings for miRNAs.[9]

Diagnosis of PCNSL Based on RNU2-1f Levels in Cerebrospinal Fluid

First, we investigated if RNU2-1f is differentially expressed in the CSF of patients with PCNSL compared with controls. The cross-reactivity of the *miR*-1246 assay with sequence fragments of human U2 snRNA has been recently reported.[12] Due to the observation that the entire mature *miR*-1246 sequence is incorporated into the human U2 snRNA sequence, we employed Qiagen *miR*-1246 qRT-PCR assays for detection of RNU2-1f.

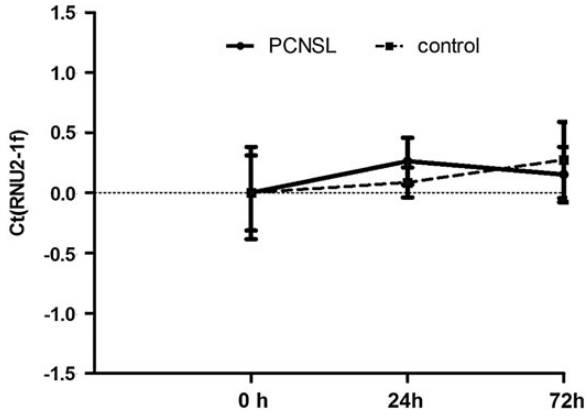

Fig. 1. Stability of endogenous RNU2-1f in cerebrospinal fluid (CSF) samples. To evaluate the thermostability, RNU2-1f was quantified in CSF samples collected from a patient with primary central nervous system lymphoma (PCNSL) and from a healthy control and stored at room temperature (22°C) for a maximum of 3 days. At times indicated, aliquots were removed and assayed for RNU2-1f by qRT-PCR. Data points were calculated by subtracting cycle threshold (Ct)-values (Ct(RNU2-1f)) of untreated samples (baseline level) from relative Ct-values of treated samples at the indicated time points. RNU2-1f yielded stable concentration upon prolonged storage of CSF at room temperature. The measurements were performed in quadruplicate.

Using RNA preparations from CSF from a patient with PCNSL, CSF from a control patient, and serum from a PCNSL patient, the Qiagen qRT-PCR assay generated analogous PCR fragments of the expected size of 80 bp (Fig. 2).

We analyzed CSF samples collected from all PCNSL patients ($n = 72$; Supplementary material, Table S1), all control patients with miscellaneous neurological disorders ($n = 12$; Supplementary material, Table S2), and all patients with CNS inflammation ($n = 35$; Supplementary material, Table S2). Results of these measurements indicated that RNU2-1f showed significantly increased levels (decreased Ct-values) in the CSF of PCNSL patients compared with CSF from both groups of control patients (Table 1). MiRNA and RNU2-1f expression data were normalized utilizing *miR*-24 levels in individual CSF specimens and reported as relative expression levels ($REL_{RNU2\text{-}1f}$), as previously detailed.[9] To detect *miR*-21 and *miR*-24 concentration, TaqMan qRT-PCR assays were employed, as previously described.[9] *MiR*-24 exhibited likely uniform expression levels and a sufficient abundance in CSF of PCNSL and control patients (Table 1).

Next, relative expression levels (RELs) of RNU2-1f were analyzed group-wise, and receiver-operating characteristics (ROC) curves were plotted (Fig. 3A). As shown in Fig. 3B, excellent separation between the groups of PCNSL and control patients was observed with area under the curve (AUC) of 0.909. Corresponding to this analysis, the cutoff CSF REL with the highest accuracy for RNU2-1f was determined as follows: REL of 5.7 with 68.1% sensitivity and 91.4% specificity. The analysis of *miR*-21 with the determined cutoff (REL 8.0) has been previously reported.[9,11] Accordingly, the data are not presented again.

To increase the specificity of discrimination of PCNSL patients from controls, we combined $REL_{RNU2\text{-}1f}$ with $REL_{miR\text{-}21}$ by adding both values up (Fig. 3C). Consequently, the sum of $REL_{RNU2\text{-}1f}$ and $REL_{miR\text{-}21}$ was analyzed group-wise, and ROC curves were plotted (Fig. 3C). As shown in Fig. 3D, excellent separation was observed between the groups of PCNSL and control patients,with an AUC of 0.987. Corresponding to this analysis, cutoff CSF REL with the highest accuracy for $REL_{RNU2\text{-}1f} + REL_{miR\text{-}21}$ was determined as follows: REL of 7.3 with 91.7% sensitivity and 95.7% specificity.

RNU2-1f During the Disease Course of PCNSL

In order to evaluate the possible role of RNU2-1f as a biomarker for disease course, longitudinal studies of RNU2-1f expression levels were performed in the CSF of 9 patients with PCNSL. For this purpose, sequential CSF samples were collected at diagnosis and at different time points during disease course by means of lumbar puncture prior to chemotherapy or at relapse and by CSF sample collection from Ommaya reservoirs (which are routinely used for intraventricular chemotherapy in our treatment protocol).[3,18] The minimal interval between the collected samples was 2 weeks.

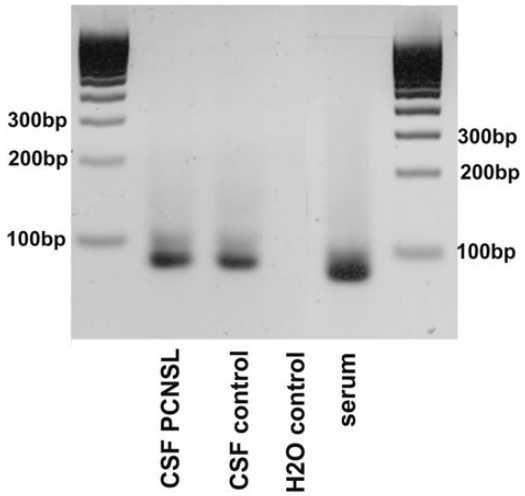

Fig. 2. Gel electrophoresis of PCR amplification products obtained by qRT-PCR with the Qiagen miR-1246 assay using cDNA from cerebrospinal fluid (CSF) of a patient with primary central nervous system lymphoma (PCNSL) and from CSF of a control patient. Serum was used as template for the positive control reaction. H_2O served as negative control. In CSF, the product of 80 bp corresponding to RNU2-1 fragments was found. The identity of the PCR product in serum was previously confirmed by sequencing.

Depending on the disease course, patients were classified into 2 groups: (1) ongoing complete remission of PCNSL ($n = 5$) or (2) recurrent PCNSL ($n = 4$).

Longitudinal REL data of RNU2-1f in the CSF of all 9 patients paralleled well with the clinical courses (Fig. 4). Five PCNSL patients who achieved a persistent complete remission showed a distinct decrease in RNU2-1f expression levels (patients I-V; Fig. 4A). In 3 patients (patients VI, VII, and VIII), transient responses to chemotherapy followed by progressive PCNSL were demonstrated in consecutive MRIs. In accordance, CSF RNU2-1f levels increased during disease progression as determined by qRT-PCR (Fig. 4B). The CSF sample of patient VIII from the time of remission was not available. Finally, increasing levels of RNU2-1f in CSF were observed in one patient who relapsed shortly after a complete remission (patient IX; Fig. 4B). Interestingly, the expression level of RNU2-1f in CSF paralleled the tumor volume as measured in MRIs (Supplementary material, Table S3). These data support the diagnostic value of expression level of RNU2-1f in CSF for monitoring the course of disease. In contrast to RNU2-1f in CSF, standard CSF parameters such WBC and protein concentration did not show consistent changes related to the PCNSL status during treatment and follow-up (Supplementary material, Table S3).

RNU2-1f Levels in Serum

Next, we investigated if expression levels of RNU2-1f in serum differ between PCNSL patients ($n = 14$) and control patients with various neurological disorders (headache, epilepsy, syncope, and stroke; $n = 8$). However, we found no significant differences in expression levels of RNU2-1f in serum as determined by means of qRT-PCR: Ct 22.01 (PCNSL) versus Ct 21.34 (controls) ($P = .39$) (Table 1). Hence, RNU2-1 fragments derived from PCNSL seem to be limited to the CNS compartment including

Table 1. RNU2-1f and *miR*-24 expression in cerebrosphinal fluid and serum of patients with primary central nervous system lymphoma versus control patients

	CSF PCNSL ($n = 72$)		CSF Control ($n = 46$)		*P* Value	Serum PCNSL ($n = 14$)		Serum Controls ($n = 8$)		*P* Value
	Ct[a]	SD[b]	Ct	SD		Ct[a]	SD[b]	Ct	SD	
RNU2-1f	24.78	0.32	29.07	0.33	*<.0001*[c]	22.01	0.43	21.34	0.64	*.39*[c]
miR-24	31.67	0.25	31.30	0.32	*.85*[c]	22.57	0.22	23.18	0.54	*.48*[c]

Abbreviations: CSF, cerebrospinal fluid; Ct, cycle threshold; PCNSL, primary central nervous system lymphoma; SD, standard deviation.
[a]Data are means of CT values (group-wise).
[b]Standard deviation.
[c]The *P* value is for comparison of expression levels of RNU2-1f among PCNSL patients and control patients and was calculated using the Mann-Whitney *U* test.

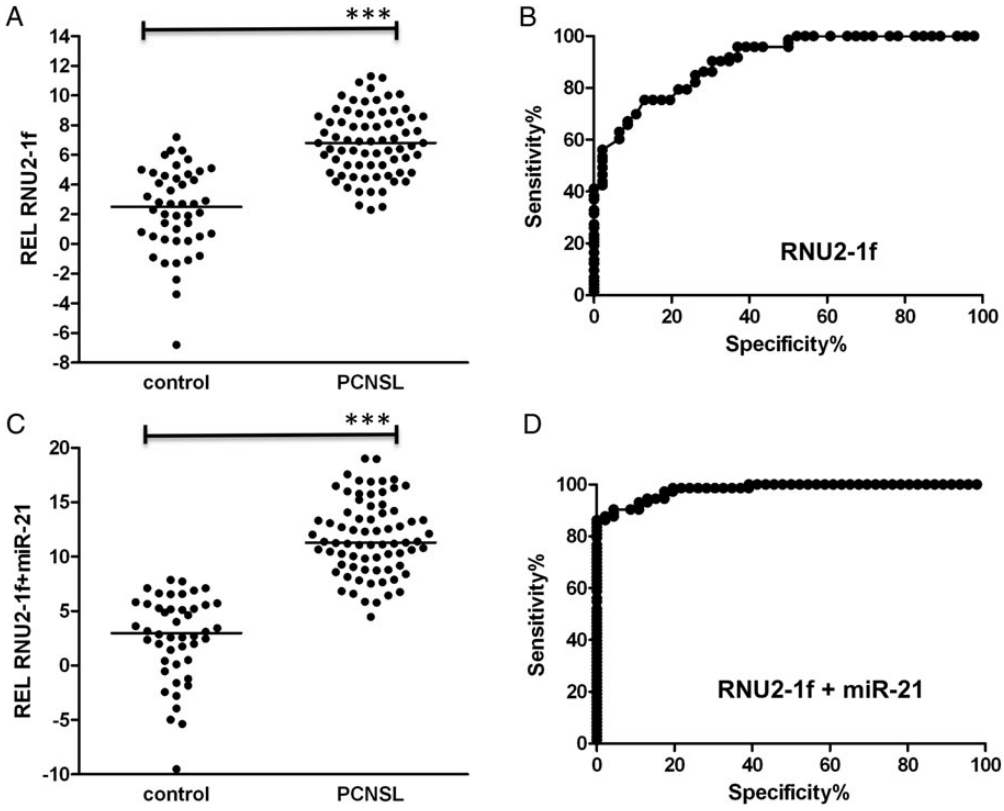

Fig. 3. (A) Scatter plots of expression levels of RNU2-1f in cerebrospinal fluid (CSF) samples from patients with primary central nervous system lymphoma (PCNSL) ($n = 72$) compared with control patients ($n = 47$), including subgroups of patients with miscellaneous ($n = 12$) and with inflammatory ($n = 35$) CNS disorders. Relative expression levels (RELs) of RNU2-1f (y-axis) are normalized to *miR*-24. The black horizontal lines represent median REL values. Group-wise *P* values are indicated as determined in Kruskal-Wallis tests with Dunn's multiple comparisons (***$P < .001$). (B) Receiver-operating characteristic (ROC) curve analyses using relative expression level of RNU2-1f in CSF with an area under the curve (AUC) of 0.909 (95% CI: 0.86–0.96). (C) Scatter plots of expression levels of RNU2-1f + *miR*-21 in CSF samples from patients with PCNSL ($n = 72$), compared with control patients ($n = 47$). Relative expression levels (REL) of RNU2-1f + *miR*-21 (y-axis) are normalized to miR-24. The black horizontal lines represent median REL values. (B) ROC curve analyses using relative expression level of RNU2-1f + miR-21 in CSF with an AUC of 0.987 (95% CI: 0.973–1.000).

CSF, or the difference was blurred by dilution in blood that exhibited a markedly higher volume than CSF.

Discussion

We previously reported that a subgroup of noncoding RNAs is present in CSF.[9] Moreover, characteristic miRNA alterations have been reported to be specific for different kinds of CNS malignancies such as PCNSL and glioma. Combined miRNA analyses demonstrated high sensitivity and specificity in diagnosis of PCNSL and glioma.[9,10] It has also been shown that miRNAs can be used as a biomarker for monitoring the course of disease in PCNSL.[11]

Considering these findings, we raised the question whether RNA molecules representing other subgroups of noncoding RNA are also detectable in CSF and whether these molecules are specifically dysregulated in CSF of PCNSL patients. In previous studies, we reported about fragments of a noncoding U2 small nuclear RNA circulating in serum and bile as a novel diagnostic biomarker for pancreatic ductal adenocarcinoma, colorectal cancer, and cholangiocellular carcinoma.[12,13] Additionally, the increase of circulating RNU2-1f expression was demonstrated for patients with different kinds of malignancies: multiple myeloma, ovarian cancer, esophageal, and gastrointestinal malignancy.[19–22]

Setting out from this point, we found that RNU2-1f is detectable in CSF as well. The qRT-PCR assay from CSF and serum generated the same PCR fragments of 80 bp size (Fig. 2). Moreover, our results demonstrated that RNU2-1 fragments circulating in CSF might serve as novel biomarkers for CNS tumors such as PCNSL. We found that the levels of RNU2-1f, as measured in qRT-PCR assays, were significantly increased in CSF samples from patients with PCNSL (Table 1). RNU2-1f CSF levels could distinguish, with considerable sensitivity (68.1%) and specificity (91.5%), patients with PCNSL from other neurological disorders. RNU2-1f had a significant diagnostic value for PCNSL and yielded an AUC of 0.91 in ROC analyses (Fig. 3).

Based on the promising data of our previous study, we investigated the diagnostic value of a combination of RNU2-1f and *miR*-21 and demonstrated that it provides, when compared with RNU2-1f, superior separation of PCNSL and controls with

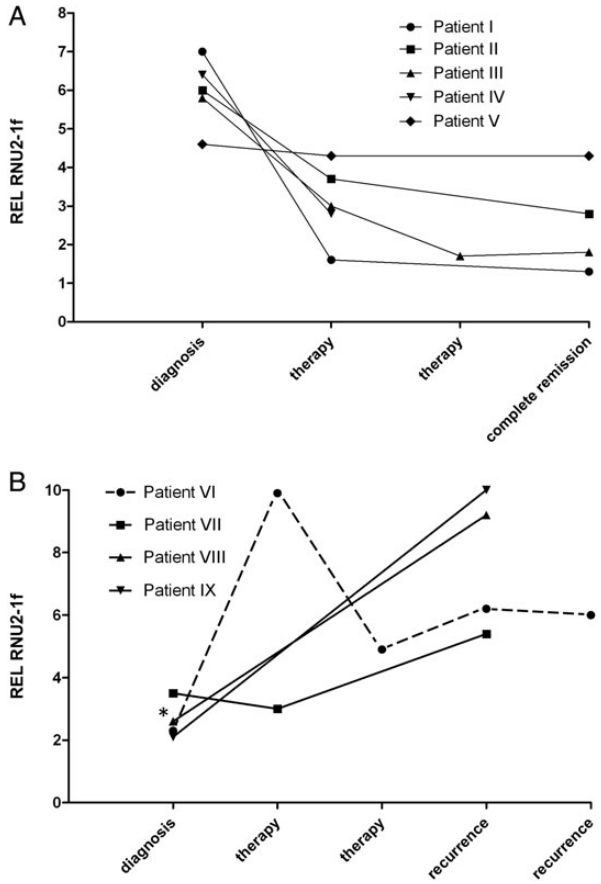

Fig. 4. CSF RNU2-1f expression levels in time courses during primary central nervous system lymphoma (PCNSL) disease of 9 patients. (A) Five patients (I–V) with complete remission, and (B) 3 patients (VI–VIII) with recurrence after initial remission, and one patient (IX) relapsing after complete remission. The time intervals between the samplings are given in Supplementary material, Table S3. *The first sample of patient IX was collected during initial lymphoma remission.

a sensitivity of 91.7% and specificity of 95.7% (AUC of 0.987 in ROC). On one hand, the diagnostic value of $REL_{RNU2\text{-}1f} + REL_{miR\text{-}21}$ seems to be comparable with the previously reported combined analysis of *miR*-21, *miR*-19b, and *miR*-92 (95.7% sensitivity, 96.7% specificity).[9] On the other hand, the determination of $REL_{RNU2\text{-}1f} + REL_{miR\text{-}21}$ level appears to be easier to perform because only 3 markers need to be quantified: RNU2-1f, *miR*-21; and *miR*-24 vs *miR*-21, *miR*-19b, *miR*-92, and *miR*-24 as previously described.[9]

Furthermore, we analyzed RNU2-1f levels in the CSF of individual PCNSL patients at different time points following diagnosis, and we demonstrated that the expression of RNU2-1f seems to correspond to the course of disease (Fig. 4, Supplementary material, Table S3). Interestingly, the CSF RNU2-1f levels appeared to parallel the tumor volume as measured by MRI (Supplementary material, Table S3). Notably, in contrast to RNU2-1f, standard CSF parameters such as WBC and protein concentration did not show consistent changes in agreement to the PCNSL status during treatment and follow-up (Supplementary material, Table S3).

Due to the heterogeneous courses of disease and the limited number of available samples, these data needs validation in a larger cohort sample and should be interpreted with caution.

Pospisil et al and our own study have investigated expression levels of PCNSL biomarker miRNAs in serum and found no significant differences in expression levels of miRNA in serum from PCNSL patients compared with serum from control patients.[23,24] On the other hand, Mao et al demonstrated increased concentration of *miR*-21 in the serum of patients with PCNSL compared with controls.[25] Twelve microRNA candidates were identified by next-generation sequencing of blood samples and were shown to be prognostic biomarkers for PCNSL patients.[26] In the current study, we investigated whether the distribution of snRNAs is also limited to the CNS compartment. Therefore, we compared the expression of RNU2–1f in the serum of patients with PCNSL with the serum of controls. In a manner similar to that for miRNAs, we found no differences between the 2 groups (Table 1). Thus, RNU2-1 fragments, as well as miRNAs derived from PCNSL, are limited to the CNS compartment including CSF.

We also demonstrated high stability of RNU2-1f in CSF towards various storage settings. We had previously observed a distinct stability of RNU2-1f in serum towards nuclease and proteinase treatment.[12] This resistance may be caused by the inclusion of RNU2-1f in apoptotic bodies or binding to Argonaute2.[12,20]

To our knowledge, this is the first observation of deregulated small nuclear RNA expression in CSF samples from patients with PCNSL. Considering the high diagnostic value of RNU2-1f as a biomarker, especially combined with a known PCNSL biomarker *miR*-21, we anticipate RNU2-1f analyses to be an important step to precisely characterizing CSF markers for PCNSL. Furthermore, this study provides the first evidence that fragments of RNU2-1 in CSF could be used as indicators of treatment response. Relevant limitations of our study are the small sample size of patients in the study part. Our finding of RNU2-1f and combination of RNU2-1f with *miR*-21 as promising CSF biomarkers for differentiation of PCNSL and for therapy response definitely needs to be validated in a larger study.

Supplementary Material

Supplementary material is available at *Neuro-Oncology Journal* online (http://neuro-oncology.oxfordjournals.org/).

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Conflict of interest statement. The authors declare no competing financial interests.

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