

Clinical association of baseline levels of conjugated dienes in low-density lipoprotein and nitric oxide with aggressive B-cell non-Hodgkin lymphoma and their relationship with immunoglobulins and Th1-to-Th2 ratio

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Objective: The aim of this study was to highlight the clinical association of baseline levels of conjugated dienes in low-density lipoprotein (LDL-BCD) and nitric oxide (NO) with immunoglobulins (Igs) and T helper (Th)1/Th2 ratio in patients with newly diagnosed B-cell non-Hodgkin lymphoma (NHL).

Patients and methods: Thirty-two newly diagnosed patients with aggressive B-cell NHL and 25 age-, sex-, and body-mass-index-matched healthy controls were randomly selected for a cross-sectional case-control study conducted at the Hematology Department of Tlemcen Medical Centre University (northwest of Algeria).

Results: Circulating levels of LDL-BCD and NO and those of IgA and IgM were significantly higher in patients than in controls. The levels of Th1/Th2 ratio and plasma total antioxidant capacity were significantly lower in patients compared with controls, while malondialdehyde and protein carbonyl levels were significantly higher in patients. B-cell NHL was significantly associated with high levels of LDL-BCD from 25th to 75th percentile (25th percentile: relative risk [RR] =2.26, 95% confidence interval [CI] 1.42–3.59, $P=0.014$; 50th percentile: RR =2.84, 95% CI 1.72–4.68, $P<0.001$; 75th percentile: RR =5.43, 95% CI 2.58–11.42, $P<0.001$). Similarly, the disease was significantly associated with high levels of NO production from 25th to 75th percentile (25th percentile: RR =2.07, 95% CI 1.25–3.44, $P=0.024$; 50th percentile: RR =2.78, 95% CI 1.63–4.72, $P<0.001$; 75th percentile: RR =4.68, 95% CI 2.21–9.91, $P<0.001$). Moreover, LDL-BCD levels were positively and significantly correlated with interferon (IFN)- γ , whereas NO levels were inversely and significantly correlated with IFN- γ and Th1/Th2 ratio.

Conclusion: LDL-BCD and NO production seem to be associated with aggressive B-cell NHL and alteration of Th1/Th2 ratio. Our results have to be examined using ex vivo mechanistic studies leading to further investigations of these parameters, with an interest in the link between Epstein-Barr virus infection and NO and immunoglobulins.

Keywords: aggressive B-cell non-Hodgkin lymphoma, LDL-BCD, NO production

Introduction

Non-Hodgkin lymphoma (NHL) is a nonspecific term that includes a spectrum of lymphoproliferative malignant diseases with different clinical and histological appearances.¹ Two clinical forms of NHL can be distinguished on the basis of their growth

rate: aggressive (fast-growing) or indolent (slow-growing).² In addition, NHL can be formed not only from an uncontrollable proliferation of either B or T mononuclear lymphoid cells but also from natural killer cells.³

Although the exact causal factors for NHL are not yet well understood, many cancers may develop from chronic irritation and inflammation.⁴ The strong link between cancer malignancy and chronic inflammation would be due, in part, to the release of many mediators that can induce increased cell proliferation, mutagenesis, oncogenic transformation, and tumor angiogenesis.⁵ These mediators include especially eicosanoids, cytokines, chemokines, and reactive oxygen species (ROS) and/or radical species derived from nitric oxide (NO). NO and its derivatives may promote oncogenesis through damage to DNA and proteins, inhibition of apoptosis, mutation of DNA, and cellular repair functions such as p53 and also via promotion of angiogenesis.⁶ It is therefore becoming crucial to fully highlight the link of NO with immune and inflammatory biomarkers in NHL.

Numerous studies have been conducted to show the role of oxidized low-density lipoprotein (ox-LDL) in inflammatory and immune responses. Ox-LDL can induce immune responses by acting as an adjuvant that activates cells of innate immunity.⁷ They can act as inducers of the expression of adhesion molecules on activated endothelium.⁸ Ox-LDLs can also display chemotactic activity for monocytes, promote their differentiation into resident macrophages, and inhibit their mobility.⁹ It has been established that the binding of ox-LDL to the scavenger receptor CD36, lectin-like ox-LDL receptor 1, and CD205 induces the synthesis of proinflammatory cytokines in human dendritic cells (DCs) leading to DC maturation and DC differentiation.¹⁰ In addition, it has been recently observed that ox-LDL activates the inflammatory pathway through nuclear factor- κ B (NF- κ B), leading to cell transformation¹¹ and that high levels of ox-LDL are associated with increased risk of cancer.¹² However, to the best of our knowledge, there has been no study that correlates ox-LDL with NHL.

Given that most NHLs are of B-cell origin, the current study aimed to highlight the association of baseline levels of conjugated dienes in low-density lipoprotein (LDL-BCD) and NO with aggressive B-cell NHL risk and to assess their link with T helper (Th)1/Th2 ratio. In this context, a cross-sectional case-control study was conducted at the Department of Hematology of Tlemcen University Hospital Centre (Algeria).

Patients and methods

Thirty-two patients with aggressive B-cell NHL and 25 age-, sex-, and body-mass-index-matched healthy controls were

randomly selected for a cross-sectional study conducted at the Hematology Department of Tlemcen Medical Centre University (northwest of Algeria). The mean age of patients (18 men, 14 women) was 52.81 years (range 25–70 years) and that of controls (13 men, 12 women) was 50.5 years (range 25–72 years). The demographic characteristics of patients and controls were recorded through a questionnaire. Histological and immunohistochemical analyses were performed in order to complete the clinical diagnosis and to determine the histologic types of NHL. The main criterion for the inclusion of cases was newly diagnosed aggressive B-cell NHL. The main exclusion criteria were NHL associated with another type of cancer, family history of cancer, indolent lymphoma, a positive serology for HIV, hepatitis C virus, and all diseases that are related to cardiac function. Informed consent was signed by all participants in this study. LDL-BCD were determined to evaluate the levels of LDL oxidation.¹³ NO production was determined by measuring the levels of nitrate (NO_3^-) and nitrite (NO_2^-), with the sensitive colorimetric Griess reaction. Immunoglobulins (Igs) were assessed as biomarkers of humoral immunity.¹⁴ Malondialdehyde (MDA) and protein carbonyl (PC) were determined as markers for assessing oxidative stress,^{15,16} while the concentration of total antioxidant capacity (TAC) and albumin were measured as markers of antioxidative defense.¹⁷ This work was approved by the institutional ethics board of University of Tlemcen.

Sample preparation

Venous blood samples were drawn in the morning between 8 am and 9 am after an overnight fast. The blood was collected in sterile tubes without coagulant for Igs, interferon (IFN)- γ , interleukin (IL)-4, protein fractions, NO, β -lipoproteins, and LDL-BCD analyses and with coagulant for TAC, MDA (ethylenediaminetetraacetic acid-containing tube), and PC (heparin-containing tube) assays. The tubes were centrifuged within 20 minutes, and then aliquoted and stored at -20°C . Lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) were collected from the individual's personal health record.

Immunological and biochemical analyses

Cytokine assay and Th1/Th2 ratio

The Th1/Th2 ratio was estimated from the IFN- γ /IL-4 ratio. The two cytokines were quantified in serum from patients as well as from healthy controls by appropriate human Cytokine sandwich enzyme-linked immunosorbent assay (ELISA) kits according to the instructions of the manufacturer

(R&D Systems, Inc., Minneapolis, MN, USA). Limits of detection were 8 pg/mL for IFN- γ and 10 pg/mL for IL-4.

IgG assay

IgG A, M, and G were measured quantitatively by single radial immunodiffusion method using specific antisera and standard samples from Cypress Diagnostics (Langdorp, Belgium).

Albumin assay

Albumin measurement was carried out by protein zone electrophoresis performed on cellulose acetate plate using a commercial kit (Helena Laboratories, Beaumont, TX, USA). Protein bands were visualized with Ponceau S Stain 5526 (Helena Laboratories). Electrophoretic patterns were analyzed by densitometry using NIH ImageJ software. Albumin content level in grams per liter was determined using serum total protein (TPROT) concentration. TPROT assay was measured by modified Biuret method using Thermo Scientific kit (Thermo Fisher Scientific, Waltham, MA, USA).

NO assay

Serum nitrite and nitrate (NO_x, NO₂⁻ and NO₃⁻) levels were measured as an indirect marker of in vivo NO formation by Griess assay.¹⁸ Serum was first deproteinized with trichloroacetic acid. After centrifugation, the clear supernatant was added to the vanadium (III) chloride to reduce nitrate to nitrite. This was followed by addition of the Griess reagent that converts nitrite into a pink-colored azo compound. The absorbance was then measured at 520 nm, and NO concentrations were determined in comparison to the standard curve prepared from sodium nitrate (NaNO₃).

TAC measurement

Plasma TAC was measured according to the kit radicalaux libres (Spiral/KIRIAL, Dijon, France) biological test based on the hemolysis induced by radical attack.^{19,20}

Protein oxidation analysis

The levels of protein oxidation were determined by measuring PCs using ELISA kit, based on the detection of 2,4-dinitrophenylhydrazine (Biocell carbonyl protein ELISA kit, ALX-850-312-KI01; Axxora Deutschland GmbH, Lorrach, Germany).

Lipid peroxidation assay

The determination of plasma lipid peroxidation as MDA was measured at 535 nm using the thiobarbituric assay as described earlier.²¹

Serum β -lipoprotein assay

Serum β -lipoprotein (LDL) was measured by Helena lipoprotein electrophoresis on a cellulose acetate plate which had been presoaked in a Tris-barbital buffer at pH 8.8 (Helena Laboratories). The electrophoretic bands were stained using a methanol solution of Oil Red O (Sigma-Aldrich Co., St Louis, MO, USA).

LDL-BCD assay

Serum LDLs were first isolated by precipitation with heparin-trisodium citrate buffer as described earlier.²² The measurement of levels of LDL oxidation products was performed on the insoluble pellet by resuspension of precipitated lipoproteins in 1 mL of 0.1 M Na-phosphate buffer, pH 7.4, containing 0.9% NaCl.²³ LDL-BCD samples (100 μ L) were measured as an indicator of circulating ox-LDL in vivo as reported²⁴ and described earlier in detail.²³ The absorption spectrum was recorded at room temperature on a spectrophotometer ultraviolet/visible (Perkin-Elmer Lambda 800). The absorbance of each sample was read at 233 nm.

Statistical analysis

Data analyses were carried out using SPSS 16.0 (Statistical Package for the Social Sciences; SPSS Inc., Chicago, IL, USA) or Epi Info 2000 (Version 1.0; Epi Info, Atlanta, GA, USA), appropriately. Depending on the results of a test of normality, the comparison of means was performed by Student's *t*-test (for normally distributed variables) or Mann-Whitney *U*-test (for variables that were not normally distributed).²⁵ The comparison of frequencies was carried out using Yates's chi-square test. Relative risk (RR) and corresponding 95% confidence interval (CI) were calculated to determine cross-sectional associations among quartiles of 25th, 50th, and 75th percentile values as cutoff points. Bivariate correlation was performed using Pearson's or Spearman's correlation coefficients, appropriately, according to the normality of the distribution. The significance level was set at $P < 0.05$.

Results

Table 1 shows the demographic and clinical characteristics of participants of the current study. The mean age, the sex ratio, and body mass index were similar between patients and controls (for all comparisons, $P > 0.05$). However, the serum levels of LDH and ALP were significantly higher in patients than in controls ($P = 0.025$ and $P = 0.030$, respectively). In addition, the histological types of B-cell lymphoma were diffuse large B-cell lymphoma (DLBCL, 59%), B-cell lymphomas, unclassifiable, with feature intermediate between Burkitt's

lymphoma (BL) and DLBCL (B-UNC/BL/DLBCL, 3%) and BL (38%). B-UNC/BL/DLBCL was diagnosed based on morphologic, molecular genetics, and immunophenotypic features, according to the World Health Organization

Table 1 Clinical and demographic characteristics of patients with newly diagnosed aggressive B-cell NHL

Variable	Controls (n=25)	Patients (n=32)	P
Age (range, years)	50.5 (25–72)	52.81 (25–70)	0.630
Sex (M/F)	13/12	18/14	0.959
BMI (kg/m ²)	23.54±0.64	23.43±0.68	0.909
LDH (U/L)	201.23±72.33	431.72±68.24	0.025
ALP (U/100 mL)	84.27±13.89	130.18±14.59	0.030
Histological types			
DLBCL (%)	–	59	–
B-UNC/BL/DLBCL (%)	–	3	–
BL (%)	–	38	–

Notes: $P < 0.05$ was considered statistically significant. Data are presented as mean \pm standard error.

Abbreviations: ALP, alkaline phosphatase; BL, Burkitt's lymphoma; B-UNC/BL/DLBCL, B-cell lymphomas, unclassifiable, with feature intermediate between BL and DLBCL; BMI, body mass index; DLBCL, diffuse large B-cell lymphoma; F, female; LDH, lactate dehydrogenase; M, male; NHL, non-Hodgkin lymphoma.

classification of tumors of hematopoietic and lymphoid tissue (2001 and revised in 2008).^{26,27}

As indicated in Figure 1, the levels of IFN- γ were significantly lower in patients than in controls ($P=0.014$), while those of IL-4 were significantly higher ($P < 0.001$). In addition, Th1/Th2 ratio, as estimated from the IFN- γ /IL-4 ratio, was significantly lower in patients compared to controls ($P=0.003$). Moreover, the serum IgA and IgM levels were significantly higher in patients than in controls ($P=0.002$ and $P < 0.001$, respectively), whereas IgG levels were similar in the two groups ($P=0.385$; Figure 2).

As indicated in Figure 3, the plasma levels of total antioxidative defense capacity and the concentration of albumin were significantly decreased in patients when compared to controls ($P=0.001$ and $P < 0.001$, respectively). However, the circulating levels of MDA and PC were significantly increased in patients compared to controls (for the two comparisons, $P=0.001$).

Although the levels of LDL are similar in both groups ($P=0.853$), those of LDL-BCD were significantly increased in

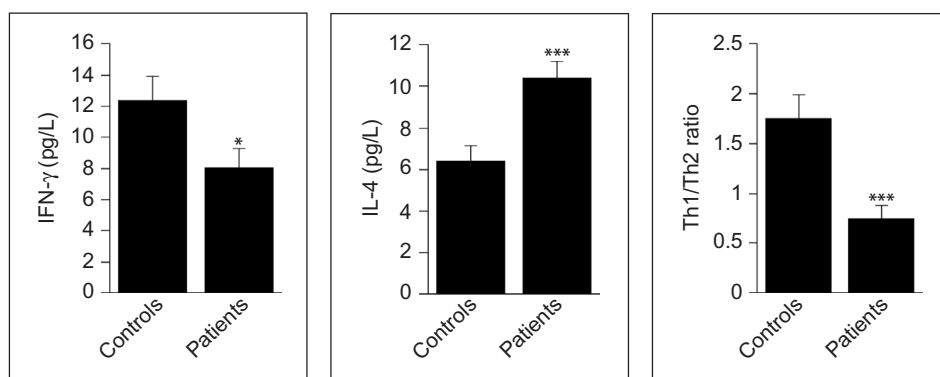


Figure 1 Serum levels of IFN- γ and IL-4 and Th1/Th2 ratio in newly diagnosed patients with aggressive B-cell NHL and matched control subjects.

Notes: * $P < 0.05$, *** $P < 0.001$. Data are presented as mean \pm standard error.

Abbreviations: IFN, interferon; IL, interleukin; NHL, non-Hodgkin lymphoma; Th, T helper.

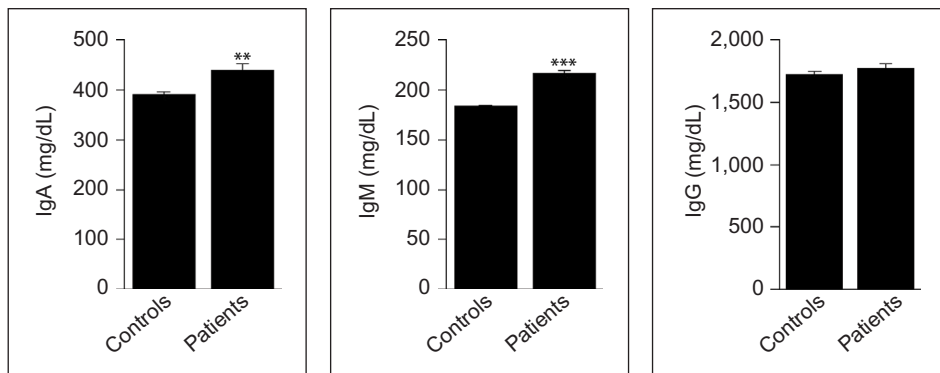


Figure 2 Serum levels of Igs A, M, and G in newly diagnosed patients with aggressive B-cell NHL and matched control subjects.

Notes: ** $P < 0.01$, *** $P < 0.001$. Data are presented as mean \pm standard error.

Abbreviations: Ig, immunoglobulin; NHL, non-Hodgkin lymphoma.

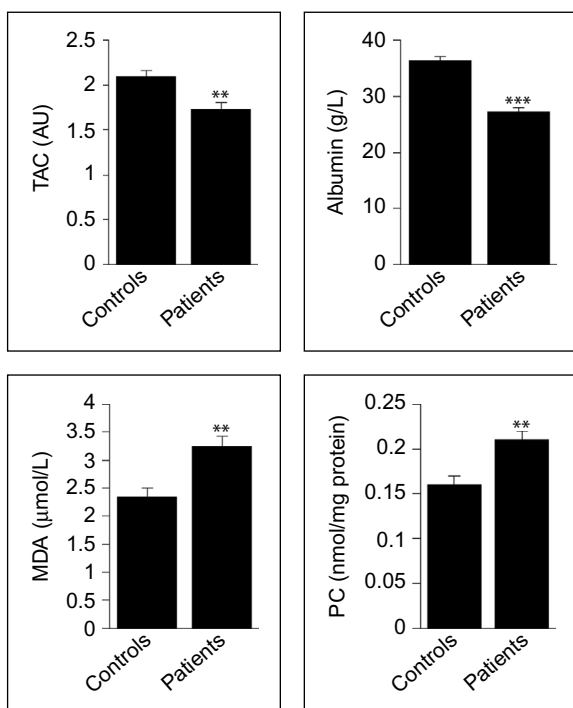


Figure 3 Oxidative stress biomarkers in patients with newly diagnosed B-cell NHL and healthy controls.

Notes: ** $P < 0.01$, *** $P < 0.001$. Data are presented as mean \pm standard error.

Abbreviations: MDA, malondialdehyde; NHL, non-Hodgkin lymphoma; PC, protein carbonyl; TAC, total antioxidant capacity.

patients compared to controls ($P < 0.001$; Figure 4). Similarly, the serum levels of NO were significantly higher in patients when compared to controls ($P < 0.001$; Figure 5).

The association analysis of LDL-BCD and NO with aggressive B-cell NHL among quartiles of 25th, 50th, and 75th percentile is highlighted in Table 2.

As indicated in Table 2, aggressive B-cell NHL was significantly associated with high levels of LDL-BCD from 25th to 75th percentile (25th percentile: RR = 2.26, 95% CI 1.42–3.59, $P = 0.014$; 50th percentile: RR = 2.84, 95% CI 1.72–4.68, $P < 0.001$; 75th percentile: RR = 5.43, 95% CI 2.58–11.42, $P < 0.001$). Similarly, the disease was significantly associated with high levels of NO production from 25th to 75th percentile (25th percentile: RR = 2.07, 95% CI 1.25–3.44, $P = 0.024$; 50th percentile: RR = 2.78, 95% CI 1.63–4.72, $P < 0.001$; 75th percentile: RR = 4.68, 95% CI 2.21–9.91, $P < 0.001$).

The correlation coefficients and their respective P -values are shown in Table 3. The levels of LDL-BCD were positively and significantly correlated with IFN- γ ($r = 0.377$, $P = 0.033$), and IgA ($r = 0.422$, $P = 0.016$), IgM ($r = 0.519$, $P = 0.002$). In addition, NO levels were positively and significantly correlated with IgM ($r = 0.461$, $P = 0.008$), but inversely and significantly correlated with IFN- γ ($r = -0.528$, $P = 0.002$) and Th1/Th2 ratio ($r = -0.354$, $P = 0.047$).

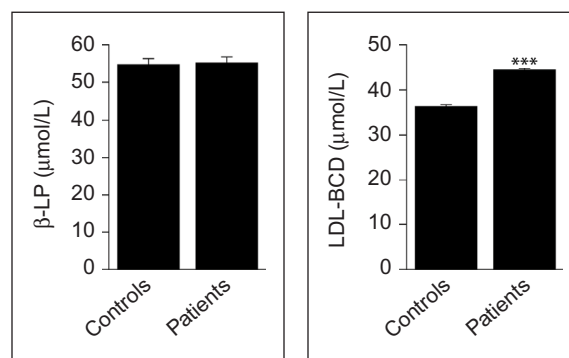


Figure 4 β -Lipoprotein and LDL-BCD levels in newly diagnosed patients with aggressive B-cell NHL and healthy controls.

Notes: *** $P < 0.001$. Data are presented as mean \pm standard error.

Abbreviations: NHL, non-Hodgkin lymphoma; LDL-BCD, baseline levels of conjugated dienes in low-density lipoprotein; β -LP, β -lipoprotein (LDL).

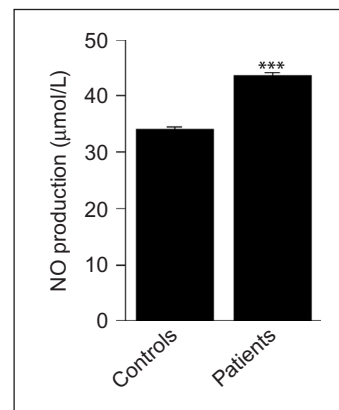


Figure 5 NO production levels in newly diagnosed patients with aggressive B-cell NHL and healthy controls.

Notes: *** $P < 0.001$. Data are presented as mean \pm standard error.

Abbreviations: NHL, non-Hodgkin lymphoma; NO, nitric oxide.

Discussion

An increase in ALP levels is frequently associated with a variety of cancers,²⁸ including NHL.^{29,30} ALP can also be suggested as a valuable prognostic biomarker for malignant tumors.³¹ Similar to ALP, we observed an increase in the levels of LDH in patients with B-cell NHL. Also of note, increased enzymatic activity of LDH leads to the release of huge amounts of lactate from pyruvic acid and therefore to the activation of proinflammatory pathways and angiogenesis. This phenomenon has been reported as Warburg effect.³² In addition, increased LDH levels have been associated with poor prognosis in patients with cancer.³³

Alteration of Th1/Th2 ratio was usually highlighted in NHL.²⁹ Low plasma concentrations of Th1-related cytokines IFN- γ have been recently shown in patients with NHL enrolled in a prospective case-control study; among them 92% were diagnosed with B-cell NHL.³⁴ Therefore, the

Table 2 Association of LDL-BCD and NO production with newly diagnosed aggressive B-cell NHL among quartiles of 25th, 50th, and 75th percentile

Quartile	Significance	LDL-BCD (μmol/L)	NO production (μmol/L)
25th percentile	RR (P/C)	2.26 (31/19)	2.07 (30/18)
	95% CI	1.42–3.59	1.25–3.44
	P	0.014	0.024
50th percentile	RR (P/C)	2.84 (30/13)	2.78 (29/12)
	95% CI	1.72–4.68	1.63–4.72
	P	<0.001	<0.001
75th percentile	RR (P/C)	5.43 (30/6)	4.68 (28/6)
	95% CI	2.58–11.42	2.21–9.91
	P	<0.001	<0.001

Note: $P < 0.05$ was considered statistically significant.

Abbreviations: CI, confidence interval; LDL-BCD, baseline levels of conjugated dienes in low-density lipoprotein; NHL, non-Hodgkin lymphoma; NO, nitric oxide; P/C, number of cases per each percentile in patients (P; n=32) and controls (C; n=25); RR, relative risk.

Table 3 Relationship of LDL-BCD and NO production with newly diagnosed aggressive B-cell NHL

Variable	Correlation coefficient	
	LDL-BCD (μmol/L)	NO production (μmol/L)
IFN-γ (pg/mL)	0.377	-0.528
P	0.033	0.002
IL-4 (pg/mL)	0.256	0.453
P	0.157	0.009
Th1/Th2 ratio	0.325	-0.354
P	0.070	0.047
IgA (mg/dL)	0.422	-0.230
P	0.016	0.205
IgM (mg/dL)	0.519	0.461
P	0.002	0.008
IgG (mg/dL)	-0.121	0.148
P	0.510	0.419
Albumin (g/L)	-0.231	-0.370
P	0.203	0.037

Notes: $P < 0.05$ was considered statistically significant.

Abbreviations: IFN, interferon; Ig, immunoglobulin; IL, interleukin; NHL, non-Hodgkin lymphoma; NO, nitric oxide; LDL-BCD, baseline levels of conjugated dienes in low-density lipoprotein; Th, T helper.

deletion or inhibition of Th1 cells may reduce the capacity of cancer cell elimination by cytotoxic CD8+ T-cells.³⁵

Serum concentrations of IgA and IgM levels were significantly higher in patients than in controls. Generation of Igs have been implicated in contributing to the disease course of multiple cancers,³⁶ and may serve as useful biomarker of disease activity and/or prognostic. Our results corroborate those of the previous study³⁷ regarding the significant increase in IgA as well as IgM levels. Both IgA and IgM could be increased early in response to invasion by bacteria

and viruses, such as Epstein–Barr virus (EBV).³⁸ Hence, numerous recent epidemiological and experimental studies have revealed that EBV is highly associated with several different types of aggressive NHL.³⁹ Therefore, our results indicate a higher concordance with inflammatory conditions that could be immunologically mediated.

Excessive ROS production can cause persistent oxidative stress that may contribute to promote genetic instability and malignant transformation of cells.⁴⁰ In the current study, increased levels of MDA and PC and decreased levels of TAC and albumin in patients point toward an oxidative stress.

MDA is one of the lipid peroxidation end products, whereas PC is a product of irreversible nonenzymatic oxidation or carbonylation of protein.⁴¹ Their excessive levels have been described in inflammatory conditions, and in various types of cancers including hematological malignancies.⁴² MDA is able to contribute to the development of human cancer by acting as a mutagen and genotoxic agent.⁴³ Of note, it has been suggested that its increased levels may have an important role in the pathogenesis of aggressive B-cell NHL.⁴⁴

TAC is considered as useful and one of the best biomarkers of overall plasma antioxidant status. Only one study on total radical scavenging ability and the risk of NHL, based on a food consumption survey, has recently reported that higher antioxidant intake as estimated by the food-frequency questionnaire-based oxygen radical absorbance capacity (ORAC) values is associated with a lower risk of NHL.⁴⁵ Albumin is described as the predominant antioxidant molecule in plasma.⁴⁶ Our findings are similar with recent results reporting lower levels of ORAC and/or albumin in patients with NHL.²⁹ In addition, several clinical trials have shown that serum albumin is a significant prognostic marker for the disease.⁴⁷

It is now well established that oxidative stress leads to LDL oxidation and vice versa.^{48,49} Our study demonstrates, for the first time, that circulating levels of LDL-BCD are increased in newly diagnosed aggressive B-cell NHL and significantly associated with the disease. In addition, LDL-BCD was significantly and positively correlated with IFN-γ, IgA, and IgM. Although the correlation does not imply causation, our results corroborate with those showing that LDL-BCD induces B-cell activation and production of antibodies and proinflammatory Th1 cytokines, such as IFN-γ.⁵⁰ In addition, it has been observed that the majority of CD5+/B-1 B-cells produce natural IgM antibodies that

recognize altered self-molecules such as ox-LDL.⁵¹ This observation is consistent with our findings regarding the correlation between LDL-BCD and IgM; nevertheless, the determination of the level of IgM anti-ox-LDL antibodies would still be interesting.

NO plays very diverse roles in physiological, neurological, and immunological functions. It may lead to different effects, which are sometimes opposed. The protective and toxic effects of NO are frequently seen in parallel.⁵² Thus, depending upon the specific situation, it can act as a mediator of apoptosis and cell death,⁵³ or otherwise opposes apoptosis and contributes to mutagenesis and carcinogenesis.^{54,55} To date, there are no data on circulating levels of NO production in patients with B-cell NHL. In this study, the levels of NO production were significantly higher in patients with newly diagnosed aggressive B-cell NHL, and strongly associated with the disease. Increased circulating levels of NO could be attributed to its role in tumor-associated inflammatory and antiviral responses.⁵⁶ Indeed, it has previously been shown that NO may be generated to counteract the reactivity of EBV.⁵⁷ Of note, during rapid cell proliferation, there is an overproduction of ROS and reactive nitrogen species resulting in oxidative stress. Protection against oxidative stress could be provided through the Warburg effect. The carcinogenic effects of excessive reactive nitrogen species and ROS can be attributed to their ability to cause DNA damage.⁵⁸ It has previously been reported that the NO activity is strongly influenced by its concentration.⁵² At high concentrations, NO is rapidly oxidized to reactive nitrogen species,⁵⁹ following its interaction with O₂ or O₂⁻.⁵⁵ The harmful effects of NO are related to its ability to form the potent cytotoxic oxidants peroxynitrite (ONOO⁻) and its conjugate acid ONOOH after interaction with the superoxide anion (O₂⁻). Interestingly, peroxynitrite was reported to play key roles in a significant proportion of various diseases, including cancers.^{60,61}

Conclusion and future prospects

In light of our results, it seems that the newly diagnosed aggressive B-cell NHL is associated not only with a marked increase in LDL-BCD and NO production but also with immune and physiological disorders, including a shift of Th1/Th2 balance toward Th2 dominance, high circulating Igs, particularly IgA and IgM, and an excessive oxidative stress. Nevertheless, our results have to be examined using ex vivo mechanistic studies leading to further investigations of these parameters, with an interest in the link between EBV infection and NO and Igs.

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Disclosure

The authors report no conflicts of interest in this work.

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