

# EVOLUTION OF IMMUNOBIOLOGICAL CHANGES AND THEIR SIGNIFICANCE FOR THE TREATMENT OF PATIENTS WITH NON-HODGKIN'S LYMPHOMA

Aliona NICORICI<sup>1</sup>✉

<sup>1</sup> Public Health Institution Institute of Oncology, Chisinau, Republic of Moldova

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## ABSTRACT

**Introduction.** Non-Hodgkin's lymphoma (NHL) shows clonal and uncontrolled proliferation of immature lymphoid precursors.

**The aim of the study.** Dynamic analysis of immunobiological and immunophenotypic parameters in the treatment of patients with NHL, and recognition of immunological remissions.

**Material and methods.** The retrospective study group consisted of 122 patients, admitted between 2012 and 2015 in the Hematology Clinic of the Republic of Moldova Oncology Institute, with a morphologically confirmed diagnosis of NHL. We assessed the predominant phenotypic line by indirect immunophenotyping and total concentration of immunoglobulin classes (A, M, G) according to treatment regimens applied to NHL patients.

**Results.** 119 patients showed CD19 antigen and CD20 increased expression of cell line B phenotype (84.4%); only 3 patients in the study showed increased CD3, CD5, resulting in the predominant T-cell phenotype line. NHL patients with both aggressive and non-aggressive primary variants after 3 cycles and 6 cycles presented a significant decrease in immunoglobulin A, M, and G indices, on average of  $7.3 \pm 0.2$  (mg/ml) ( $p < 0.05$ ), while the immunological indices of patients treated with the polychemotherapy (PTCH) scheme 2 reported statistically significant minimal decreases ( $p$

## RÉSUMÉ

**Evolution des modifications immunobiologiques et leur importance dans le traitement de patients atteints de lymphome non-Hodgkinien**

**Introduction.** Le lymphome non-Hodgkinien (LNH) montre une prolifération clonale et incontrôlée de pré-curseurs lymphoïdes immatures.

**Le but de l'étude.** Analyse dynamique des paramètres immunobiologiques et immunophénotypiques dans le traitement des patients atteints de LNH et reconnaissance des rémissions immunologiques.

**Matériel et méthodes.** Le groupe d'étude rétrospectif était composé de 122 patients, admis entre 2012 et 2015 à la clinique d'hématologie de l'Institut d'Oncologie de la République de Moldova, avec un diagnostic de LNH confirmé par la morphologie. Nous avons évalué la lignée phénotypique prédominante par immunophénotypage indirect et concentration totale des classes d'immunoglobulines (A, M, G) en fonction des schémas thérapeutiques appliqués aux patients présentant un LNH diagnostiqué.

**Résultats.** 119 patients ont présenté l'expression accrue de l'antigène CD19 et CD20 du phénotype de la lignée B (84,4%); seuls 3 patients de l'étude ont présenté une augmentation de CD3, CD5, entraînant la lignée de phénotype prédominant des cellules T avec un poids de 2,4%. Les patients LNH avec des variantes

✉ Address for correspondence:

Aliona NICORICI  
Public Health Institution Institute of Oncology, Chisinau, Republic of Moldova  
Email: [alionanicorici@yahoo.com](mailto:alionanicorici@yahoo.com); Phone 068599004

<0.05), setting an incomplete immunological remission.

**Conclusions.** Due to the information obtained by periodic monitoring of patients with NHL following the termination of conventional clinical protocols, it was possible to reveal the immunological and immunophenotypic features of the disease, for the detection of early and late recurrences, late side effects, occurrence of the second malignancy, with the evaluation of the immunological and immunophenotypic remission and identification of specific molecular targets for subsequent targeted therapy.

**Keywords:** polychemotherapy, non-Hodgkin's lymphoma, target treatment, monoclonal antibodies, immunoglobulins.

#### List of abbreviations:

NHL – Non-Hodgkin's lymphoma  
mAb – monoclonal antibodies  
PCHT – polychemotherapy  
Ig – immunoglobulins

## INTRODUCTION

Non-Hodgkin's lymphoma (NHL) represents the clonal and uncontrolled proliferation of immature lymphoid precursors, which lose their differentiation capacity and cause lymphoid organ hypertrophy<sup>1,2</sup>.

Immunological tests are currently very important for diagnosis, completing the morphological study, in the analysis of malignant lymphoproliferative diseases<sup>3,4</sup>. In addition, the results of the immunophenotypic assays can be used to identify the expression of targets for antibody therapy and to provide additional prognostic information. Also, following therapy, immunophenotyping may be a method of assessing residual minimal disease. Neoplastic mature B lymphoid cells can be distinguished from normal cells by identifying two major types of phenotypic abnormalities – the Ig light chain class restriction and the aberrant expression of antigens<sup>4,6</sup>.

Immunophenotyping is recommended as one of the ideal strategies for accurately detecting the phenotype of neoplastic proliferative lesions and can

primaires agressives et non agressives après 3 cycles et 6 cycles dans tous les groupes de traitement assignés aux schémas thérapeutiques ont noté une diminution significative des indices d'immunoglobuline A, M et G en moyenne de  $7,3 \pm 0,2$ (mg/ml) ( $p < 0,05$ ), alors que les indices immunologiques des patients traités avec le schéma de polychimiothérapie (PCHT) 2 faisaient état de diminutions minimales statistiquement significatives ( $p < 0,05$ ), ce qui établissait une rémission immunologique incomplète.

**Conclusions.** Grâce à la recherche sur le suivi périodique des patients atteints de LNH à la suite de l'arrêt des protocoles cliniques conventionnels, il a été possible de révéler les caractéristiques immunologiques et immunophénotypiques de la maladie lors de la détection de récurrences précoces et tardives, d'effets secondaires tardifs, de la survenue d'un deuxième cancer avec l'évaluation de la rémission immunologique et immunophénotypique, pour déclencher l'identification de cibles moléculaires spécifiques, en vue d'une thérapie ciblée ultérieure.

**Mots-clés:** polychimiothérapie, lymphome non-Hodgkinien, traitement ciblé, anticorps monoclonaux, immunoglobulines.

#### Liste des abréviations:

LNH – lymphome non-Hodgkinien  
AcMo – anticorps monoclonaux  
PCHT – polychimiothérapie  
Ig – immunoglobulines

help in the staging and detection of residual minimal disease<sup>7-9</sup>.

In addition to its use in diagnosis, immunobiological research can serve as a starting point in the selection of individualized therapy, as well as in the determination of the moment of occurrence and installation of immunological and immunophenotypic remissions<sup>10-12</sup>.

## THE AIM OF THE STUDY

Dynamic analysis of expression of immunobiological and immunophenotypic parameters in NHL patients, before and after treatment, to demonstrate and argue the scientific value of predictive factors in improving the long-term survival rate and the recognition of immunological and immunophenotypic remissions in the choice of treatment methods.

## MATERIAL AND METHODS

The retrospective study group consisted of 122 patients, admitted between 2012 and 2015 in

the Hematology Clinic of the Republic of Moldova Oncology Institute, Chisinau, with a morphologically confirmed diagnosis of NHL. All the patients signed an informed consent. The average age of the patients was 56.5 years; the study group included 58 men and 64 women.

The methods of direct and indirect observation have been used in peripheral blood research since the onset of the disease, following the application of specific therapy with intervals of 3 and 6 specific treatment cycles polychemotherapy, with the evaluation of humoral homeostasis indices, the assessment of the lymphoma phenotype by labeling monoclonal antibody (mAb) lymphocytes using luminescent microscopy in the dark field, comparing them for a deductive analysis.

Through the validated data at repeated hospitalizations, we recorded the influence of the response to the applied treatment, relapses and refractory post-treatment complications.

Indirect immunophenotypic investigation was performed in several steps: collection specimens for research, lymphocyte ring separation step, lymphocyte dilution step, approximate dissolution step, mAb lymphocyte tagging step, mAb labeled lymphocyte dilution step, fluorescence labeling in lymphocyte stages, visualization of malignant lymphocytes in a dark field and interpretation of the results.

According to the research methodology, immunological evidence has been investigated in patients with NHL, grouped according to treatment regimes applied and morphological types in three study groups, on both cell line B and cell line T (Table 1).

**RESULTS**

Of the 122 histopathologically diagnosed NHL patients enrolled in the study and examined by the indirect immunophenotyping method of peripheral blood research by labeling lymphocytes with mAb,

**Table 1.** Distribution of patients according to PCHT treatment.

NHL subtype	NHL patients included in the study	PCHT treatment	Treatment regimens
Aggressive LNH (lymphoblastic)	56	1. CHOP	Cyclofosfan Doxorubicin Oncocin (vincristine) Prednisolone
	12 (determined cell line by immunohistochemistry biopsy)	2. R - CHOP	Rituximab (antiCD20) Cyclofosfan Doxorubicin Oncocin (vincristine) Prednisolone
NHL nonaggressive (indolent, pro-inflammatory)	54	3. CVLP	Cyclofosfan Vinblastine Leukeran Prednisolone

**Table 2.** 100% malignant cells (B lymphocyte cell line phenotype).

No. patients	%	CD 20						ci	CD 19						ci
		abs.			%				abs.			%			
		min	med	max	min	med	max		min	med	max	min	med	max	
119	100	13	42	98	11.2%	36.2%	84.4%	25.4±0.7	9	32	75	7.75%	27.58%	64.67%	19.8±2.3

**Table 3.** Total IgA, IgM, IgG Assay PCHT Study Group Scheme N° 1.

<i>Aggressive NHL (Lymphoblastic)<sup>13, 14, 16</sup></i>			
	Primary M(mg/mL)	M(mg/mL)± after 3 cycles of PCHT	M(mg/mL)± after 6 cycles of PCHT
IgA	1.4±0.1 mg/mL	1.1±0.02 mg/mL	1.0±0.01 mg/mL
IgM	1.6±0.05 mg/mL	1.4±0.08 mg/mL	1.2±0.06 mg/mL
IgG	17.5±0.2 mg/mL	16.8±0.1 mg/mL	15.2±0.1 mg/mL
p	<0.05	<0.05	<0.01
t	2.84	0.98	1.82

**Table 4.** Results of Ig A, IgM, and IgG according to PCHT treatment scheme N° 3 in repeated cycling periods for non-aggressive NHL.

<i>Indolent NHL (prolymphocyte)<sup>13, 14, 16</sup></i>			
	<i>Primary M(mg/mL)</i>	<i>M(mg/mL)± after 3 cycles of PCHT</i>	<i>M(mg/mL)± after 6 cycles of PCHT</i>
<b>IgA</b>	1.4±0.08 mg/mL	1.2±0.04 mg/mL	1.1±0.02 mg/mL
<b>IgM</b>	1.6±0.05 mg/mL	1.4±0.08 mg/mL	1.2±0.01 mg/mL
<b>IgG</b>	19.1±0.2 mg/mL	18.8±0.2 mg/mL	15.6±0.03 mg/mL
<b>p</b>	< 0.05	< 0.05	< 0.05
<b>t</b>	2.34	1.22	1.96

**Table 5.** Results of IgA, IgM, IgG according to PCHT treatment scheme N° 2 in repeated cycles for NHL B - cell.

<i>NHL B - cell</i>			
	<i>Primary M (mg/mL)</i>	<i>M(mg/mL)± after 3 cycles of PCHT</i>	<i>M(mg/mL)± after 6 cycles of PCHT</i>
<b>IgA</b>	2.5±0.02 mg/mL	1.7±0.02 mg/mL	1.9±0.01 mg/mL
<b>IgM</b>	1.6±0.01 mg/mL	1.4±0.04 mg/mL	1.4±0.03 mg/mL
<b>IgG</b>	12.3±0.03 mg/mL	18.8±0.2 mg/mL	16.7±1.3 mg/mL
<b>p</b>	<0.05	<0.01	<0.05
<b>t</b>	2.36	1.08	1.98

119 patients showed increased affinity for the CD19, CD20 antigen, expressing the cell line B phenotype (Table 2).

As a result of the immunophenotypic investigation of the same group of NHL patients by labeling lymphocytes with CD3 and CD5 monoclonal antibodies, we determined that only 3 patients had lymphocyte T phenotype.

Thus, the total concentration of immunoglobulins (Ig): IgA, IgM and IgG in the group of patients receiving PCHT according to Scheme 1 follows a downward trend (Table 3).

54 NHL patients had indolent morphological subtypes treated with the PCHT treatment scheme 3 and presented a relatively insignificant decrease in the indices of the Ig A, IgM and IgG (Table 4).

In the 12 patients with B-cell LNH (confirmed by immunohistochemical examination) who received the PCHT scheme 2 (R-CHOP), an insignificant reduction in the immune response was observed (Table 5).

## DISCUSSION

Our study shows that B lymphoid cells have a progressive proliferation tendency, which justifies the inclusion in the treatment of NHL of specific monoclonal antibodies as targeted therapy. The proportion of B cells phenotype, encountered in 84.4% of patients enrolled in the study, did not differ significantly from the literature (over 90% of cases with B cells)<sup>11,12</sup>.

In our study, the T-cell NHL was rare, with a frequency of 2.4% versus 10% compared to literature data<sup>9, 14, 17</sup>.

Table 3 revealed the dynamics of IgA, IgM and IgG indices during PCHT in 56 patients treated according to protocol treatment scheme 1 and with repeated control of their values after 3 and 6 PCHT cycles. The results showed a significant decrease in all immunoglobulin indices, both primary and in the dynamics after the 3<sup>rd</sup> and 6<sup>th</sup> cure of PCHT.

In patients treated with the N° 1 and 3 schemes, significant decreases of the IgA, IgM, IgG classes, both primary and after 3 and 6 cycles of PCHT, have been observed. Thus, primary IgA level was 2.5±0.02 mg/mL, after 3 courses it was 1.7±0.02 mg/mL and after 6 courses it was 1.9±0.01 mg/mL, primary IgM was 1.6±0.01 mg/mL, after 3 cures it was 1.4±0.04 mg/mL, after 6 cures 1.4±0.03 mg/mL. Primary IgG was 12.3±0.03 mg/mL, after 3 courses it was 18.8±0.2 mg/mL, after 6 courses 16.7±1.3 mg/mL (Table 4).

The results obtained show that the patients in the PCHT mAb CD20 (MabThera) PCHT group (Table 5) had an attenuation of the statistically significant immune response, where p 0.05 established the occurrence of an incomplete immunological remission.

## CONCLUSIONS

- Indirect immunophenotypic complement tests, that allowed us to detect B cell line of NHL by

labeling lymphocytes with mAb CD20, CD19, revealed a significant number of malignant cells, increased in 84.4% of the patients, proving that B lymphocytes have a progressive proliferative evolution, motivating inclusion in the treatment of mAb variants of NHL cells as target therapy.

- CD3 and CD5 immunophenotypic examination in NHL patients determined the predominant T-cell phenotype in only 3 patients (2.4%), against 10% in the literature.
- Due to the information obtained by periodic monitoring of patients with NHL following the termination of conventional clinical protocols, it was possible to reveal the immunological and immunophenotypic features of the disease, for the detection of early and late recurrences, late side effects, occurrence of the second malignancy, with the evaluation of the immunological and immunophenotypic remission and identification of specific molecular targets for subsequent targeted therapy.

#### Compliance with Ethics Requirements:

„The authors declare no conflict of interest regarding this article“

„The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study“

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