

PROGNOSTIC FACTORS IN FOLLICULAR LYMPHOMA IN THE RITUXIMAB ERA: HOW TO IDENTIFY A HIGH-RISK PATIENT?

Vit Prochazka*, Tomas Papajik, Marie Jarosova, Karel Indrak

Department of Hemato-Oncology, University Hospital Olomouc, Czech Republic
E-mail: vit.prochazka@fnol.cz

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Background. Follicular lymphoma accounts for about 20–30% of non-Hodgkin's lymphomas. Clinical behaviour and overall prognosis are highly variable, ranging from indolent forms with occasional spontaneous remissions to rapidly progressive disease.

Methods and Results. Modern treatment strategies have shifted from a primarily “palliative” approach to more intensive risk-adapted therapy with the intention of achieving complete long-term remission. New targeted treatment with monoclonal antibodies (MoAb) and radioimmunoconjugates (RIT) has resulted in unprecedented improvements in treatment outcome. At the same time, a large amount of information is now available on lymphomagenesis, the role of the microenvironment of lymphomatous follicles and cytogenetic abnormalities. We can better understand the role of the patient's innate anti-lymphoma immunity. Although no standard front-line therapy has been established, increasingly more data show that risk-adapted treatment strategy have survival benefits for high-risk patients. For this reason, accurate prognostic indices are urgently needed to find optimal therapies for particular lymphoma patients. Whereas the currently used FLIPI index was established in the pre-rituximab era, the newly designed FLIPI 2 index still needs to be confirmed in prospective trials.

Conclusion. New therapeutic approaches with MoAb, RIT and other biological agents allow the population to be divided into increasing numbers of groups with different outcomes. All in all, in the near future, we will probably not use only one basic prognostic index for all populations of FL patients. New prognostic schemes should analyze patients separately and include both disease- and patient/host-related parameters.

INTRODUCTION

Follicular lymphoma (FL) accounts for about 20–30% of all non-Hodgkin's lymphomas in Western Europe and the USA¹. In Central Europe (the Czech Republic), the incidence is about 19%, as seen from the Czech Lymphoma Study Group registry data². Although FL is a clinically and biologically heterogeneous disease, the outcome for patients has dramatically improved over the last decade, especially after targeted therapy with monoclonal antibodies (MoAb) was introduced³.

Prior to the introduction of rituximab, FL was considered a chronically relapsing condition. This was reflected by a treatment strategy using low-dose chemotherapy irrespective of the particular patient's risk. Despite evidence that patient survival is influenced by the quality of remission, those claiming that high-risk patients need intensive therapy were in the minority. The therapeutic success of MoAb showed that long-term remission may be induced. There were even optimistic predictions that these patients could be cured by conventional therapy. In the rituximab era, the treatment goal has changed significantly. Primary therapy, as non-toxic as possible, should induce long-term complete remission, preferably supported by maintenance (MoAb) or consolidation (RIT) therapy. The effect and tolerable toxicity of MoAb led to the concept of chemo-

immunotherapy, i.e. combinations of previously known conventional schemes with MoAb or RIT.

The effects of an antibody itself and its concomitant or sequential administration with chemotherapy were confirmed by numerous prospective randomized clinical studies in both primary therapy and relapses^{4,7}. The impact that monoclonal antibody added to induction therapy has on the survival of patients with B-cell lymphoma was confirmed by a meta-analysis of studies⁸. Extremely valuable for prolonging remission is application of rituximab or ibritumomab-tiuxetan in post-remission (maintenance) therapy^{9–13}. A similar effect on remission duration was seen in patients on maintenance treatment with interferon-alpha^{14,15}. The successful concept of chemoimmunotherapy is overshadowed by the fact there is still no agreement as to the optimal initial chemotherapy protocol and individual European and American professional groups prefer particular (or their own) protocols. So far, no prospective randomized study comparing a larger number of induction chemotherapy schemes has been carried out. Further, there are very few prospective randomized studies to assess the impact of therapy intensification^{16–18}.

The published data suggest that the benefit of adding rituximab to induction therapy is not comparable for all risk subgroups. Progression-free survival/overall survival

were substantially more influenced in high-risk patients than in low-risk groups⁶. However, patients with a high risk according to the FLIPI still have relatively unsatisfactory results after conventional chemoimmunotherapy. In this group, the proportion of patients free from lymphoma progression is about 30% and the proportion of survivors is 50-60% at 5 years (Table 1).

The aim of a modern prognostic index is to identify patients at high risk of first-line therapy failure so that their treatment is adequate to their risk. This goal can only be achieved by appreciating the variability of patient population studied and producing a predictive tool from variables that are relevant in the particular population¹⁹.

This review aims at summarizing the state of the art of prognostic factors used in FL patients that are relevant to the choice of therapy in the chemoimmunotherapy era.

GELF 86 criteria	BNLI criteria
Nodal or extranodal tumor >7 cm	B symptom(s) or pruritus
At least 3 nodal areas involved, each with lymph node(s) $\geq$ 3 cm	Rapid generalized disease progression
Any B symptom	Marrow compromise
Significant spleen enlargement	Life-threatening organ involvement
Blood cytopenia	Renal infiltration
	Bone lesion(s)
Treatment must be initiated if at least one criterion is present	

Fig. 1. Comparison of the GELF 86 and BNLI criteria for initiating therapy in FL¹⁹.

History of prognostic indices in follicular lymphoma

The first clinical prognostic criteria related to therapeutic decision-making were recommendations issued by the Groupe d'Etude des Lymphomes Folliculaires (GELF, France) and British National Lymphoma Investigation (BNLI) group. The criteria published between 1985 and 1990 aimed to identify patients with active disease and high tumour burden already requiring treatment²⁰.

In 1983, the International Prognostic Index (IPI) was published, which has become the gold standard for pre-

dicting the prognosis of patients with aggressive B-cell lymphoma²¹. The IPI was repeatedly tested in groups of FL patients and was shown to be able to stratify patients into groups with significantly different survival. Yet in cases of FL and indolent lymphoma, the IPI is not the optimal prognostic tool because (1) it was based on a group of patients with aggressive lymphoma, (2) variables relevant to indolent lymphoma were not considered and in contrast, some factors involved in IPI do not have the same impact in FL, and (3) the distribution of patients into individual risk groups was rather uneven.

The need for a specific prognostic index for FL patients led to the initiation of an international project in 2004, which retrospectively analyzed the overall survival (OS) of 4,162 patients treated between 1985 and 1992. Eight independent prognostic parameters were identified. Of those, the following five parameters were selected to produce an index: age over 60 years, lactate dehydrogenase level above the upper limit of normal, involvement of more than 4 lymph node groups, haemoglobin level of less than 120g/l and advanced stage of the disease (Ann Arbor classification). The final tool, referred to as Follicular Lymphoma International Prognostic Index (FLIPI), is used to evenly distribute patients into three groups: low, intermediate and high risk²². The predictive value of the FLIPI was confirmed by a large number of prospective studies of both untreated^{24,25} and relapsed patients²⁶. A high FLIPI score was found to predict unfavorable outcome after autologous stem cell transplantation^{27,28} and is associated with high risk of lymphoma transformation²⁹. Although the FLIPI score is used globally as the gold standard, its historical and methodological limitations must be considered. First, the FLIPI was created in the pre-rituximab era, that is, at the time when only few patients were treated with intensive (high-dose) chemotherapy and autologous stem cell transplantation and a large proportion of patients did not receive anthracycline-regimens. In these patient groups, the predictive value of the index may be limited. Another problem is the FLIPI endpoint, i.e. the overall survival. It is possible that if the progression-free survival (PFS) was assessed, the score would include other variables as well. Moreover, from a current perspective, PFS is a much more practical

Table 1. Comparison of the survival of patients with high risk of disease treated with conventional chemotherapy and chemoimmunotherapy in individual studies.

Study	Induction regimen	Progression-free survival	Overall survival
FLIPI ²²	chemo	NA	52.5% (at 5 years)
ILI ²³	chemo	NA	36% (at 5 years)
FLIPI2 (ref. ³¹)	R-chemo	29.0% (at 5 years)	59.0% (at 5 years)
Marcus et al. ⁵	R-CVP	26 months (median)	NA
Hiddemann et al. ⁶	R-CHOP	67% (at 2 years)	92% (at 2 years)

tool for comparing the individual treatment modalities in indolent lymphoma. With better (longer) survival of FL patients, studies with OS as the endpoint would take a rather long time. A third disadvantage is that due to unavailable data, the FLIPI did not test many other well-known robust prognostic parameters (beta-2 microglobulin level).

Variable	Adverse factor	P	RR	95% CI
Sex	Male	.001	1.33	1.14-1.56
Age	> 60 years	< 10 ⁻³	2.40	2.05-2.81
Ann Arbor stage	III-IV	< 10 ⁻³	1.66	1.26-2.19
Bone marrow	Involved	.001	1.37	1.14-1.64
Number of nodal sites	> 4	.001	1.32	1.11-1.56
Hemoglobin level	< 120 g/L	< 10 ⁻³	1.59	1.31-1.92
PB lymphocyte count	< 1 × 10 ⁹ /L	.008	1.27	1.06-1.52
LDH	> ULN	< 10 ⁻³	1.50	1.26-1.77

RR indicates relative risk (of death); CI, confidence interval; PB, peripheral blood; LDH, lactate dehydrogenase; and ULN, upper limit of normal.

Fig. 2. Results of the multivariate analysis of prognostic factors in the production of the FLIPI²².

Risk group	Number of factors*	Distribution of patients, %	5-year OS, % (SE)	10-year OS, % (SE)	RR	95% CI
Low	0-1	36	90.6 (1.2)	70.7 (2.7)	1.0	NA
Intermediate	2	37	77.6 (1.6)	50.9 (2.7)	2.3	1.9-2.8
High	≥ 3	27	52.5 (2.3)	35.5 (2.8)	4.3	3.5-5.3

N = 1795. OS indicates overall survival; SE, standard error; CI, confidence interval; RR, relative risk (of death), and NA, not applicable.

*Factors adversely affecting survival in the FLIPI include age greater than 60 years; Ann Arbor stage III-IV; number of nodal sites greater than 4; serum LDH level greater than the upper limit of normal; and hemoglobin level less than 120 g/L.

Fig. 3. Risk groups as defined by the FLIPI²².

The above reasons led to an effort to create a new index comprising all the relevant modern prognostic factors and primarily concerned with the progression-free survival. In 2009, a working group led by Professor Solal-Célgny, one of the authors of the original FLIPI, published a draft of a new index, FLIPI 2 (ref.³⁰). Prospective data from 832 patients were analyzed. Univariate analysis of 15 clinical and laboratory parameters identified five independent variables. These included two factors already contained in the FLIPI (age more than 60 years and serum haemoglobin less than 120 g/l) and three new factors: longest diameter of the largest involved node,

bone marrow involvement and the aforementioned beta-2 microglobulin level. Compared with the FLIPI, FLIPI 2 places stress on as precise assessment of tumour mass as possible. The FLIPI 2 also stratifies patients into three groups, albeit according to a different number of risk factors: low (0), intermediate (1-2) and high (3 or more) risk (Fig. 4). Despite this approach, the FLIPI 2 has certain weaknesses. Unlike FLIPI, the distribution into groups is less even (20% vs. 52% vs. 27%). The study population comprised only 59% of patients treated with rituximab and the median follow-up was short (38 months). Although subanalyses confirmed the predictive value of FLIPI 2 in the rituximab-treated population too³¹, the definite value of the FLIPI 2 will only be confirmed by prospective studies.

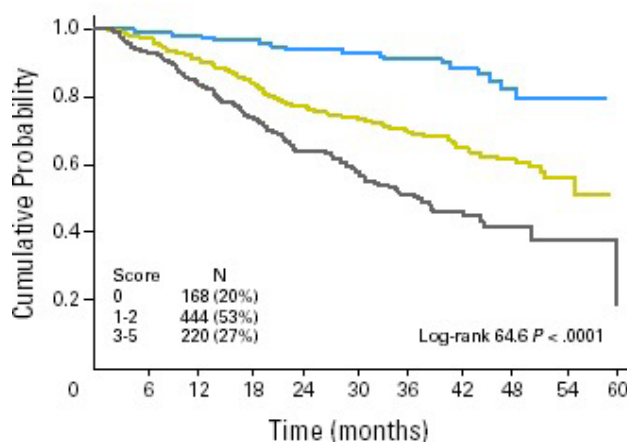


Fig. 5. Progression-free survival curves stratified according to the FLIPI 2 risk groups³⁰.

Prognostic value of the quality of remission

Treatment response is a key prognostic factor for the overall survival of FL patients. Numerous studies in both the pre-rituximab and the current era have convincingly shown that a satisfactory treatment outcome cannot be achieved without complete remission^{32,33}. It was the high complete remission rate that contributed to the success of the concept of chemoimmunotherapy, initially in the form of longer progression-free survival and recently as increasing evidence of the impact on the patients' overall survival⁵⁻⁷.

The effect of modern therapy places higher demands on assessing the depth (quality) of remission. The original conventional criteria published by Cheson et al. in 1999 (ref.³⁴) look insufficient for patients receiving modern

Risk Group	No. of Factors	Patients (%)	PFS				HR	95% CI
			3-Year		5-Year			
			%	SE	%	SE		
Low	0	20	90.9	2.4	79.5	5.0	1.00	—
Intermediate	1-2	53	69.3	2.4	51.2	5.7	3.19	2.00 to 5.15
High	3-5	27	51.3	3.7	18.8	13	5.76	3.53 to 9.40
High v intermediate							1.81	1.40 to 2.33

Abbreviations: FLIPI2, Follicular Lymphoma International Prognostic Index; PFS, progression-free survival; HR, hazard ratio.

Fig. 4. Outcome and risk of lymphoma progression according a risk groups as defined by FLIPI 2 (ref.³⁰).

therapy. This is due to the inadequate use of modern laboratory methods (bone marrow examination only) and the absence of the use of sensitive molecular genetic methods. Induction of the so-called molecular remission in FL patients, i.e. the absence of the *bcl-2*/IgH transcript in bone marrow or peripheral blood examination, has a significant predictive value. The impact of molecular remission detected by RT-PCR has been confirmed both in patients treated with conventional CHOP therapy^{35,37} and in those receiving high-dose chemotherapy with autologous stem cell transplantation^{16,38}. Patients who achieve complete remission according to the conventional criteria but remain *bcl-2*/IgH-positive in bone marrow are at a higher risk of lymphoma relapse, regardless whether they were or were not given maintenance therapy^{39,40}.

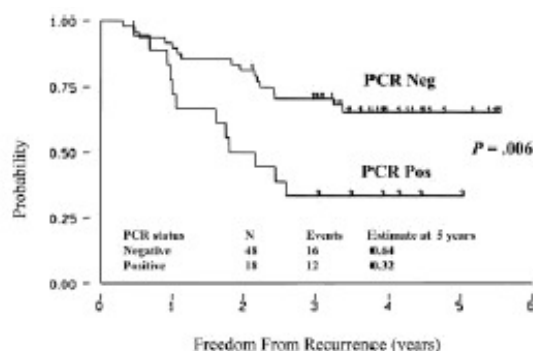


Fig. 6. Time to treatment failure in FL patients treated with CHOP or R-CHOP. Stratification according to the presence of the *bcl-2*/IgH transcript in bone marrow after therapy³⁵.

The possibility of molecular detection of residual disease goes hand in hand with modern methods for the detection of tumour mass, in particular 18-fluorodeoxyglucose positron emission tomography (FDG-PET). Imaging of viable tumour tissue is a brand new modality in oncology. Induction of post-treatment PET negativity is associated with a significantly better prognosis in patients with diffuse large cell lymphoma and Hodgkin's lymphoma^{41,42}. A new generation of hybrid PET/CT scanners provides an unprecedented quality of imaging by fusing a morphological CT scan and functional (metabolic) image from a PET detector⁴³. The introduction of this diagnostic modality required development of new "revised" criteria for treatment response⁴⁴. Although the use of PET/CT in FL is still in its infancy, the available studies suggest that it is of great value in the identification of patients with FL transformation to aggressive lymphoma^{45,46}. On the other hand, the initial assumption that the level of FDG accumulation closely correlates with the level of aggressiveness (histologic subtype) of lymphoma was not confirmed^{47,48}. Moreover, FDG-PET is probably unable to reliably differentiate bone marrow involvement⁴⁹. The predictive value of achieving PET negativity in FL patients is subject to study. However, FDG-PET/CT is likely to be valuable especially in patients with advanced, prognostically unfavourable disease who are candidates for intensive chemotherapy^{50,52}.

Prognostic value of cytogenetic abnormalities

A genetic abnormality typical for follicular lymphoma is translocation of the *BCL-2* gene to the immunoglobulin heavy chain gene enhancer region, leading to overexpression of the *Bcl-2* oncogene and subsequently to overproduction of the *bcl-2* protein. Overexpression of the *BCL2* is in rare cases caused by variant translocations such as the *t*(2;18) or *t*(18;22). This genetic events are considered to be so crucial in the process of clonal evolution of FL that the translocation is used as one of the supportive diagnostic features^{53,54}. In 1991, Limpens et al. demonstrated the presence of sporadic *t*(14;18)-bearing B cells in the blood and lymphoid tissues of healthy individuals in the absence of clinically manifest FL⁵⁵.

Fluorescent in situ hybridization (FISH) is capable of detecting *t*(14;18) in up to 90% of FL patients. In cases that until recently were considered *t*(14;18)-negative, FL can be detected by the so-called cryptic translocation⁵⁶.

Similar studies of cases with the absence of *t*(14;18) have shown significant differences between the two groups at the molecular genetic and histological levels too. A team led by A. Rosenwald studied 184 FL patients, of whom 17 were identified as *t*(14;18)-negative. Gene expression profiling and comparative genomic hybridization showed gains/amplifications of the *BCL2* gene locus in *t*(14;18)-positive FL, whereas activated B cell-like, *NFκB*, proliferation and bystander cell signatures were enriched in *t*(14;18)-negative FL. These genetic abnormalities correlated with immunohistochemical results since *t*(14;18)-negative FL showed weak or absent CD10 expression and increased Ki-67 proliferation index was present in 91% of cases. Despite the findings, no differences in the clinical course were observed between the two FL forms⁵⁷.

Most *t*(14;18)-positive FL cases are associated with several additional chromosomal abnormalities, some of which were found to have a clear prognostic value: 6q and 17p deletions are associated with unfavourable prognosis and a high risk of transformation. On the other hand, +der18q, +7 and +8 are associated with an indolent clinical course^{58,59}. In case of *t*(14;18)-negative FL, a variant form with 1q36 deletion and favourable prognosis has been reported⁶⁰.

Value of histological changes and role of the lymph node microenvironment

The lymph node microenvironment is made up of immune system cells that are not a part of the tumour clone but play a key role in the pathogenesis for the clinical picture of FL. Tumour elements in FL are in active interaction, especially with tissue macrophages and CD4 T cells. The interaction between lymphoma cells and the lymph node microenvironment seems to have a significant impact on lymphoma behaviour, treatment response and eventually the patient's overall prognosis^{61,62}.

Studies of gene expression profiling in FL identified two subgroups of patients based on different expression profiles of nonmalignant tumour-infiltrating cells⁶³. Type 1 immune response gene signature is associated with overex-

pression of genes encoding T cell markers (CD7, CD8B1, STAT4) and genes expressed in macrophages (ACTN1, TNFSF3B). This signature is associated with a good prognosis. On the other hand, type 2 immune response is associated with expression of genes of dendritic cells and/or macrophages (FCGR1A, CCR1) and with a poor prognosis⁶⁴. Some of these genes seem to be very strong predictors, such as CD7 for type 1 immune response or CCR1 for type 2 immune response. The immune response profiles divide the population into quartiles with different overall survival (Fig. 7).

Although gene expression profiling studies provide impressive data, their use in routine practice is severely limited by the technical requirements. Therefore, attention is focused on studies of the microenvironment using immunohistochemical methods.

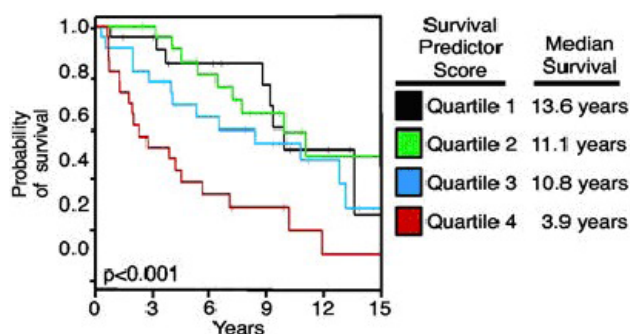


Fig. 7. Prognostic value of individual immune response gene expression profiles in FL⁶³.

The most studied populations of microenvironment cells are T cells and macrophages. The first studies showed a strong negative predictive value of large numbers of tumour-associated macrophages (TAM) (ref.⁶⁵). These findings were true for patients treated without rituximab. By contrast, this prognostic marker loses its value⁶⁶ or, paradoxically, becomes positive⁶⁷, which is explained by the role of TAM in antibody-dependent cell-mediated cytotoxicity (ADCC). A large number of TAM in the lymphatic tissue may result in more effective monoclonal antibody therapy.

An important population in lymph node tissue is the T cell population with a normal phenotype. About 75% of CD3+ cells are CD4+ T cells, the remained are mainly CD8+ cells and a very small number of CD4+ CD8+ cells⁶⁸. Some CD4+ cells play an important role in the pathogenesis of FL. For example, germinal centre cells carrying CD40 ligand bind CD40 on FL cell surface, protecting the tumour cells from apoptosis⁶⁹. Regulatory CD4+ CD25+ T cells (Tregs) play an important role in the evolution of the process of lymphoma transformation. Tregs regulate the activation of T cells and establish homeostasis in the immune system by acting upon CD4+ and CD8+ cells after their encounter with antigen^{70,71}. Tregs act through contact with the other T cells and through production of cytokines (interleukin-10 and TGF-beta). A recently published article pointed to the key role of localization of Tregs in patient prognosis. Diffuse infiltration of lymph nodes was associated with a good prognosis

while perifollicular infiltration suggested a poor prognosis⁷².

Value of the patient's antitumor immunity

Despite results from the overwhelming majority of studies suggesting the superiority of regimens with monoclonal antibodies, it is clear that a proportion of patients do not fully benefit from chemoimmunotherapy. This may be due to two reasons: limited effect of immunotherapy itself in particular patients with a large tumour mass and insufficient intensity of concomitant chemotherapy⁷³.

Given the fact that improved treatment outcomes were significantly contributed to by activation of the patient's immune mechanisms, it is possible that variability in the activity of non-specific antitumor immunity may cause variability in the effect of immunotherapy.

Indolent lymphomas are highly immunogenic tumours. The relations between the patient's organism, his/her antitumor immunity and lymphoma have been repeatedly demonstrated. Examples may be spontaneous remission reported in patients with early follicular lymphoma and a significant effect of immunotherapy with interferon on better overall survival of the patients^{15,74,75}. For many decades, properties of the tumour were stressed. Today, however, equal attention is paid to individual genetic predispositions of the patient (gene polymorphisms) that are reflected in the strength of interaction between the host's immune system and the tumour. These properties gain importance in the case of targeted therapy with monoclonal antibodies which is less effective without the contribution of the patient's own cytotoxic mechanisms. The most studied monoclonal antibody is rituximab.

Rituximab is an IgG₁-type chimeric monoclonal antibody specifically targeted against the CD20 antigen, which is expressed by mature normal B cells and, with varied density, by cells in almost 90% of malignant B-cell lymphomas. Based on in vitro experiments, several hypotheses have been formulated concerning the in vivo mechanism of action of the anti-CD20 antibody. These include complement-dependent cellular cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC) and direct induction of apoptosis^{76,77}. The role of CDC was studied in tissue cultures and mouse models⁷⁸, but in the case of ADCC, it seems that in vivo studies of the activity of autologous cytotoxic mechanisms may predict the effect of immunotherapy.

ADCC is mediated by effector cells of the immune system after the Fc fragment of an opsonizing antibody (e.g. rituximab) is bound to the low-affinity receptor for immunoglobulin (FcγR). There are 3 classes (FcγRI, FcγRII and FcγRIII) and 8 subclasses of immunoglobulin receptors. Of the subtypes, those important for ADCC are FcγRIIa (CD32), expressed on macrophages and granulocytes, and especially FcγRIIIa (CD16), present on macrophages and NK cells. Genes encoding Fc receptors are located on the first chromosome and show functional allelic polymorphism leading to structural and thus functional diversity of the encoded proteins (receptors). Certain polymorphisms have been identi-

fied as genetic predispositions leading to a higher risk of autoimmune or infectious diseases^{79,80}. The most studied allelic variation is FCGR3A gene dimorphism, encoding the amino acids phenylalanine (FcγRIIIa-158F) or valine (FcγRIIIa-158V) at position 158. The valine-containing homozygous form of FcγRIIIa was shown to be able to bind the IgG₁ molecule more strongly than the form with phenylalanine, resulting in more pronounced degranulation and a stronger cytotoxic effect of NK cells⁸¹. A similar effect was observed in the case of FCGR2A gene polymorphism, namely the allelic variant encoding histidin at position 131 (FcγRIIa-131H). Fc receptor affinity to rituximab seems to be determined by FCGR3A gene polymorphism⁸².

Value of Fc receptor polymorphism in clinical studies

The relationship between FCGR3A gene polymorphism and treatment response to rituximab was studied in patients with indolent aggressive lymphomas treated with both monotherapy with anti-CD20 antibody and chemoimmunotherapy.

Cartron et al.⁸³ studied the relationship between FCGR3A gene polymorphism and treatment response to primary therapy with rituximab in FL patients. Patients with the FcγRIIIa-158V homozygous form achieved significantly more remissions, both complete and molecular. An independent prognostic effect of V/V homozygosity on treatment response was confirmed by multivariate analysis.

A more detailed analysis of factors participating in the activity of ADCC was carried out by Weng et al.⁸⁴ in a group of 87 FL patients. In addition to the benefit of FCGR3A and FCGR2A gene polymorphism, in vitro activity of ADCC was studied in isolated tumour cells. Cellular cytotoxicity was assessed using a radioisotope method of measuring the release of the isotope of chromium (⁵¹Cr) from labelled tumour cells. No relationship was found between in vivo activity of ADCC and quality of treatment response, or between ADCC and expression of the CD20 molecule on lymphoma cells. The effect of FCGR3A gene polymorphism on the quality of achieved (molecular) remission was tested in a group of 34 patients newly diagnosed with FL and treated with prognostically stratified chemoimmunotherapy. No difference in molecular remission rates were found between the individual subgroups. Interestingly, however, patients with the FcγgammaRIIIa 158 F/F homozygous form had significantly higher FLIPI scores at the time of diagnosis than F/V heterozygotes and FcγgammaRIIIa 158 V/V homozygotes⁸⁵. An important study on the role of FcγgammaRIIIa and FcγgammaRIIa polymorphisms in patients treated sequentially with CHOP chemotherapy and rituximab was published by Professor Rambaldi's team⁸⁶. No differences in the quality of treatment response or progression-free survival were found between individual groups.

Value of antibody-dependent cellular cytotoxicity (ADCC)

Mechanisms of ADCC represent a very effective tool of non-specific anti-tumour immunity. The immunoglobulin Fc fragment binding to the high-affinity receptor for

effector cytotoxic cells (FcR) leads to the release of cytotoxic substances (perforin, granzyme B) from granules and formation of pores in the target cell membrane⁸⁷. For many years, ADCC was studied only in vitro. A breakthrough in the research on the activity of effector T and NK cells was made by introducing flow cytometry, a technique capable of visualizing degranulated, and thus active, cells using the CD107a molecule⁸⁸. Also described was the dependence of the level of degranulation of cytotoxic cells on the dose (concentration) of the administered antibody (rituximab, alemtuzumab). The population of effector cells was cytometrically defined, with the majority carrying the following phenotypes: CD56dim, CD69+, NKG2D+, NKp30 neg., NKp46 neg. and CD94 neg.⁸⁹.

This study enabled in vivo investigation of the activity of cytotoxic mechanisms. Convincing evidence has been presented that the administration of rituximab immediately increases the activity of cytotoxic T and NK cells in patients with CD20+ B-cell non-Hodgkin's lymphoma⁹⁰.

CONCLUSION

Follicular lymphoma is a very heterogeneous disease. The introduction of monoclonal antibodies into clinical practice has resulted in better survival rates in all patient risk groups. However, patients with active disease and a large tumour mass still have an unsatisfactory prognosis following conventional chemoimmunotherapy. Modern prognostic schemes should be aimed at correct identification of such patients.

To achieve more detailed stratification of patients in the chemoimmunotherapy era, FLIPI 2, a novel prognostic index, was developed. However, this has not been sufficiently validated by prospective studies.

Advances in research on the tumour cell genome have provided much information on the role of particular cytogenetic changes, especially in the case of t(14;18)-positive FL. An important tool is detection of minimal residual disease using molecular biology methods. There is convincing evidence about the role of the achievement of molecular remission, particularly in patients with advanced FL. On the other hand, the value of FDG-PET/CT examination, especially the contribution of post-treatment PET negativity, remains unclear.

Significant advances have been made in the research on non-malignant cell of the lymphoid tissue environment. In particular, the presence and character of Tregs may contribute to the assessment of prognosis in the future, especially after the immunohistochemical methods are standardized.

The last decade has brought new insights into gene polymorphism and the patient's antitumor immunity. Genetic predisposition has been shown to have an impact on the effect of therapy with monoclonal antibodies.

It may be concluded that in clinical practice, prognosis estimation is still based on the older FLIPI score. Therefore, in high-risk patients who are candidates for intensive therapy, clinical decision-making should include as broad spectrum of additional risk factors as possible,

irrespective of the fact that many of them need to be confirmed by prospective studies. This should result in an individualized therapeutic approach to the patient, reflecting the character of his/her disease to the maximum extent possible.

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