High Sensitivity Quantitative Allele Specific RHOA G17V PCR for Diagnosis, Staging and Minimal Residual Disease Assessment in Angioimmunoblastic T-Cell Lymphoma

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Abstract: Angioimmunoblastic T-cell lymphoma (AITL) is a T-cell lymphoma, characterized by abundant polymorphocellular infiltrate of lymph nodes with the small number of tumor CD4+ Tfh-cells. AITL could often be misdiagnosed as reactive processes and other lymphomas, including Hodgkin's lymphoma and diffuse large B-cell lymphoma (DLBCL). We used quantitative allele-specific PCR with LNA (locked nucleotide acid) modified primers (qAS-PCR-LNA) for RHOA G17V mutation assay. Sensitivity of determination (0.02%) was sufficient for minimal residual disease (MRD) monitoring and evaluation of tumor cell number in different tissues. Method proposed demonstrated sensitivity superior to histology and PCR-based clonality determination. RHOA G17V mutation in lymph nodes was detected in 53% (32 of 62) patients with AITL. In control group (n-110) we have revealed RHOA G17V mutation in 3 patients with Hodgkin's lymphoma (HL) and 1 patient with diffuse large B-cell lymphoma (DLBCL). Three patients with HL had clonal CD4+ T-lymphocytes population with aberrant immunophenotype in blood and clonal rearrangements of TCRG and/or TCRB genes in lymph nodes. We have shown that RHOA G17V can be used as a screening marker for patients with lymphadenopathy to exclude AITL or PTCL NOS. The persistence of tumor cells with RHOA G17V mutation was shown in most patients (12 of 16 -75%) with AITL after the induction chemotherapy and during the maintenance therapy (5 of 7 - 71.4%). Therefore qAS-PCR-LNA can be enrolled into standard protocols for management of patients with AITL to assess the effectiveness and the duration of antitumor therapy.

Keywords: Angioimmunoblastic T-cell lymphoma, PCR; RHOA, Point mutation, Hodgkin's lymphoma, Diffuse large B-cell lymphoma, Minimal residual disease.

INTRODUCTION

Angioimmunoblastic T-cell lymphoma (AITL) presents a certain complexity for routine diagnosis. This lymphoma was considered a non-tumor disease for a long time due to unusual clinical autoimmune manifestations and abundant reactive component [1-2]. With the spread of immunophenotyping and molecular diagnostics, it became apparent that this disease is a T-cell lymphoma. Though small number of tumor CD4 + T cells with markers of follicular T-helpers (CD10, CXCL13, BCL-6, PD-1, ICOS) is found by the morphological study, reactive component usually predominates and may be represented by B- and T-lymphocytes, plasma cells, histiocytes, eosinophils and immunoblasts (some may look like Hodgkin or Reed-Sternberg cells) or a network of follicular dendritic cells [3-5]. There are objective difficulties in the differential diagnosis of AITL and Hodgkin's lymphoma (HL), diffuse large B-cell lymphoma (DLBCL), and reactive proliferation in lymph nodes with expansion of the paracortical areas [4-6]. There are numerous questions related to AITL staging, identification of prognostic groups, required treatment duration and evaluation of treatment efficacy [7-11]. Recently discovered point somatic RHOA G17V mutation is present in 53-71% of angioimmunoblastic T-cell lymphomas and 13-18% peripheral T-cell lymphoma not otherwise specified (PTCL-NOS) [12-15]. RHOA G17V mutation leads to an amino acid substitution in the GTP-binding region of the RHOA protein and its inactivation, which disrupts the transmission of the signal from the T-cell receptor that can be a trigger mechanism of AITL development [13, 16]. It is shown that unlike point mutations in the TET2 and DNMT3A genes RHOA G17V mutation in AITL is detected in tumor cells only, but not in other hematopoietic cells, thus allowing quantitative assessment of tumor cells [12]. Still it is not completely clear if this marker is specific for AITL exclusively. We used allele-specific PCR with LNA (locked nucleotide acid) modified primers (qAS-PCR-LNA) with increased sensitivity of RHOA G17V mutation determination. Substantial numbers of patients with AITL, HL including elderly, DLBCL, reactive changes of lymph nodes, indolent B-cell lymphomas had been tested proving the feasibility of this marker for diagnosis and residual tumor clone monitoring in AITL.

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MATERIALS AND METHODS

Patients

The study included 62 patients with AITL, diagnosed according to WHO criteria, 2017 [3]. The median age was 62 years (29-87), the ratio m:f – 37:25. The control group included patients with Hodgkin's lymphoma <35 years old (n - 35), ≥35 years old (n - 26), DLBCL (n - 15), reactive changes of lymph nodes (n - 14), indolent B-cell lymphomas – chronic lymphocytic leukemia (CLL) and follicular lymphoma (FL) (n - 20). Characteristics of patients and samples can be found in Table **1**.

To verify the diagnosis, histological and immunohistochemical (IHC) studies of a tumor sections were performed with an extended antibody panel (CD2, CD3ε, CD4, CD5, CD7, CD8, CD10, CD20, CD21, CD23, CD30, PD1, CXCL13, BCL6, PAX-5, Ki67, EBER (in situ hybridization)). Flow cytometry was performed on a FACS Canto II device (BD Biosciences) using monoclonal antibodies to CD3, CD16, CD56, CD2, CD5, CD7, CD4, CD8, CD10, CD279. T-and B-cell clonality was assessed using BIOMED-2 protocol for TCRG, TCRB, IGH genes rearrangements [17]. The fragment analysis of PCR products was performed on an automatic nucleic acid analyzer ABI PRISM 3130 Genetic Analyzer (Applied Biosystems, USA).

Quantification of RHOA G17V by Allele-Specific PCR

Quantitative allele-specific (qAS) TaqMan Real-Time PCR assays were designed with unmodified forward primers: WT TGGTGATGGAGCCTGTAG, MT TGGTGATGGAGCCTGTAT, and with LNA modified nucleotide at the 3' end (in brackets) WT-LNA TGGTGATGGAGCCTGTA(G), MT-LNA TGGTGATGGAGCCTGTA(T). For both assays fluorescent probe and reverse primer were the same: FAM-CAAGGACCAGTTCCCAGAGG-BHQ1, GCTTTCCATCCACCTCGATA. Figure **1** (Supplementary material) contains schematic representation of allele-specific PCR with conventional and LNA-modified primers. 200-400 ng of DNA was added to 25 µl of the reaction mixture (Syntol, Russia) containing 10 pmol of WT or MT forward primer, 10 pmol of the reverse primer, 7.5 pmol of the probe. The PCR analysis was carried out in triplicates (3WT + 3MT). qAS-PCR and data analysis were performed on the Step One Real-Time PCR System (Applied Biosystems, USA). For qAS-PCR with unmodified primers PCR conditions were: preliminary denaturation of 95° (5 min), 50 PCR cycles - 95°(30 s), 56° (15 s), 54° (15 s), 72° (30 s); for qAS-PCR-LNA: preliminary denaturation of 95° (5 min), 50 PCR cycles - 95°(30 s), 62° (30 s), 72° (30 s).

Sensitivity of Determination, Quantification of the Mutant Allele Frequency

The number of tumor cells /the mutant allele frequency*/* was calculated relatively to amplification of wild-type primer (WT) according to the formula: $N = 50 / (2^{\Delta Ct})$, where N - mutant allele frequency in percentages; $\Delta Ct = Ct (mt) - Ct (wt)$; Ct (mt) and Ct (wt) are the threshold cycles with the mutant and wild type primers, respectively. The linearity of measurement was confirmed by a standard dilution of tumor DNA in normal DNA (Figure **1A**). Since the mutant-specific primers always give minor nonspecific amplification in non-tumor samples, estimation of cut-off level is essential to provide maximal sensitivity of tumor cells determination without compromising the specificity

Table 1: Summary Characteristics of Patients and Samples. LN – Lymph Nodes, BM - Bone Marrow, PB - Peripheral Blood

Diagnosis	Patients (n)	Age (min-max/median)	Male/ Female	Samples
AITL	62	29-87 /62	37/25	LN, BM, PB, skin
HL <35 years	35 (22 primary HL; 13 relapsed HL)	19-34 /27	12/23	LN (n - 33); mediastinum (n - 2)
HL (≥35 years)	26 (19 primary HL; 7 relapsed HL)	36-75 /56	15/11	LN (n - 24); spleen (n - 2)
DLBCL	15	20-68/ 52	8/7	LN
Reactive changes of LN	14	17-53/ 30	4/10	LN
FL, CLL	20	43-74/ 57	14/6	LN

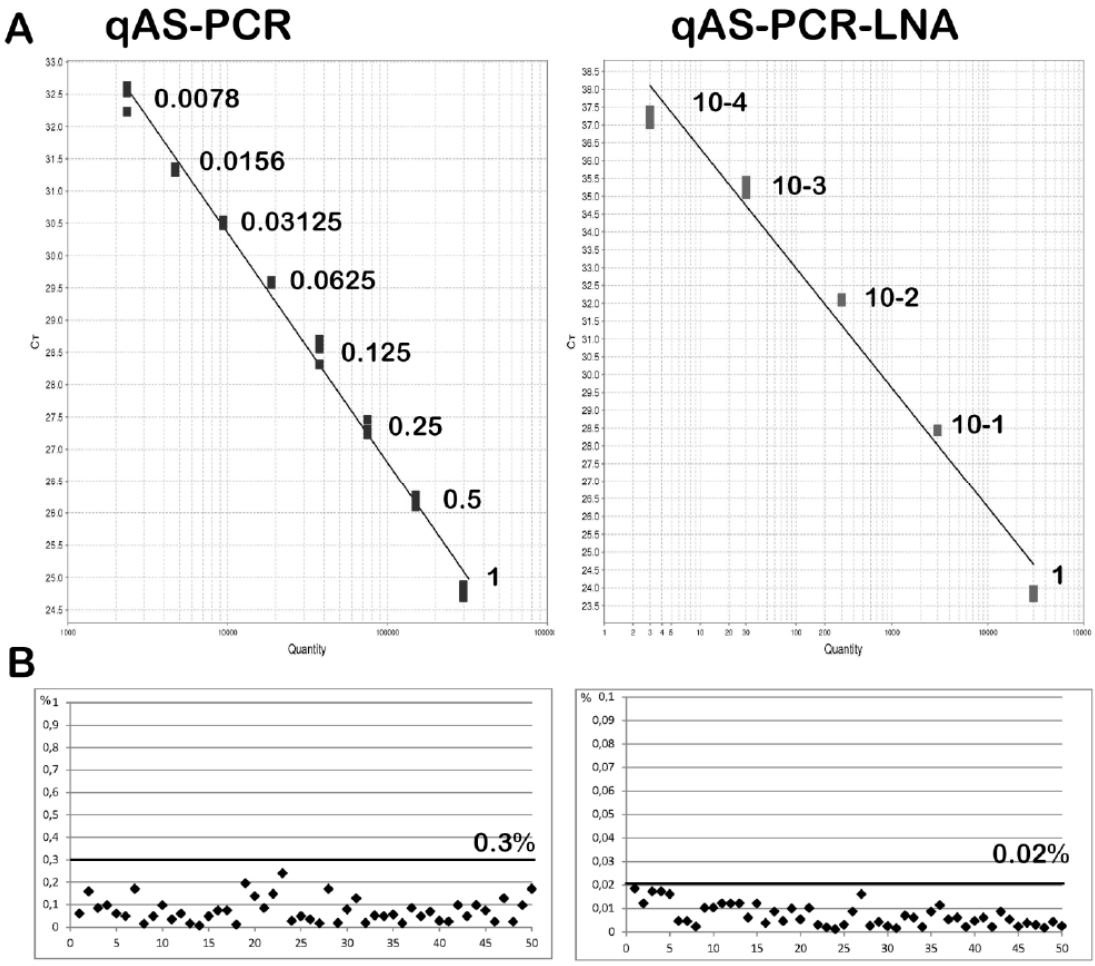

Figure 1: Real-time quantitative allele-specific polymerase chain reaction data for RHOA G17V detection (qAS–PCR and qAS-PCR-LNA methods). **A**. Standard curve based on serial dilutions of tumor DNA in normal. X – relative quantity; Y - cycle threshold (CT); **B**. The sensitivity of methods based on the study of negative control samples (n-50). X - patient number; Y - percentage of cells with the mutation (%).

(increased numbers of false-positive results). The required cut-off levels for both qAS-PCR and qAS-PCR-LNA techniques were optimized in experiments on negative controls (Figure **1B**). Calculated by standard curve and negative control testing (n - 50), the sensitivities of qAS-PCR and qAS-PCR-LNA methods were 0.3% and 0.02%, respectively. A more specific amplification was observed with LNA primers and ΔCt was 12-14 cycles in negative samples, whereas for normal primers ΔCt was 8-9 cycles. Standard curves showed that counting of cells with the mutation in the ranges of sensitivity is possible by both methods.

CD4+, CD8+Cells Selection

CD4 + and CD8 + lymphocyte fractions were isolated from 4 patients by MACS selection kit (Miltenyi Biotec, Germany) according to the manufacturer's protocol.

Statistical Analysis

Statistical processing of results was performed using the Stat Plus program (Analyst Soft Inc., USA, Version v6, http://www.analystsoft.com/en).

RESULTS

RHOA G17V mutation was detected in 53% (32 of 62) patients with AITL. In one case, 1.5% cells with RHOA mutation were detected in the lymph node biopsy 10 years before the development of complete clinical and morphological picture of AITL (Table **2**). Interestingly, the morphological and IHC pattern in the initial biopsy corresponded to the reactive changes with the expansion of the paracortical zone. Same length of rearranged TCRG gene fragments were revealed in the both biopsies, indicating identical clones of tumor cells in both lymph nodes (Figure **2**).

Table 2: Patient N, female, 58 y.o. Cells with RHOA Mutation were Detected in the Lymph Node Biopsy 10 Years Before the Development of Complete Clinical and Morphological Picture of AITL. * Clonal Rearrangements Coincided in all Tissues. LN – Lymph Nodes, BM - Bone Marrow, PB - Peripheral Blood

Study	LN1 in 2007	LN2 in 2018	PB in 2018	BM in 2018
RHOA	1,5%	29%	19%	12%
Clonality testing*	TCRG+/- TCRB- IgH-	TCRG+ TCRB+ IgH-	TCRG+ TCRB+ IgH-	TCRG+ TCRB+ IgH-
Histology/ IHC	Reactive changes	AITL	-	-

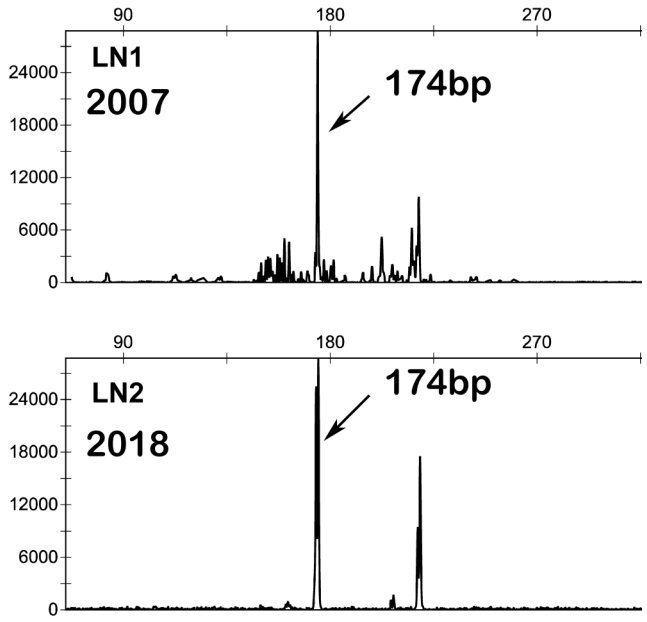

Figure 2: Patient N, female, 58 y.o. TCRG monoclonal rearrangements coincided in the first lymph node LN1 and the second lymph node LN2 with 10 years interval. Cells with RHOA G17V mutation are revealed in LN1 at the amount of 1.5% and LN2 in at the amount of 29%. X - size of PCR products (bp), Y – relative fluorescence units (RFU).

We estimated the number of mutated cells in various tissues in 32 patients with AITL positive for RHOA G17V mutation. The percentage of RHOA G17V positive cells varied significantly between samples (Figure **3** and Table **2** of Supplementary Material). The number of cells with RHOA mutation was higher in lymph nodes – 8.33% (0.5 - 47%) and skin biopsies – 4.12% (0.22 - 19%) than in blood and bone marrow (Table **3**). Circulating tumor cells were found in all patients examined (n - 22) with an average content of 2.3% (0.04 - 19%). In 92% of patients, RHOA G17V mutated cells were found in BM. The number of cells was significantly lower in BM than in blood - 0.98% (0.02 - 12%). Positive correlation between the number of mutated cells in the blood and BM (n =19, rs = 0,7344, p = 0,0002) was observed. Histological and IHC study revealed bone marrow infiltration in only 63% of these patients. BM involvement (estimated by the analysis of histological and IHC data) and the number of cells with RHOA G17V mutation in blood or BM detected by PCR were not significantly correlated.

Table 3: Percentage of Cells with a RHOA G17V Mutation in Different Tissues of Patients with AITL Positive for RHOA G17V Mutation. LN - Lymph Nodes, PB - Peripheral Blood; BM - Bone Marrow

Samples	LN	PB	BM	Skin
Number of positive samples / total	29/29	21/21	24/26	8/8
Average percentage of cells with mutation (%)	8.33	2.39	0.98	4.12
Min-max percentage of cells with mutation (%)	0.5-47	0.04-19	0.02-12	0.22-19

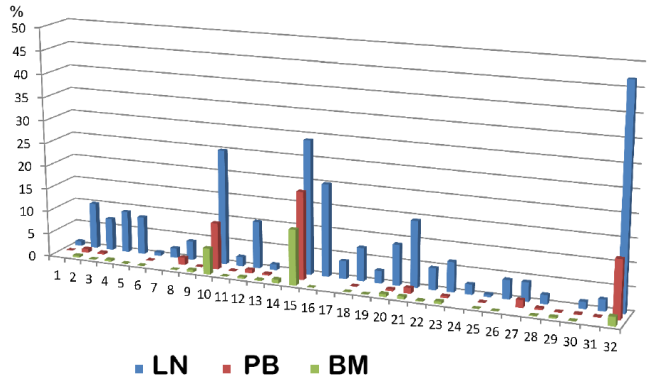

Figure 3: Quantitation of RHOA G17V mutant allele frequency in different samples of patients with AITL positive for RHOA G17V mutation (n - 32): in lymph nodes (LN) - blue, peripheral blood (PB) – red; bone marrow (BM) – green. X - patient number; Y - percentage of cells with the mutation (%).

In 6 patients with more than 1% tumor cells in blood we observed a marked dissemination of the tumor and it was clinically manifested as the enlargement of all groups of peripheral and visceral lymphatic nodes,

bone marrow involvement, multiorgan failure, and general decline in clinical status. When several lymph nodes were investigated for a single patient, the amounts of RHOA G17V positive cells and the results of T-cell clonality determination could vary considerably for different samples (Figure **4**). RHOA G17V mutation was not found in control group of patients with Hodgkin's lymphoma, DLBCL, reactive changes in lymph nodes, indolent B-cell lymphomas (n - 110), except for 4 patients (Figure **5**).

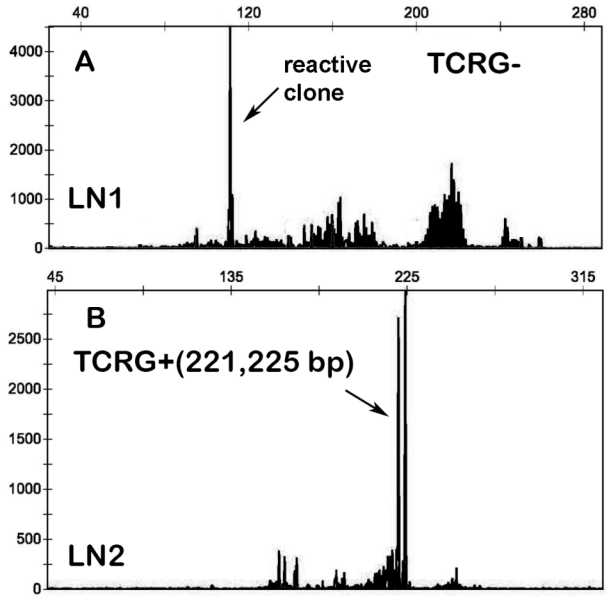

Figure 4: Results of 2 LN biopsies with monthly intervals: neck LN (A) and inguinal LN (B), with no therapy. In the neck LN, reactive T-cell clonality and a low percentage (2.5%) of cells with a RHOA G17V mutation was detected, in contrast to inguinal LN, which contains 16% of cells with a RHOA G17V mutation and clonal TCRG rearrangements. X - size of PCR products (bp), Y – relative fluorescence units (RFU).

We revealed different amount of cells with mutation in 3 patients with HL (8.8, 2.3, 4%) and 1 patient with DLBCL (0.24%). Circulating clonal CD4+ T-lymphocytes with aberrant immunophenotype and clonal rearrangements of TCRG and/or TCRB genes in lymph nodes were found in 3 HL patients with RHOA G17V mutation.

Case 1. Patient M, male, 38 years old. There was a significant increase in cervical, subclavian, axillary, inguinal and ileac lymph nodes (up to 3 cm) and retroperitoneal lymph nodes (up to 4-5 cm). Reactive lymphoproliferation was found in two biopsies of the cervical lymph nodes based on histology and IHC. After a biopsy of the retroperitoneal lymph node the diagnosis of Hodgkin lymphoma, mixed-cellularity subtype was verified. Significant numbers of RHOA G17V positive cells were found in all three biopsies: 3.47; 1.6; 3.96%. Monoclonal rearrangements of TCRG, TCRB and IGH were detected only in the retroperitoneal lymph node (Table **4**). RHOA G17V mutation was found in 1.41% of blood cells and in 0.36% of bone marrow cells. Flow cytometry revealed a population of lymphocytes in blood with CD3- CD5+ CD4+ CD10+/- CD279+ immunophenotype. The study of selected blood cells showed that CD4+ lymphocytes population contained cells with RHOA G17V mutation (0.4%) and the corresponding clonal rearrangements of TCRB.

Case 2. Patient K, male, 65 y.o. In this patient with a diagnosis of classic Hodgkin's lymphoma, IIIA, nodular sclerosis, we revealed a small number of cells with RHOA G17V mutation in lymph node biopsies at the first and second relapse at 1 year interval in the

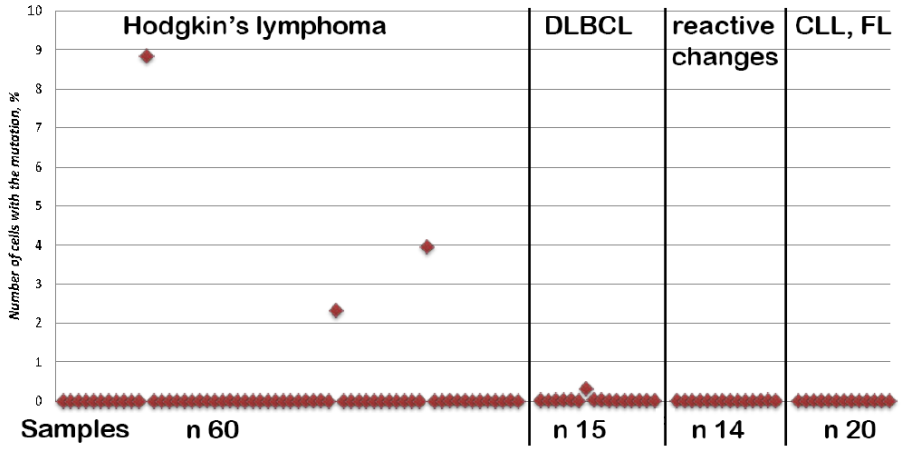

Figure 5: Results of RHOA G17V mutation testing of lymph nodes in the control group of patients with Hodgkin's lymphoma, DLBCL, reactive changes of lymph nodes, indolent B-cell lymphomas (n-110). Four positive patients revealed. X – number of analyzed patients; Y - percentage of cells with the mutation (%).

Table 4: Results of Laboratory Examination of Patient #1 with Hodgkin's Lymphoma and RHOA G17V Mutation. LN1 and LN2 – Cervical Lymphatic Nodes, LN3– Retroperitoneal Lymphatic Nodes. *Aberrant Clone was Revealed in the Amount of 2.251% of Lymphocytes and 0.295% of All Events. Nd - no Data, neg – Negative Result

Study	LN1 Cervical	LN2 Cervical	LN3	Blood	BM	Blood and Selected Cells (during therapy)		
						Whole	CD4+	CD8+
RHOA	3.47%	1.6%	3.96%	1.41%	0.36%	0.06%	0.4%	neg
Clonality testing	TCRG- TCRB+ IgH-	TCRG- TCRB+ IgH-	TCRG+ TCRB+ IgH+	TCRG+ IgH-	TCRG- TCRB- IgH-	TCRB-	TCRB+	TCRB-
Histology/ IHC/ Immunophenotype	reactive LN	reactive LN	HL	nd	nd	*CD3-CD5+CD4+CD10+/- CD279+		
Cytogenetics	nd	Trisomy 3	Trisomy 3	nd	nd	nd		

amount of 0.14% and 2.32%, respectively (Table **5**). Clonal rearrangements of TCRG and TCRB were detected only at the second relapse. A population of lymphocytes with immunophenotype CD3+ CD4+ CD10+ CD279- was detected in blood in the amount of 0.2% of lymphocytes and 0.04% of all events. CD4+/CD8+ selection of blood cells showed that RHOA G17V mutation was present in 0.16% of CD4+ lymphocytes.

Case 3. Patient L, male, 75 y.o. was diagnosed with classic Hodgkin's lymphoma, IVA, nodular sclerosis, with involvement of cervical lymph nodes and liver. 8.8% of cells with RHOA G17V mutation and clonal rearrangements of TCRG in lymph node, lymphocytes with immunophenotype CD3- CD4+ CD5+ CD2+ CD10- CD279- in blood (1.2% of lymphocytes and 0.33% of all events) and in bone marrow (1.66% of lymphocytes and 0.173% of all events) were found.

Taking into account the molecular and immunophenotyping data, a repeated morphological and extended IHC examination of the biopsy material was carried out for these patients, as a result the diagnosis of Hodgkin's lymphoma was confirmed again. These patients are currently left under surveillance and the formation of a tumor T-cell clone with the development of AITL as a secondary lymphoma could be speculated for these cases.

Monitoring of Minimal Residual Disease During the Course of Therapy

Bone marrow (BM) and peripheral blood samples from 16 patients with AITL who achieved clinical and hematologic remission were studied at the diagnosis and during the therapy. All patients received CHOP-like induction therapy (6 courses) and then 2-3 years of maintenance therapy with small doses of cytotoxic or immunomodulatory drugs. MRD was tested before the start of therapy, after completion of induction chemotherapy (6 months from the beginning of therapy), and then every six months during the maintenance therapy, also in some patients 3-5 years

Table 5: Results of Laboratory Examination of Patient #2 with Hodgkin's Lymphoma and RHOA G17V Mutation. LN1 - Lymphatic Node at the Initial Diagnosis; LN2 and LN3 - Lymphatic Nodes at the First and Second Relapses. *Aberrant Clone was Revealed in the Amount of 0.2% of Lymphocytes and 0.04% of all Events. Nd- no Data, neg – Negative Result

Study	LN 1 2012	LN2 2014	LN3 2015	Blood and Selected Cells		
				Whole	CD4+	CD4-
RHOA	nd	0.14%	2.32%	neg	0.16%	neg
Clonality testing	nd	TCRG- TCRB- IgH-	TCRG+ TCRB+ IgH-	TCRG- TCRB-		
Histology/ IHC/ Immunophenotype	HL	HL	HL	*CD3+CD4+CD10+CD279-		

after the onset of the disease. The numbers of detectable cells with the mutation before and during the therapy were higher in the blood comparing to that in BM (Figure **6**).

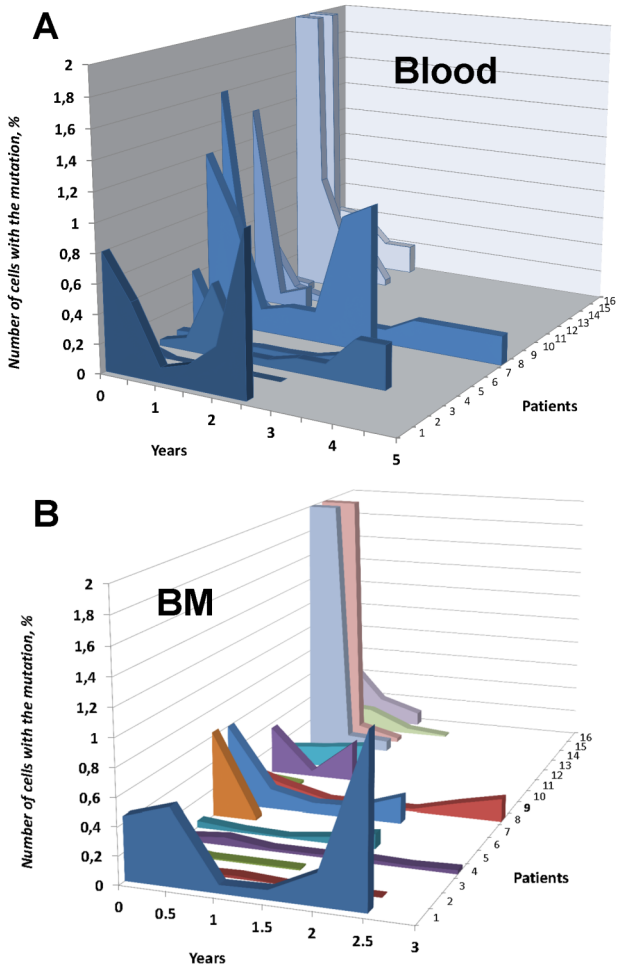

Figure 6: Monitoring of RHOA G17V mutation during the course of therapy in blood and bone marrow (BM). Z - patient number; Y - percentage of cells with the mutation (%); X - time from the beginning of treatment (years).

All examined patients had complete or partial molecular response and achieved clinical remission after induction chemotherapy. However, 12 of 16 patients had a tumor clone (0.03-2% of the total number of cells) after completion of induction chemotherapy in the blood and/or BM. During the maintenance therapy 7 patients were examined. The number of tumor cells in PB/BM was undetectable in two patients (≤0.02%), and increased up to 0.1-1.1% in five other patients. In two patients flow cytometry of blood sample confirmed the presence of tumor cells with a specific immunophenotype in the amount of 2.3% of lymphocytes (0.3% of all events) and 4.8% of lymphocytes (0.4% of all events), that practically coincided with the quantitative determination of RHOA G17V mutant allele frequency (0.2 and 0.15%, respectively). In two patients (case #1 and #7) we observed a significant increase in the number of cells with the RHOA G17V mutation in the blood (Figure **6A**) that coincided with the development of clinical relapse. In this way, an increasing number of cells with a RHOA G17V mutation may be a predictor of disease recurrence.

DISCUSSION

We used LNA (locked nucleotide acid) modified allele-specific primers, providing increased PCR sensitivity (0.02%) due to a greater binding ability in the complementary interactions [18-21]. Using this method, it was shown that all patients with AITL have circulating tumor cells (100%) and most patients (92%) have bone marrow involvement. Histological/IHC study revealed bone marrow infiltration in only 63% of patients with AITL positive for RHOA G17V, that is most likely due to lower sensitivity of the histological examination compared with PCR methods. We assume that a large number of circulating tumor cells with RHOA G17V mutation in blood, compared with BM, is a result of generalized lymph node involvement in AITL, while BM infiltration is secondary and therefore not so prominent. Conventional PCR methods of T-cell clonality testing showed positive results in lymph nodes in 88% (51 of 58) of AITL patients studied. Presence of circulating tumor cells or BM involvement proved by T-cell clonal peaks identical to those from lymph nodes was found in 38.6% (22 of 57) only. This was not surprising, since the sensitivity of conventional T-cell clonality assays are not exceeding the level of 1% of tumor cells in bulk T-lymphocyte population. In all T-cell monoclonal blood and BM samples the numbers RHOA G17V positive cells were higher than 0.4% (Table **2** of Supplementary Material). A statistically significant correlation was found between the T-cell clonality (with clonal peaks identical to those from LN) and the number of RHOA G17V positive cells ($n = 23$, $r_s = 0.8137$, $p = 0.000002$ for BM samples and $n = 26$, $r_s = 0.6919$, $p = 0.00009$ for blood samples). Given the low content of tumor cells in bone marrow (an average 0.98%), as well as in lymph nodes of some patients (5 of 29 patients had less than 2% of tumor cells in lymph nodes), the results of PCR clonality testing also cannot be considered as a sufficiently sensitive and reliable method for AITL diagnosis. Previously, we have shown that lymph nodes only in 78% of AITL patients have distinct clonal TCRG or TCRB rearrangements at the time of diagnosis, and only 24% of patients had a clear

monoclonal pattern in the primary bone marrow samples coinciding with that in the lymph nodes [22]. In addition to a high level (20-45%) of B-cell clonality detection in AITL [22-27], the results of clonality testing may be surprising. Here we show that somatic RHOA G17V point mutation is a specific marker of AITL (specificity 96.4%) in the differential diagnosis with Hodgkin's lymphoma, DLBCL, reactive changes of lymph nodes and indolent B-cell lymphomas. Differential diagnosis of these diseases with AITL is very relevant, as AITL is characterized by the presence of B-cell immunoblasts, including Hodgkin-Reed-Sternberg-like cells and massive infiltration by reactive lymphocytes [27-30]. Revealing the RHOA G17V mutation in HL, reactive processes or DLBCL will help to identify patients with a high risk of developing AITL, and to detect a tumor clone before the manifestation of the disease. In one case, we detected RHOA G17V mutation 10 years before the complete clinical onset of AITL, which proves the extremely slow rate of tumor growth and progression in AITL at the initial stages. In 3 of 61 cases (4,9%) with HL we revealed RHOA G17V mutation, clonal rearrangements of TCR genes in blood and lymph nodes, circulating lymphocytes with aberrant immunophenotype of follicular T-helpers CD4+CD10+ or CD3- CD4+ CD10-. The presence of these clones was not interpreted as the pathomorphologic diagnosis of AITL or PTCL NOS, but may give rise to the development of a secondary tumor and requires further research. It is known that secondary B-cell lymphomas, including DLBCL and HL may occur in 12-23% of AITL patients within few months to several years after the initial manifestation of the disease [31-34]. A single case of DLBCL and subsequent development of AITL in 6 months was described [34]. It is suggested that frequent development of B-cell tumors in AITL can be due to chronic antigenic stimulation with Epstein-Barr virus (EBV) and/or alternatively to marked secondary immunodeficiency caused by EBV, chemotherapy and lymphoma itself [34-36].

Data on the presence of RHOA G17V in different lymphomas are limited: it is known that it can be observed in 13-18% of cases of peripheral T-cell lymphoma not otherwise specified (PTCL NOS), in 3% of adult T-cell leukemia [13, 37-38]. Also a single case reported with secondary DLBCL arising from PTCL NOS positive for RHOA G17V [39]. In this study we show that the sensitivity of qAS-PCR-LNA (0.02%) is sufficient for residual tumor clone detection in most patients after induction chemotherapy or in the process of maintenance therapy. Since prolonged disease-free survival usually observed in 20% of AITL patients only and approximately 2/3 of patients develop relapses in the first 2-3 years after completion of therapy [6-11], the data obtained may be beneficial for therapy improvement.

CONCLUSION

Quantification of cells with RHOA G17V mutation is necessary for AITL diagnosis and monitoring. qAS-PCR-LNA has sensitivity superior to that of histological and clonality testing (PCR based) methods. RHOA G17V mutation is a specific marker of AITL for differential diagnosis with Hodgkin's lymphoma, reactive proliferation in lymph nodes and DLBCL. RHOA G17V detection can be used as a screening method in patients with any lymphadenopathy for the exclusion of AITL or PTCL NOS. Sensitivity of determination (0.02%) is sufficient for MRD monitoring. Tumor cells with RHOA G17V mutation persist in most patients with AITL after the induction chemotherapy and during the maintenance therapy. qAS-PCR-LNA can be enrolled into standard protocols for management of patients with AITL to assess the efficacy and control the duration of treatment.

CONFLICTS OF INTEREST

Nothing to disclose. The authors declare no conflicts of interest.

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