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ORIGINAL ARTICLE

Evaluation of cerebrospinal clonal gene rearrangement in newly diagnosed non-Hodgkin's lymphoma patients

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Abstract

Overt central nervous system (CNS) involvement in aggressive non-Hodgkin's lymphoma (NHL) is rare at diagnosis. Much effort is put to identify risk factors for occult CNS involvement, and the risk assessment of CNS relapse. Prophylactic treatment carries risk of adverse events and its efficacy is not clear. Detection of cerebrospinal fluid molecular gene rearrangement (GRR) as a method to detect occult disease has been studied in acute leukemia and primary CNS lymphoma. To date, the capacity of a positive GRR in newly diagnosed NHL patients to predict CNS relapse has not been addressed. We retrospectively studied the prognostic value of GRR in cerebrospinal fluid samples of 148 newly diagnosed patients with high grade NHL. We demonstrate that positive GRR at diagnosis does not affect PFS or OS and did not predict CNS relapse. However, although numbers were small, repeated positive samples (≥ 2) correlated with a higher risk for CNS relapse ($p = 0.048$), possibly stressing the need for an aggressive preventive approach.

Keywords Non-Hodgkin lymphoma · Gene rearrangement · CSF · Central nervous system (CNS)

Introduction

Somatic V-(D)-J gene rearrangement (GRR) of the immunoglobulin heavy chain (IgH) and the T cell receptor chain (TCR) which occurs during the maturation of B cells and T cells provide a unique clonal signature. GRR has been utilized to improve diagnostic accuracy in cases with uncertain histology suspected for lymphoma [1]. GRR is able to detect occult bone marrow (BM) involvement by lymphoma. Notably, histologic negative cases with positive GRR in the BM had a worse progression-free survival (PFS) and overall survival (OS) [2]. A previous analysis by our group has found GRR in the CSF of patients with isolated CNS lymphoma to strongly correlate with disease occurrence. Concordant results for both cytology and PCR studies were present in 46 (73%) of the samples. Sixty-seven percent of patients considered to be in an active disease phase, but with no cytology or neuro-imaging studies demonstrating leptomeningeal involvement, showed a positive GRR result. In 95% of patients responding to treatment, GRR was negative [3]. In patients with acute lymphoblastic leukemia, testing for CSF GRR was shown to be superior to cytology for the detection of occult disease [4, 5]. The risk of CNS involvement in non-Hodgkin's lymphoma varies by type and certain recognized risk factors, such as bone marrow involvement, extra-nodal sites as well as others, generating a score termed CNS international prognostic index (CNS-IPI), ranking patients to low, intermediate, and high risk for CNS relapse [6]. To date, no study evaluated the role of CSF GRR at diagnosis in predicting CNS involvement, late relapse, and decisions regarding CNS directed therapy. In the current study, we evaluated the prognostic value of CSF GRR of patients with newly diagnosed NHL, with no evidence for CNS involvement by neuro-imaging or cytology studies. Follow-up samples from repeated CSF tested were also analyzed. We correlated GRR results with clinical data at presentation including known risk factors for CNS involvement, response to treatment, and systemic and CNS relapse rate.

Boaz Nachmias, Veronica Sandler, Neta Goldschmidt and Moshe E. Gatt contributed equally to this work.

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Patients and methods

The retrospective cohort included aggressive non-Hodgkin lymphoma patients who were treated at Hadassah Medical Center between 1999 and 2012. Patient cohort included patients at high risk for CNS involvement, as those were designated for CSF evaluation and prophylactic treatment according to common practice guidelines. Patients diagnosed with CNS involvement (primary CNS lymphoma, or as part of a systemic disease), i.e., diagnosed parenchymal CNS disease or cytology proven leptomeningeal disease, were excluded. Patients had CSF cytology and GRR analysis at diagnosis and upon each subsequent intrathecal (IT) treatment. Thus, a patient may have had more than one sample tested. A lumbar puncture (LP) was performed at the discretion of the treating physician, to assess for the presence of lymphoma and to administer chemotherapeutic prophylaxis with methotrexate (12 mg) or cytarabine (50 mg). Patient records were reviewed for demographics and disease characteristics. Stage, ECOG performance status, and IPI (international prognostic index) were determined as previously described [7]. Standard criteria were used to evaluate response to treatment [8]. A complete response (CR) had to be maintained for 6 months to be termed CR. CSF is routinely analyzed in our center for cell count and cytology (both by H&E staining reviewed by pathologists and by Wright Giemza stains reviewed by hematologists), protein and glucose levels, and PCR analysis for GRR.

The retrospective data collection was approved by the local ethics committee in accordance with the Helsinki declaration standards and provided informed consent to participate in the study.

Detection of clonal GRR by PCR analysis

DNA was extracted from the CSF using the QIAamp DNA Mini Kit (Qiagen). The average DNA concentration in extracted samples was 6.03 ng/μl (SD ± 5.62; total volume 50 μl) as detected by NanoDropOne (Thermo Scientific). Five-microliter DNA was subjected for each PCR reaction. A positive, negative, and no DNA (water blank) controls were included in all experiments. The method sensitivity was previously assessed for mixed genomic DNA: this method was able to detect one malignant cell (from a cell line) diluted in 10^4–10^5 in normal peripheral lymphocytes [3]. PCR for gene rearrangement of B cells was performed using the following primers: JH–antisense, VH1, VH2, VH3, VH4, VH5, VH6–sense, as previously described [3]. The screening for TCR-γ rearrangement was performed using a set of single and duplex PCR reactions [9].

Statistical analysis

The characteristics of patients with a positive and negative GRR results were compared using T test for categorical variables and Mann–Whitney *U* test for continuous variables as the data was not normally distributed. Kruskal-Wallis test was used for comparison of 3 groups or more. Correlation of clinical characteristics, clinical outcome, and CSF GRR status at diagnosis were calculated using v2 and Fisher exact tests. The Kaplan–Meier curves and Log-Rank test were used to determine progression-free survival (PFS) and overall survival (OS). PFS and OS were determined from the date of diagnosis until progression or relapse for PFS and until last follow-up or death for OS.

Results

Patients

The study cohort included 374 sample obtained from 148 aggressive NHL patients; diffuse large B cell lymphoma (DLBCL) (n = 109), Burkitt lymphoma (BL) (n = 6), Mantle cell lymphoma (MCL) (n = 4), and peripheral T cell lymphoma (PTCL) (n = 29). Patient baseline characteristics are shown in Table 1. Fifty-eight women and 90 men were included and the median age was 54 years (range 28–88 years).

Table 1 Patient baseline characteristics

Gender	
Male	60.9% (n = 90)
Female	39.1% (n = 58)
Median age	54 (28–88 years)
Disease	
DLBCL	73.7% (n = 109)
BL	4% (n = 6)
MCL	2.7% (n = 4)
PTCL	19.6% (n = 29)
BM involvement	63.5% (n = 94)
Stage IV	73% (n = 108)
EN sites	75.6% (n = 112)
Paranasal/epidural/testicular involvement	5.4% (n = 8)
Treatment	
CHOP based	90% (n = 133)
High-dose cytosar	6.7% (n = 10)
Other	3.3% (n = 5)
Rituximab (of B cell lymphoma)	97% (n = 116)
HDMTX	47.2% (n = 70)
Median IT injections	3.4 (SD = 1.55)

DLBCL, diffuse large B cell lymphoma; *BL*, Burkitt lymphoma; *MCL*, mantle cell lymphoma; *PTCL*, peripheral T cell lymphoma; *BM*, bone marrow; *EN*, extra-nodal; *CHOP*, cyclophosphamide, adriamycin, vincristine, prednisone; *HDMTX*, high-dose methotrexate; *IT*, intrathecal

Most patients (n = 108, 72.3%) had a stage 4 disease. Thirty seven percent of patients had bone marrow involvement, and 63.5% had extra-nodal involvement. All patients were considered high risk for CNS involvement by the treating physician and were referred for CSF evaluation. Each patient had a median of 3 (range 1–7) CSF samples for analysis, at diagnosis and during treatment the disease course. Accurate retrospective implementation of the current CNS-IPI [6] on DLBCL patients in our cohort was feasible for 100 of 109 patients. Seventy-five percent of the patients had an intermediate to high risk of CNS disease. However, there was no difference between the two groups (p = 0.9). The majority received CHOP based therapy (91%) and 80% of the B cell NHLs received rituximab. Seventy (47%) of the patients received additional CNS systemic prophylaxis with high-dose methotrexate (HDMTX), and all patients received prophylaxis IT injections, with a median of 3 treatments (range 0–7).

Correlation of the GRR status at diagnoses, risk factors, response to treatment, and PFS

Patients were stratified according to the GRR results (Fig. 1). Of 109 DLBCL patients, 40 (36%) had a positive GRR result, and of the other 39 aggressive NHL patients, 16 (41%) had a positive GRR. No statistically significant factor of any demographic or clinical finding was found in correlation with positive GRR result, including disease stage and BM involvement and CNS-IPI (Table 2). Abnormal CSF protein and glucose levels did not correlate significantly with either GRR-positive samples.

Treatment protocols also did not significantly differ between the GRR-positive and GRR-negative groups: CHOP-based protocols (91% vs. 89%, p = 0.34), HDMTX treatment (53% vs 46%, p = 0.24), rituximab (95% vs. 98% of only the B cell lymphoma subgroup), and median number of IT treatments (3.4 vs 3, p = 0.11), respectively (Table 2).

Overall hematologic response to therapy was similar between the GRR-positive and GRR-negative groups (Table 3, CR 87.5% vs. 81.5, p = 0.65, respectively) and no significant difference was found in the median PFS (75 m vs. 80 m, p = 0.85) and OS (140 m vs. 91 m, p = 0.79) between the GRR-positive and GRR-negative groups, respectively (Fig. 2). Nine patients had CNS relapse. The GRR status did not correlate with time to CNS relapse (123 days vs 211 days p = 0.96 in positive vs. negative GRR, respectively) (Fig. 3a).

Prophylactic HDMTX treatment reduces risk of systemic but not CNS relapse in DLBCL patients

Of the 109 DLBCL patients, 47 received prophylactic HDMTX. Prophylactic high-dose methotrexate has been administered as 2 treatments of 3.5 mg/m2 over 3 h. Treatments were usually given at the interval of the initial R-CHOP treatments, 14 days from the administration of R-CHOP, allowing for uninterrupted R-CHOP treatment schedule. The risk for CNS relapse did not differ between patients receiving HDMTX in comparison to those not receiving the HDMTX (9% vs. 7.4%, p = 1, respectively, Table 4). All of the patients experiencing a CNS relapse received treatment with rituximab. Treatment with rituximab was not found to be significantly different between systemic relapsed patients with or without the addition of HDMTX treatment (87% vs. 71% respectively, p = 0.87). The risk of systemic relapse was lower among patients receiving HDMTX compared with patients who did not (29% vs 57.4%, p = 0.003, respectively, Table 4).

Risk of CNS relapse is higher in patients with two or more positive GRR samples

The median sample tested per patient was 3 (range 1–7). Of the overall study patient population, 19 patients had two or more positive GRR samples. When we compared the risk for CNS relapse between patients with a single positive GRR to patients with two or more positive GRR samples, a significantly higher rate of CNS relapse was detected in patients with two or more positive samples (Fig. 3b, 2.7% vs. 15.7%, p = 0.048, respectively). No difference in the number of IT treatments was noted between the two groups. No difference in systemic relapse or OS was noted between the two groups (Fig. 3c, d). Similarly, in the subgroup of DLBCL patients only, two or more GRR-positive samples showed a trend towards a higher CNS relapse rate (p = 0.07). When comparing only DLBCL patients with intermediate-HR CNS-IPI, patients with two or more GRR-positive samples had a statistically

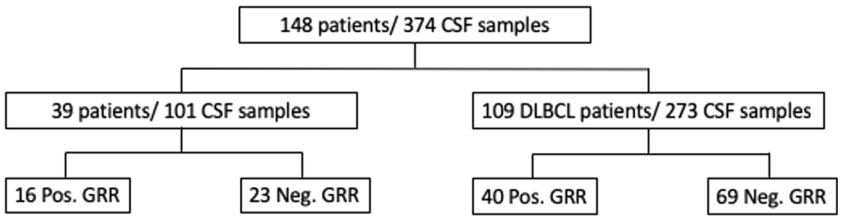

Fig. 1 Study scheme

Table 2 Patient's characteristics according to the GRR status

Patient characters		Pos. GRR	Neg. GRR	*p* value
Age (years)		54	55	0.66
Type	DLBCL	71.4% (*n* = 40)	75% (*n* = 69)	0.63
	BL	3.6% (*n* = 2)	4.3% (*n* = 4)	
	MCL	3.6% (*n* = 2)	2.1% (*n* = 2)	
	PTCL	21.4% (*n* = 12)	18.5% (*n* = 17)	
Stage	I–II	16%	14.3%	0.83
	III–IV	84%	85.7%	
BM involvement		41.1%	34.1	0.12
EN involvement		80.4%	72.9%	0.22
CNS-IPI risk	Low	27%	23.5%	0.9
	Intermediate	50%	51%	
	High	23%	25.5%	
Treatment protocol	CHOP based	91.1%	89.1%	0.34
	HD cytosar	8.9%	5.4%	
IT injections (median)		3.43 (0–6)	3.01 (1–7)	0.11
HDMTX		53.6%	43.5%	0.24
Rituximab (of B cell lymphoma)		95% (*n* = 42)	98% (*n* = 74)	0.39
CSF	Glucose (mmol/L)	3.65	4.14	0.41
	Protein (mg/L)	500	489	0.79
	RBC)/mm^3)	67	64	0.61

GRR, gene rearrangement (B cell receptor or t cell receptor); *DLBCL*, diffuse large B cell lymphoma; *BL*, Burkitt lymphoma; *MCL*, mantle cell lymphoma; *PTCL*, peripheral T cell lymphoma; *BM*, bone marrow; *EN*, extra-nodal; *HDMTX*, high-dose methotrexate; *IT*, intrathecal

significant higher rate for CNS relapse compared with 0–1 positive samples (37.5% vs. 8.3%, $p = 0.04$, respectively).

Discussion

Occult CNS involvement is often undetectable or missed at diagnosis of NHL. Risk factor scales, such as the CNS-IPI, were developed to identify the patients at high risk for CNS involvement and offer indications for additional CNS directed preventive therapy [6]. Unfortunately, some patients are underestimated as having a low risk for CNS relapse, or may receive unnecessary prophylactic treatment with additional excess toxicity. Moreover, the value of methotrexate augmented therapy is not fully validated [10]. CSF cytology is often used to assess involvement, but usually is positive only upon overt CNS disease and requires a significant number of cells. Others have utilized flow cytometry analysis of CSF and demonstrated a superior sensitivity compared with cytology [11, 12]. Detection of clonal GRR has been studied due to its high specificity and sensitivity, as a method to detect occult disease, or monitor minimal residual disease [4]. Our group has previously studied CSF GRR in patients with primary CNS lymphoma. We found that the specificity of this test is very high (97%) when evaluating and monitoring active CNS lymphoma, and it is comparable to the specificity of a positive cytology. However, the sensitivity was only 54%, indicating that multiple evaluations may be required to verify the presence of active disease. Comparing the sensitivity of both cytology and the PCR testing for GRR, it is clear that PCR is superior to cytology, whose sensitivity was only 25% [3]. Several other studies examined the feasibility of GRR detection in CSF samples from systemic lymphoma patients; however, no clinical correlation of the data was presented [13]. To the best of our knowledge, this is the first report that evaluates screening for occult CNS involvement by GRR in newly diagnosed NHL patients. Our cohort included 148 patients, evaluated 374 samples over a 13-year period, all considered with no evidence of involvement by standard cytology technics. Over 80% of the patients were with advanced stage

Table 3 Response to therapy according to GRR status

Response to therapy	Pos. GRR	Neg. GRR	*p* value
CR	87.5% (*n* = 49)	81.5% (*n* = 75)	0.65
PR	3.6% (*n* = 2)	8.7% (*n* = 8)	
SD	0	1.1% (*n* = 1)	
PD	8.9% (*n* = 5)	8.7% (*n* = 8)	

GRR, gene rearrangement (B cell receptor or t cell receptor); *CR*, complete remission; *PR*, partial remission; *SD*, stable disease; *PD*, progressive disease

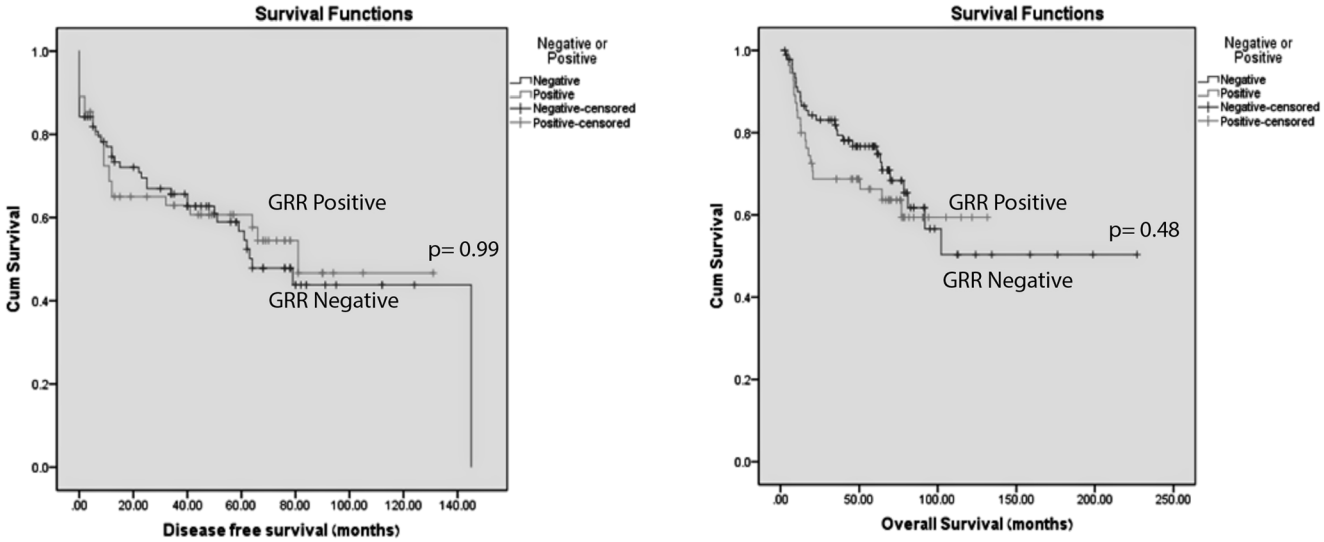

Fig. 2 Disease-free survival and overall survival according to GRR status

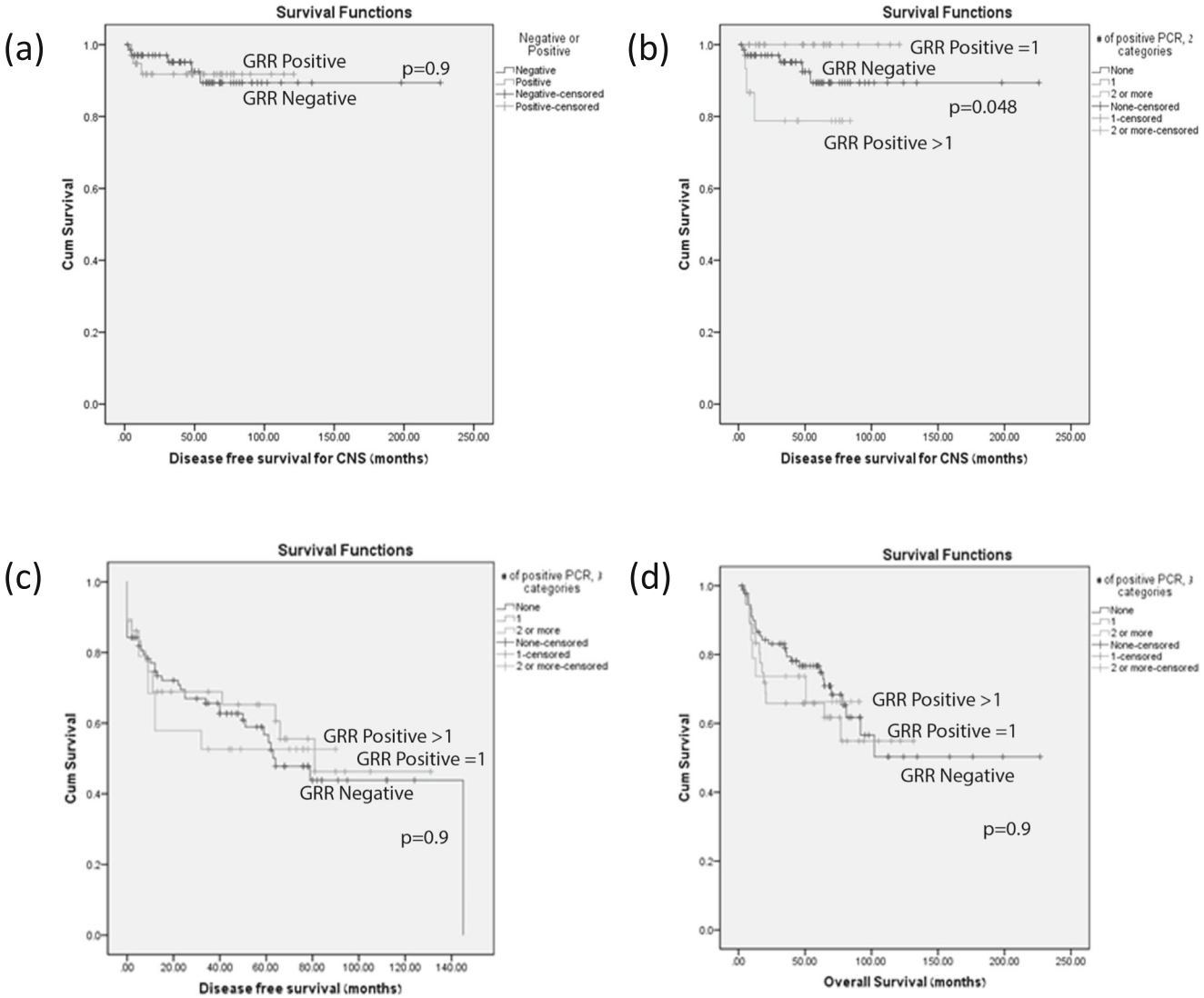

Fig. 3 **a** Disease-free survival for CNS according to GRR status. **b** Disease-free survival for CNS survival according to number of positive GRR samples. **c**, **d** Disease-free survival and OS according to number of positive GRR samples

Table 4 CNS and systemic relapse rate according to HDMTX treatment in DLBCL patients

HDMTX treatment	CNS relapse	*p* value	Systemic relapse	*p* value
Yes	9% ($n = 5$)	1.0	29% ($n = 16$)	0.003
No	7.4% ($n = 4$)		57.4% ($n = 31$)	

DLBCL, diffuse large B cell lymphoma; *BL*, Burkitt lymphoma; *HDMTX*, high-dose methotrexate; *CNS*, central nervous system

disease, EN involvement and 40% had BM involvement. While, the criteria for CNS prophylaxis were not well define during the study period, all patients were deemed with a high risk for CNS relapse and thus underwent LP at diagnosis, including CSF GRR analysis, and received chemotherapy preventive treatment. Accordingly, implanting the CNS-IPI [6] on our cohort confirms that most of the patients were considered intermediate to high risk for CNS relapse. The study design creates a clear selection bias for patients at high risk for CNS relapse, and the results should be viewed in this regard.

When we compared the patient's characteristics at diagnosis between those with GRR-positive and GRR-negative samples, no statistically significant difference was noted in correlation to the known risk factors for CNS involvement. This discrepancy might be in part due to this selection bias for high-risk patients. It is likely that low risk patients will have significantly less GRR-positive CSF samples; however, this was not tested. Similarly, the response to the systemic treatment, PFS, and OS did not differ between the two groups.

Six percent of the patients developed CNS relapse, consistent with that reported in the literature [6]. Our findings highlight that a single positive CSF GRR is neither sensitive nor specific to predict CNS relapse. Repeated positive CSF GRR (≥ 2) showed a statistically significant higher rate of CNS relapse. The sensitivity of two or more positive tests to predict CNS relapse was low at 33% (7.49%–70.07%, 95% CI); however, the specificity was 88.5% (81.98%–93.28%, 95% CI).

This may suggest that patients with two consecutive positive samples were resistant to the IT preventive chemotherapy, whereas those with a single positive GRR represent the subgroup that responded well to this protective therapy. This hypothesis is further strengthened when calculating the CNS-IPI which was higher ($p = 0.04$) for this subgroup. Another possibility is that a single positive GRR may be a false-positive test, and only a repeated positive GRR confirms the validity of the assessment.

Results of the CSF GRR were not readily available for the treating physician and did not affect real-time decisions regarding IT treatments, precluding the possibility of selection bias of positive patients.

CNS prophylaxis often includes systemic HDMTX. Several studies provided evidence for the use of HDMX as prophylaxis for CNS relapse. In a single arm study of 65 high risk for CNS disease DLBCL patients, the addition of HDMTX (3.5 g/m2) to R-CHOP resulted in a low rate of CNS relapse as compared with historical similar patient. PFS and OS were similar [14]. Another study, compared R-CHOP combined with either IT MTX or IT MTX and 2 cycles of systemic HDMTX to a more aggressive protocol (hyper-CVAD or CODOX-M/IVAC (cyclophosphamide, vincristine, doxorubicin, and high-dose methotrexate; alternating with ifosfamide, etoposide, and cytarabine) with IT methotrexate. The rate of CNS relapse was significantly lower in the groups incorporating systemic CNS prophylaxis, again advocating this approach [10]. Ferreri et al. retrospectively evaluated the benefit of CNS prophylaxis in high-risk CNS-IPI patients, and again demonstrated a risk reduction of CNS relapse [15]. Other studies reported no added benefit for HDMTX in reducing the CNS relapse rate [16], suggesting that better control of the systemic disease is achieved [17, 18]. Of the 109 DLBCL patients in our study, 55 received HDMTX. We did not observe any reduction in CNS relapse rate in patients who received HDMTX treatment. Notably, however, in accord with similar results published by our group [19], HDMTX decreased systemic relapse. Small numbers and lack of randomization preclude clear conclusions on the utility of this approach.

In conclusions, the focus of our work was to study the ability of positive CSF GRR at diagnosis to predict CNS relapse. Our findings highlight that a single positive CSF GRR is neither sensitive nor specific to predict CNS relapse. Repeated positive tests are more specific for CNS relapse and might in fact suggest a resistant disease, possibly stressing the need for an aggressive preventive approach.

Compliance with ethical standards

The retrospective data collection was approved by the local ethics committee in accordance with the Helsinki declaration standards and provided informed consent to participate in the study. All patients gave their informed consent prior to their inclusion in the study.

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