



Original Article

Prognostic Impact of Baseline Immunologic Profile in Aggressive B-cell non-Hodgkin's Lymphomas

Safaa Ramadan^{1,2}, Giusy Ceparano¹, Alessandro Cignetti³, Simona Sammassimo¹, Vincenzo Bagnardi⁴, Eleonora Pagan⁴, Daniela Gottardi³, Stefano Fiori⁵, Rita Passerini⁶, Tommaso Radice¹, Giuseppe Saglio³ and Corrado Tarella^{1,7}.

¹ Division of Onco-Hematology, European Institute of Oncology, IRCCS, Milano, Italy.

² NCI-Cairo University, Egypt, Cairo, Egypt.

³ Divisione Universitaria di Ematologia e Terapie Cellulari, A.O. Ordine Mauriziano, Torino, Italy.

⁴ Department of Statistics and Quantitative Methods, University of Milan-Bicocca, Milano, Italy.

⁵ Haemolymphopathology Unit, European Institute of Oncology IRCCS, Milan, Italy.

⁶ Divisione di Medicina di Laboratorio, European Institute of Oncology, Milano, Italy.

⁷ Dipartimento Universitario di Scienze della Salute (DISS), Università di Milano, Italy.

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Abstract. Host immune homeostasis as an independent prognostic indicator has been inadequately evaluated in aggressive non-Hodgkin's lymphomas (NHL). The present study addresses the prognostic significance in aggressive NHLs of the immunologic profile evaluated by pretreatment serum levels of immunoglobulins (Ig) and lymphocyte-monocyte ratio (LMR). In this series of 90 patients with aggressive lymphoma, the median level for IgG was 1,024mg/dl (range 436-2236), and for LMR was 2.2 (range 0.2-13.8). CR rate was higher with IgG levels $\geq 1,024$ mg/dL (91% vs 77% p=0.059). LMR ≤ 2.2 was associated with lower 1-year PFS (73% vs. 92%, p 0.016). Patients with good/very good R-IPI showed a reduced PFS if IgG or LMR was low, while patients with poor R-IPI did better if LMR or IgG levels were high. We combined both parameters with the R-IPI and produced a four-risk prognostic score showing one-year PFS of 95% (95% CI 68%-99%), 100% (95% CI 100%-100%), 73% (95% CI 52%-86%), and 59% (95% CI 31%-79%), in patients with zero, one, two and three risk factors, respectively. The results indicate for the first time the value of baseline serum Ig levels in the prognostic assessment of aggressive lymphoma.

Keywords: Immunoglobulin; IGG; Prognosis; Aggressive B-cell Non-Hodgkin's Lymphoma; Lymphocyte-monocyte ratio.

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Correspondence to: Prof. Corrado Tarella. Onco-hematology Division, European Institute of Oncology IRCCS, Milan, Italy; & Dip. Universitario Scienze della Salute (DISS), Università di Milano, Italy, Via Ripamonti 435, 20141 Milan, Italy. Tel: +39 02-574896. E-mail: corrado.tarella@unimi.it

Introduction. Several immune biomarkers have been recognized as having a prognostic impact on the clinical outcome in patients with Hodgkin and non-Hodgkin lymphomas. Some of them are the absolute lymphocyte (ALC) and monocyte count (AMC) as well as the

lymphocyte-monocyte ratio (LMR) at diagnosis.^{1,2} The latter was documented as an independent prognostic factor from the IPI prognostic score in patients with DLBCL treated with chemo-immunotherapy.³

Serum immunoglobulin (Ig) levels can mirror immune homeostasis and may be of prognostic relevance in hematologic malignancies. Low levels of serum Ig have a well-documented adverse prognostic role in various indolent lymphomas.^{4,5} Interestingly, pre-transplant hypogammaglobulinemia had a negative impact on RFS in patients with DLBCL undergoing autologous transplant, with 18-month RFS 44% if the levels of IgG <600mg/dl, and 63% if higher.⁶ To our knowledge, there are no other reports on the prognostic impact of immunoglobulin levels at diagnosis in patients with aggressive NHL. Therefore, we evaluated the prognostic role of LMR and pretreatment levels of immunoglobulins in aggressive NHL patients. In addition, we investigated the role of these factors in the current standard prognostic score system, the R-IPI score. Finally, we developed a scoring system that includes patients' immunologic profile and R-IPI to optimize their outcome prediction.

Methods. A retrospective analysis has been performed in aggressive B-cell NHL patients diagnosed and managed at two Hematology Centers, at the Mauriziano Hospital in Torino and the European Institute of Oncology in Milan, Italy, between April 2014 and October 2018. Eligible for this study were newly diagnosed patients with a histologically proven aggressive B-cell lymphoma, including diffuse large B-cell lymphoma (DLBCL, n=71), plasmablastic lymphoma (n=1), primary mediastinal large B-cell lymphoma (PMLCL, n=17), and Burkitt's lymphoma (n=1).

All patients were treated with chemo-immunotherapy according to the Center guidelines, after giving informed consent. The study has been approved by the local Ethical Committee in Milan. PFS and O.S. were assessed using the Kaplan-Meier method and compared between groups using the log-rank test. Hazard ratios were calculated using univariate and multivariate Cox proportional hazard models. Analyses were performed with SAS software, version 9.4 (SAS Institute, Cary, North Carolina, USA).

Results. Overall, 90 patients were eligible for the study, but 89 were evaluable for response (one patient died due to sepsis immediately after the first cycle). At baseline, the median serum immunoglobulin levels was 1,024mg/dL (range 436-2,236) for IgG, 191mg/dL (range 15-510) for IgA and 91mg/dL (range 15-1462) for IgM. Median ALC was 1,300/mm³ (range 230-3,990), median AMC was 600/mm³ (range 100-1,440) and median LMR was 2.2 (range 0.2-13.8).

IgM levels were higher in females compared to males (median 102mg/dL, vs 81mg/dL, respectively, P=0.038). Patients with no bone marrow (B.M.) infiltration showed higher IgG and IgA levels than patients with B.M. involvement. There was no association between Ig

levels and LMR.

A trend of higher CR rates was seen in patients with IgG levels $\geq 1,024$ mg/dL (91.3% vs 76.7%, respectively p=0.059) and in patients with LMR > 2.2 (91.1% vs 77.3%, p=0.073).

At a median follow up of 16 months, the 1-year O.S. and PFS of the whole series were 92% (95% CI: 83%-96%) and 83% (95% CI: 73%-89%), respectively.

All risk factors included in the revised IPI (R-IPI) score (i.e., age > 60, advanced stage, high ECOG PS, 2 or more extranodal sites, high LDH) were associated with a worse prognosis (**Table 1**). PFS was significantly lower in patients with poor R-IPI score compared to the other two groups (**Figure 1A**, 73% vs. 91%, p=0.018). Patients with IgG lower than 1,024mg/dl had lower 1-year PFS (73% vs 91%, respectively p=0.135), and patients with LMR ≤ 2.2 had also inferior 1-year PFS (73% vs 92%, respectively p=0.016).

The prognostic role of IgG and LMR on PFS was further investigated according to the R-IPI score. Having a low IgG at diagnosis (<1,024mg/dl) worsened the 1-year PFS in good or very good R-IPI patients (84% vs. 96%). Interestingly, the poor R-IPI risk group with high IgG levels had 1-year PFS similar to the good risk group with low IgG levels (87% vs. 84%). The lowest PFS (63%, 95% CI 40%-80%) was seen in the poor R-IPI group with low IgG (**Figure 1B**). Similarly, a low LMR ratio strongly worsened the PFS of all R-IPI risk groups (**Figure 1C**).

Poor R-IPI, low IgG, and low LMR were then assessed in a new 4-level risk score, taking 1 point for each adverse factor. The 1-year PFS was 95% (95% CI 68%-99%) in patients with zero risk factors, and 100% (95% CI 100%-100%), 73% (95% CI 52%-86%), 59% (95% CI 31%-79%) in patients with one, two and three risk factors, respectively (**Figure 1D**).

Discussion. Besides cell-mediated immunity, humoral factors, mainly antibody-mediated immunity, have a role in controlling neoplastic cell development and expansion.^{7,8} In the present study, the prognostic role and the clinical implications of serum Ig levels at baseline, along with the pattern of circulating lymphocytes and monocytes, were investigated in aggressive NHL patients. Although the follow-up period was limited to 16 months, the estimated 1-year O.S. (92%) and PFS (83%) look quite promising.

Overall, hypogammaglobulinemia was recorded at baseline in only eight patients (9%), which is less than the 15% reported in a previous study.⁹ Interestingly, four out of these eight patients had primary refractory disease with rapidly fatal outcome. Among the remaining 82 patients, more than half had Ig values towards the low normal range, according to the reference laboratory values, and that is in line with similar results previously reported in NHLs.¹⁰ Therefore, we chose to use the median immunoglobulin values at diagnosis

Table 1. Univariate and multivariate analysis on PFS according to the revised International Prognostic Index (R-IPI) and immunological parameters.

	N	Events	1-yr PFS (95% CI)	p-value ^a	H.R. (95% CI) Univariate analysis ^b	H.R.** (95% CI) Multivariate analysis ^c
All patients	90	18	83% (73%-89%)	-	-	-
R-IPI single parameters						
Age at diagnosis						
≤ 60 years	37	3	95% (80%-99%)	0.045	Ref.	-
> 60 years	53	15	76% (61%-85%)		3.30 (1.02-11.4)	
Stage at diagnosis						
I/II	43	5	86% (70%-94%)	0.194	Ref.	-
III/IV	47	13	80% (66%-89%)		1.95 (0.70-5.49)	
ECOG PS at diagnosis						
0/1	56	7	91% (79%-97%)	0.016	Ref.	-
2 or more	34	11	70% (51%-82%)		3.18 (1.17-8.61)	
Number of extranodal sites						
0 or 1	66	10	88% (77%-94%)	0.097	Ref.	-
2 or more sites	24	8	70% (47%-85%)		2.16 (0.85-5.52)	
LDH level at diagnosis						
≤ 1 × normal	41	5	85% (68%-94%)	0.158	Ref.	-
> 1 × normal	49	13	81% (66%-89%)		2.09 (0.73-5.93)	
R-IPI total score						
Very good (0 IPI factors)	10	1	90% (47%-99%)	0.018	Ref.	Ref.
Good (1 or 2 IPI factors)	42	3	92% (77%-97%)		0.63 (0.07-6.13)	
Poor (>2 IPI factors)	38	14	73% (56%-85%)		3.05 (0.40-23.4)	3.00 (0.92-9.80)
Immunological parameters						
IgG at diagnosis						
< 1024 (median)	44	12	73% (56%-84%)	0.135	Ref.	Ref.
≥ 1024	46	6	93% (78%-98%)		0.48 (0.18-1.29)	0.60 (0.22-1.64)
IgA at diagnosis						
< 191 (median)	44	11	77% (60%-87%)	0.393	Ref.	-
≥ 191	46	7	88% (74%-95%)		0.66 (0.26-1.72)	
IgM at diagnosis						
< 91 (median)	45	10	75% (61%-85%)	0.616	Ref.	-
≥ 91	45	8	87% (73%-94%)		0.79 (0.31-2.00)	
L/M ratio at diagnosis						
≤ 2.2 (median)	45	13	73% (57%-84%)	0.016	Ref.	Ref.
>2.2	45	5	92% (78%-97%)		0.30 (0.11-0.85)	0.46 (0.15-1.36)
New risk score ^d						
0 points	21	1	95% (68%-99%)	0.011	Ref.	-
1 point	26	2	100% (100%-100%)		1.90 (0.17-21.0)	
2 points	28	8	73% (52%-86%)		6.67 (0.83-53.4)	
3 points	15	7	59% (31%-79%)		11.1 (1.36-90.2)	

^a log-rank test. ^b estimated using a Cox regression model. ^c estimated using a multivariate Cox regression model including the revised IPI total score and the immunological parameters with a log-rank p-value < 0.20. ^d Poor R-IPI, low IgG and low LMR were considered in a new 4-level risk score, taking 1 point for each adverse factor. *Abbreviations:* P.S.: Performance Status; L/M ratio: circulating Lymphocyte to Monocyte ratio

corresponding to IgG 1,024mg/dL to categorize patients with high or low IgG levels in correlative analysis. In univariate analysis, patients with IgG value <1024mg/dL had a worse 1-year PFS than patients with

higher levels.

In the present series, patients with LMR ≤2.2 had an inferior 1-year PFS. Baseline LMR was matched with IgG levels, and the two parameters were not correlated.

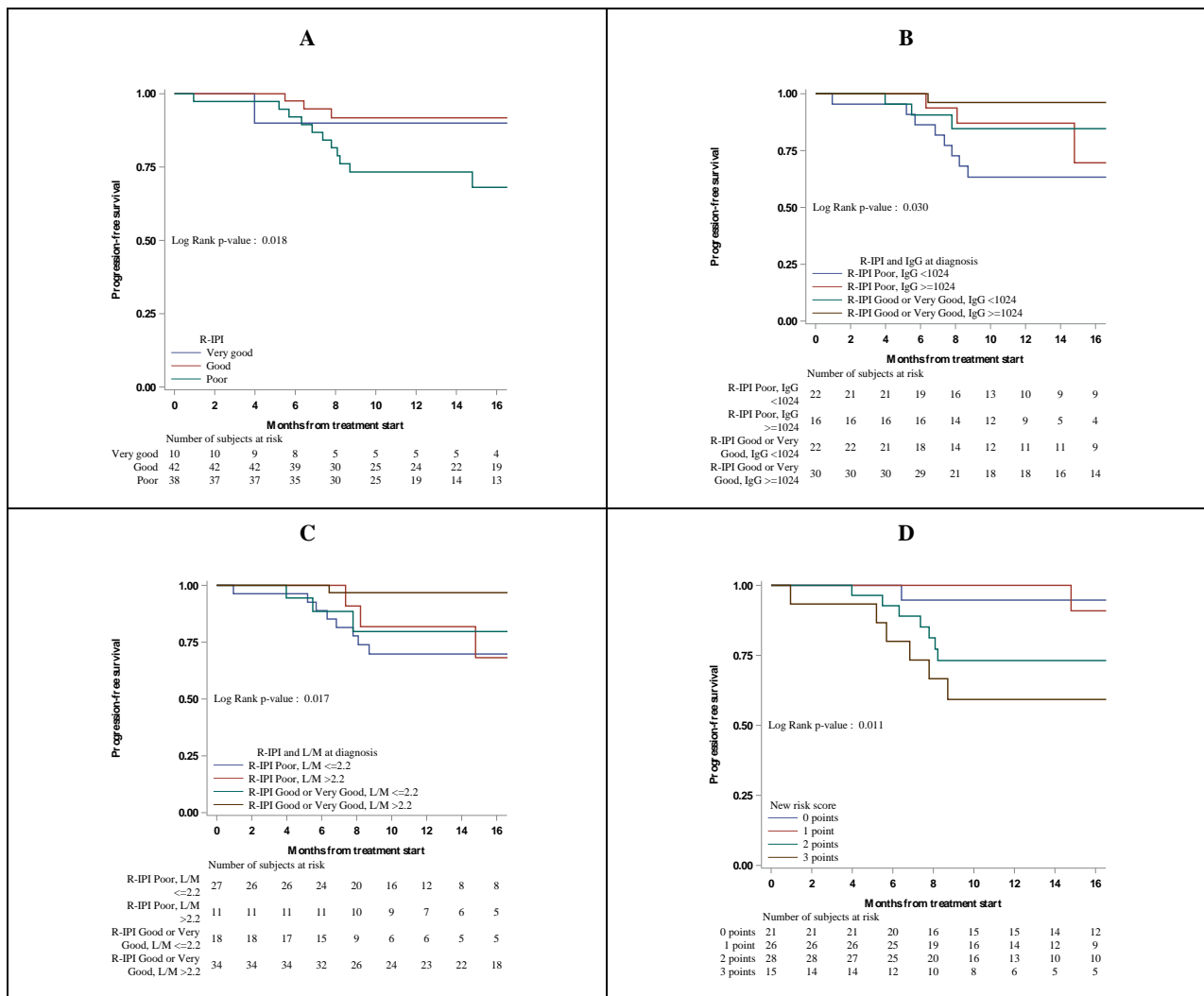


Figure 1. Progression-free survival, by R-IPI risk group (**Panel A**), by R-IPI risk group and IgG at diagnosis (**Panel B**), by R-IPI risk group and Lymphocytes/Monocytes ratio at diagnosis (**Panel C**), and by the 3-factors (poor R-IPI - low LMR - low IgG levels) new risk score (**Panel D**).

This finding could be explained by the fact that low LMR was due to high absolute monocytic count in 11 out of 45 (24.5%) patients. Besides, IgG levels were low only in 60% of patients with lymphopenic versus 40% of those with lymphocytes in the normal range. Due to the lack of correlation between IgG levels and LMR at baseline, both parameters were assessed in combination with the R-IPI. Patients with good/very good R-IPI showed a reduced PFS if associated with either low IgG or low LMR. Patients with poor R-IPI did better if they had either high LMR or high IgG levels. Dismal outcome was seen in those patients with poor IPI and low levels of either IgG or LMR. Based on these observations, a novel clinical and immunological prognostic score was developed. The score distinguished four groups with different 1-year PFS ranging from 95% if no or one risk factor was present to 59% if the three factors were present.

Our study introduces the baseline serum immunoglobulin levels in the prognostic assessment of aggressive lymphoma patients. We are aware of some

weak points of the study: in particular, the retrospective analysis and the relatively small number of patients, with the inclusion of different aggressive lymphoma subtypes, although most of them were DLBCL; lastly, the follow up time is short. However, C.R. achievement and the 1-year PFS are considered reliable surrogate endpoints to predict the ultimate outcome in DLBCL.^{11,12}

Conclusions. Our results suggest that the addition of baseline immunologic profile to R-IPI optimizes the prognostic stratification of patients with aggressive B-NHL and identifies more distinctly patients at high risk of poor outcome. Additional studies on the role of Ig levels in the development of aggressive B-cell lymphoma are advised.

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References:

1. Porrata LF, Ristow K, Colgan JP, Habermann TM, Witzig TE, Inwards DJ, Ansell SM, Micallef IN, Johnston PB, Nowakowski GS, Thompson C, Markovic SN. Peripheral blood lymphocyte/monocyte ratio at diagnosis and survival in classical Hodgkin's lymphoma. *Haematol*. 2012; 97(2): 262-269. <https://doi.org/10.3324/haematol.2011.050138> PMID:21993683 PMCid:PMC3269488
2. Wilcox RA, Ristow K, Habermann TM, Inwards DJ, Micallef IN, Johnston PB, Colgan JP, Nowakowski GS, Ansell SM, Witzig TE, Markovic SN, Porrata LF. The absolute monocyte and lymphocyte prognostic score predicts survival and identifies high-risk patients in diffuse large-B-cell lymphoma. *Leukemia*. 2011; 25(09): 1502-1509. <https://doi.org/10.1038/leu.2011.112> PMID:21606957
3. Rambaldi A, Boschini C, Gritti G, Delaini F, Oldani, E, Rossi A, Barbui AM, Caracciolo D, Ladetto M, Gueli A, De Crescenzo A, Passera R, Devizzi L, Patti C, Gianni AM, Tarella C. The lymphocyte to monocyte ratio improves the IPI-risk definition of diffuse large B-cell lymphoma when rituximab is added to chemotherapy. *Am J Hematol*. 2013; 88(12): 1062-1067. <https://doi.org/10.1002/ajh.23566> PMID:23940056
4. Atilla E, Atilla PA, Civriz Bozdog S, Toprak SK, Topcuoglu P, Ilhan O, Ozcan M, Arslan O. Does hypogammaglobulinemia at diagnosis effects survival and infection risk in chronic lymphocytic leukemia (CLL)? *Blood*. 2016; 128: 5577. <https://doi.org/10.1182/blood.V128.22.5577.5577>
5. Fischer T, Ni A, Soumerai JD, Alperovich A, Batlevi CL, Younes A, Zelenetz AD. Natural history of hypogammaglobulinemia in patients with follicular lymphoma and the impact of anti-CD20-based therapy. *Blood*. 2017; 130: 4054.
6. Bolwell BJ, Kalaycio M, Sobecks R, Andresen S, Rybicki L, Kuczkowski E, Bates J, Summers K, Bernhard L, Cherni K, Baker J, Brown S, Pohlman B. Autologous transplantation for Diffuse Large B Cell Lymphoma: pre-transplant hypogammaglobulinemia is a predictor for early toxicities. *Blood*. 2004; 104(11): 908. <https://doi.org/10.1182/blood.V104.11.908.908>
7. Upadhyay R, Hammerich L, Peng P, Brown B, Merad M, Brody JD. Lymphoma: immune evasion strategies. *Cancers (Basel)*. 2015; 7(2): 736-62. <https://doi.org/10.3390/cancers7020736> PMID:25941795 PMCid:PMC4491682
8. Lo Nigro C, Macagno M, Sangiolo D, Bertolaccini L, Aglietta M, Merlano MC. NK-mediated antibody-dependent cell-mediated cytotoxicity in solid tumors: biological evidence and clinical perspectives. *Ann Transl Med*. 2019; 7(5): 105. <https://doi.org/10.21037/atm.2019.01.42> PMID:31019955 PMCid:PMC6462666
9. Casulo C, Maragulia J, Zelenetz AD. Incidence of hypogammaglobulinemia in patients receiving Rituximab and the use of intravenous immunoglobulin for recurrent infections. *Clin Lymphoma Myeloma Leuk*. 2013; 13(2):106-11. <https://doi.org/10.1016/j.clml.2012.11.011> PMID:23276889 PMCid:PMC4035033
10. Biggar RJ, Christiansen M, Rostgaard K, Smedby KE, Adami HO, Glimelius B, Hjalgrim H, Melbye M. Immunoglobulin subclass levels in patients with non-Hodgkin lymphoma. *Int J Cancer*. 2009; 124(11): 2616-20. <https://doi.org/10.1002/ijc.24245> PMID:19235925
11. Shi Q, Schmitz N, Ou FS, Dixon JG, Cunningham D, Pfreundschuh M, Seymour JF, Jaeger U, Habermann TM, Haioun C, Tilly H, Ghesquieres H, Merli F, Ziepert M, Herbrecht R, Flament J, Fu T, Coiffier B, Flowers CR. Progression-free survival as a surrogate end point for overall survival in first-line diffuse large B-cell lymphoma: an individual patient-level analysis of multiple randomized trials (SEAL). *JCO*. 2018; 36(25): 2593-2602. <https://doi.org/10.1200/JCO.2018.77.9124> PMID:29975624 PMCid:PMC6532366
12. Tarella C, Gueli A, Delaini F, Rossi A, Barbui AM, Gritti G, Boschini C, Caracciolo D, Bruna R, Ruella M, Gottardi D, Passera R, Rambaldi A. Rate of primary refractory disease in B and T-cell non-Hodgkin's lymphoma: correlation with long-term survival. *PLoS One*. 2014; 9(9): e106745. <https://doi.org/10.1371/journal.pone.0106745> PMID:25255081 PMCid:PMC4177839