

**Prognostic factors and the clinical course of
follicular lymphoma in the FDG-PET and
rituximab era**

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Abstract

Follicular lymphoma (FL) is classically described as an incurable disease characterised by a distinctive clinical course, with repeated relapses and remissions. A progressive shortening of the duration of each remission and an increased relapse rate after each response is typically described. Major recent developments in the management of patients with FL have been the introduction of haematopoietic stem cell transplant (HSCT) and of rituximab treatment, resulting in a significant improvement in the overall survival of patients diagnosed with FL in the recent era. Another major advance in the 21st century is the introduction of 18-fluoro-deoxyglucose positron emission tomography (FDG-PET) for the staging of patients with lymphoma, which has proven to be more accurate than standard computer tomography. A new prognostic score, the Follicular Lymphoma International Prognostic Index 2 (FLIPI2) has been designed in the setting of these innovations.

The aim of this thesis is to examine how the introduction of such advances has impacted on the management and on the clinical course of patients with FL. It will specifically address the impact that FDG-PET has on the staging, management and prognosis of FL and it will compare the recently designed FLIPI2 in newly diagnosed FL patients with the pre-rituximab prognostic score (FLIPI). As mentioned, the introduction of rituximab and HSCT has contributed to the significant improvement in the outcome of patients with FL. This thesis will demonstrate how these developments have changed not only the outcome but also the clinical course of patients with FL and will assess the impact that prior treatment with rituximab has on the outcome of patients undergoing a HSCT.

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List of abbreviations

18-fluoro-deoxyglucose	FDG
18-fluoro-deoxyglucose positron emission tomography	FDG-PET
18-fluoro-deoxyglucose positron emission tomography and low dose computerized tomography	FDG-PET/CT
Acute myeloid leukaemia	AML
Autologous stem cell rescue	ASCR
BCNU, etoposide, cytarabine, melphalan	BEAM
Best response	BR
Beta-2-microglobulin	B2M
Bone marrow	BM
Bone marrow aspirate and trephine	BMB
British National Lymphoma Investigation	BNLI
Complete response	CR
Computerised tomography	CT
Contrast enhanced computerised tomography	CeCT
Cyclophosphamide, doxorubicin, etoposide, prednisone	CHVP
Disease-free survival	DFS
Eastern Cooperative Oncology Group	ECOG
European Group for Blood and Bone Marrow Transplantation	EBMT
European Society of Medical Oncology	ESMO
Event-free survival	EFS
First complete remission	CR1
First partial remission	PR1

First very good partial remission	VGPR1
Follicular lymphoma	FL
Follicular Lymphoma International Prognostic Index	FLIPI
Follicular Lymphoma International Prognostic Index 2	FLIPI2
Group d'Etude des Lymphomes Folliculaires	GELF
Haemopoietic stem cell transplant	HSCT
High power field	HPF
High-dose therapy	HDT
Incidence of relapse	IR
International Harmonization Project	IHP
International Prognostic Index	IPI
Involved field radiotherapy	IFRT
Lactate dehydrogenase	LDH
Lymph node	LN
Maximum standardised uptake value	SUVmax
Mechlorethamine, vincristine, prednisolone, procarbazine	MOPP
Mediastinal blood pool	MBP
Monoclonal antibody	MoAb
National Comprehensive Cancer Network	NCCN
National Institute for Health and Clinical Excellence	NICE
Non-relapse mortality	NRM
Overall survival	OS
Partial response	PR
Performance status	PS
Prednisolone, methotrexate, doxorubicin, cyclophosphamide, etoposide	ProMACE

Progression-free survival	PFS
Radiotherapy	RT
Receiver operating characteristic	ROC
Reduced-intensity conditioning	RIC
Region of interest	ROI
Relapse-free survival	RFS
Response rate	RR
Rituximab, cyclophosphamide, vincristine, doxorubicin, prednisolone	R-CHOP
Rituximab, cyclophosphamide, vincristine, prednisolone	R-CVP
Rituximab, fludarabine, cyclophosphamide, mitroxastrone	R-FCM
Rituximab, mitroxastrone, cyclophosphamide, prednisolone	R-MCP
Stable disease	SD
Standardised uptake value	SUV
Standardised uptake value average	SUVav
Secondary myelodysplastic syndrome/ acute myeloid leukaemia	sMDS/AML
Time-to-treatment	TT
Total-body irradiation	TBI
Treatment related mortality	TRM
Upper limit of normal	ULN
Very good partial response	VGPR
Watchful waiting	WW

CHAPTER ONE

Introduction

1.1 Historical perspective

Lymphomas were not recognised as a separate disease entity until 1862 when the term 'pseudo leukaemia' was utilised to differentiate these cases from leukaemia (1). Follicular lymphoma (FL), as a subtype, was first recognised as Brill-Symmers disease, named after the two authors that first described it (2-3). Both authors described the disease as a benign chronic disorder characterised by generalised lymphadenopathy, spleen involvement and clinical response to radiotherapy. Gall *et al* in 1941 described the clinical course and pathological features of FL, disputing the benign nature of FL, reporting its ability to transform to a more aggressive disease in some patients (4-5).

1.2 Epidemiology and pathology of FL

Non Hodgkin lymphomas (NHL) account for 2% of malignancies in the UK. FL is the second commonest subtype of NHL with an annual incidence in the UK of 3-5/100,00, which increases with age (6). The median age at diagnosis is situated in the 6th decade of life.

FL is included amongst the so-called 'low-grade' NHL and originates from follicle centre B-cells. FL cells express surface immunoglobulin and are characteristically CD5-, CD10+, CD19+, CD20+, CD22+ and CD79a+. Genetically it is characterised by the presence of the chromosomal translocation t(14;18) in 85% of cases, resulting in the over-expression of BCL2, an anti-apoptotic protein (7). According to the latest WHO classification FL is graded by counting the absolute number of centroblasts in ten neoplastic follicles per 40x high power field (HPF) (table 1). Grade 1 and 2 are recommended to be reported together as low grade. Grade 3b is considered to be more aggressive and is considered to be equivalent to diffuse large B-cell lymphoma (DLBCL) and thus it is treated as DLBCL (8).

Table 1: Follicular lymphoma grading (WHO 2008)

Grade	Definition
Grade 1-2 (low grade)	0- 15 centroblasts per HPF
Grade 1	0-5 centroblasts per HPF
Grade 2	6-15 centroblasts per HPF
Grade 3	>15 centroblasts per HPF
Grade 3a	Centrocytes present
Grade 3b	Solid sheets of centroblasts

1.3 Natural history and clinical course of FL

1.3.1 Natural history, clinical course and presentation of FL

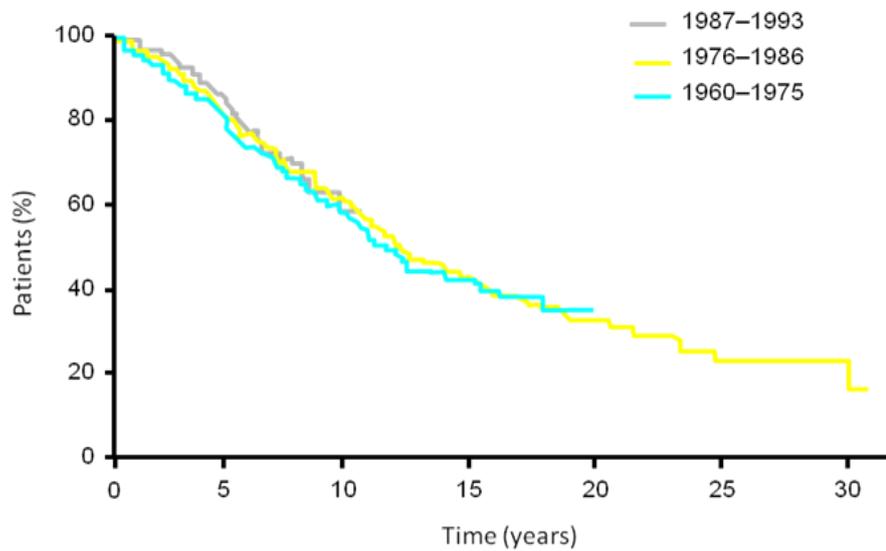
Patients diagnosed with FL are usually asymptomatic, in spite of the fact that the majority present in an advanced stage at diagnosis, with generalised lymph node (LN) involvement. Bone marrow (BM) is also frequently involved at presentation in up to 60% of patients. Less common extra-nodal sites include the gastrointestinal tract, skin, breast and testis (8). Symptoms can be related to the bulk or the site of the disease (if compressing vital structures), in addition to B-symptoms (fever, night sweats or unexplained loss of weight), but the performance status is normal in more than 90% of patients (1).

FL remains incurable and as a result reoccurrence after treatment is the norm in most patients. There is no difference in the overall survival (OS) of patients initially managed expectantly and those patients treated at diagnosis. Therefore, patients can be managed expectantly at diagnosis if asymptomatic; however, most patients will eventually require treatment: only a small proportion of patients (10-19%) will never require treatment (9). FL is characterised by its responsiveness to treatment, so the majority of patients will respond to first-line therapy. However, it is also characterised

by a typical pattern of relapses after each remission. With each relapse the likelihood of response to treatment and the duration of the remission decreases, as the disease becomes more resistant to treatment (10).

1.3.2 Outcome of patients with FL over time

Improvement in the OS of FL patients treated by combination chemotherapy regimens in comparison to single agent chemotherapy remained elusive for years, irrespective of the year of diagnosis, despite the achievement of better response rates and longer disease-free survival (DFS) (11) (figure 1). An improvement in the OS of patients with FL depending on the year of diagnosis was demonstrated for the first time by the analysis of the SEER data, showing an improvement in the median OS of FL patients diagnosed in the 1993 - 1989 era (93 months) compared with those diagnosed in the 1983 - 1989 era (84 months) (12). Subsequent studies by the SWOG and M.D. Anderson Cancer Center compared the progression-free survival (PFS) and the OS of patients treated on their trials over 30 years (13-14). Both studies showed an improvement of OS by around 30% for patients treated in the 1990 era compared to patients treated in the 1970 era. This improvement in OS was attributed to the success of improved sequential treatment, better supportive care and, importantly, the inclusion of monoclonal antibody (MoAb) treatment. Thus, the current expected median OS of patients in the rituximab era is between 10 and 13 years.

Figure 1: OS remained unchanged over three decades

Adapted from Horning S, *Semin Oncol* 1993; 20(5 Suppl 5):75.

1.4 Staging and the role of FDG-PET in patients with FL

1.4.1 The importance of staging

Staging is the process of identifying the extent of a disease at presentation. It provides the baseline assessment against which post treatment investigations should be compared with to assess response to therapy (1). The stage at diagnosis in patients with FL influences their management and is one of the most important prognostic factors. Furthermore, accurate staging enables comparisons of different populations treated in different centres, and allows comparisons amongst series and different clinical trials. Although the Ann Arbor staging system was initially designed specifically for Hodgkin lymphoma (HL) (15-17) it is utilised in the staging of all subtypes of lymphoma including FL. The classification was modified at the Cotswold meeting to include the recognition of bulky disease ($\geq 10\text{cm}$ denoted by X) and to recommend the inclusion of

computerised tomography (CT) to evaluate intrathoracic and infradiaphragmatic LN (18). Notably, it has not undergone any changes since then.

1.4.2 Imaging investigations and bone marrow biopsy

The investigations utilised to determine the stage of a patient diagnosed with FL have changed over the decades as imaging technology has advanced. Before the advent of CT and its introduction into medical practice, several other procedures were employed, including frontal and lateral chest x-rays, lymphangiography, staging laparotomy and splenectomy, skeletal surveys and isotope liver and spleen scanning (19). Since its introduction into clinical practice, the CT has become the dominant imaging investigation in the staging of patients with lymphoma. It is a non-invasive, fast investigation when compared to lymphangiography and laparotomy. In addition it gives an overall view of the extent of disease thereby eliminating the need for multiple investigations (19). Furthermore, it is an easily reproducible investigation that can be utilised in the assessment of response to treatment. The major limitation of CT results from its dependence on size criteria. Consequently the presence of disease in normal sized LNs is difficult to identify, as is the presence of diffuse involvement in normal sized liver/spleen and BM disease. One of the advantages of lymphangiography in this regard was its ability to identify presence of disease in normal sized LN (19).

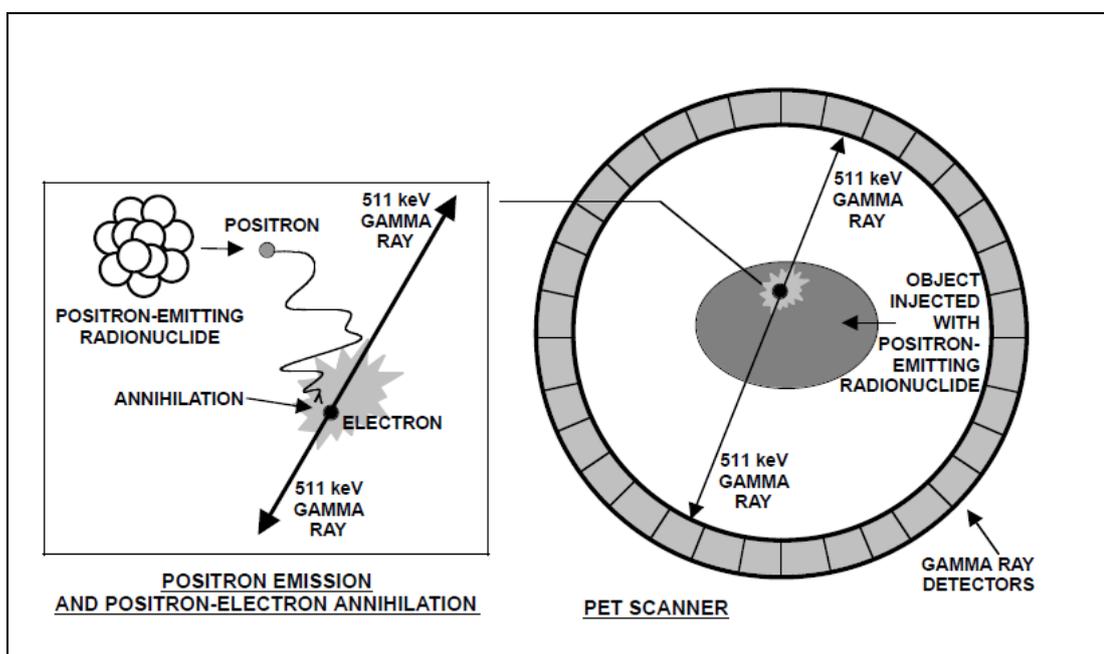
Bone marrow aspirate and trephine (BMB) is the current gold standard for the assessment of BM involvement and is an essential part of staging in FL. The BM is involved in 40-60 % of patients presenting with FL (20). The presence of disease in the BM leads to a change in the stage, prognosis and frequently the management of patients with FL. However, BMB is an unpleasant procedure that can potentially miss disease not present in the biopsy area, or if the biopsy is inadequate in quality.

Performing a bilateral BMB can increase the sensitivity of the procedure by 10-22%; nevertheless, given its morbidity, most centres, including St Bartholomew's Hospital, perform only unilateral BMB.

1.4.3 18-fluoro-deoxyglucose positron-emission tomography (FDG-PET)

Positron-emission tomography (PET) is a nuclear medicine functional imaging modality. It utilises a biological compound labelled with a positron-emitting radionuclide to produce an image which reflects a specific metabolic activity in the body. The PET detectors surrounding the patient are designed to detect the annihilation photons that are emitted simultaneously in opposite directions as a result of the interaction of ejected positrons from the radionuclide with electrons in the surrounding tissue (figure 2). This allows PET to localise the points of origin of these photons and reconstruct an image with this information. The number of signals received by the PET detectors and the resulting intensity depicted in the 3D image reconstructed depends on the uptake, concentration and excretion of the positron emitting compound in the body (21).

Figure 2: Interaction of released positron with electron, and detection of annihilation photons by PET (22)



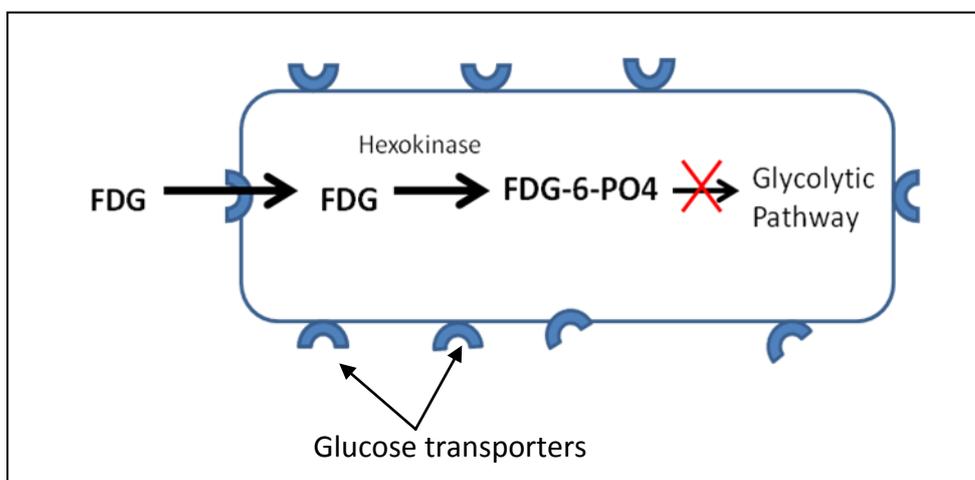
1.4.4 Warburg effect and 18-fluoro-deoxyglucose

Cancer cells have a higher rate of glucose uptake and metabolism than normal cells.

This is due to the reliance of cancer cells on aerobic glycolysis even in the presence of oxygen. ATP production is faster via aerobic glycolysis but requires more glucose than oxidative phosphorylation (the pathway utilised in normal cells in the presence of oxygen). This unique metabolic characteristic of cancer cells that differentiates them from normal cells was first reported by Otto Warburg and is known as the 'Warburg effect' (23).

18-fluoro-deoxyglucose (FDG) is composed of deoxyglucose (a glucose analogue) attached to a radioactive molecule 18-fluorine. After administering FDG intravenously it is preferentially taken up via glucose transporters which are over expressed in cancer cells and it is phosphorylated to FDG-6-phosphate by hexokinase enzyme. FDG-6-phosphate is not able to enter glycolysis and is trapped in the cells (figure 3). As it decays, positrons are emitted and the resulting photons will be detected by the PET scanner (24). The more FDG is taken up in a tissue the more photons originating from that area will be detected by the PET scanner. FDG was first utilised in humans in 1976, since then has become the dominant PET tracer in use clinically (25).

Figure 3: Uptake of FDG via glucose transporters on the cell membrane



1.4.5 Analysis of PET images: visual and quantitative interpretation

FDG-PET can be interpreted either by visual analysis, semi-quantitative or quantitative analysis. The most widespread and popular method in the routine practice is visual analysis. Visual analysis relies on the difference in the uptake of a tracer between the uptake in a lesion and the background uptake, where the uptake is not explained by the normal distribution of the tracer (26-27). Visual analysis of PET is currently the dominant method for reporting PET in patients with either HL or NHL, as recommended by the International Harmonization Project (IHP) (27). Visual analysis is dependent on the reporting physician in assessing whether a lesion with enhanced uptake is pathological or not: this is acknowledged as the main flaw of visual analysis as it makes it relatively subjective. The reference background being used by the physician is also an important factor, as this can be the surrounding background of the lesion, the mediastinal blood pool (MBP) or the liver (28). A 'positive' scan is defined by the presence of one or more lesions with increased uptake relative to the chosen background that is unaccounted for by physiological uptake. A 'negative' scan is where no abnormal uptake is seen relative to the chosen background.

One of the hallmarks of PET is that it enables the quantification of metabolic uptake in lesions, which is not possible with any other imaging modality. There are different quantitative methods: some are complex and require dynamic data acquisition and blood analysis for tracer levels (29) but this type of analysis is usually limited to the research of new PET tracers. Standardised uptake value (SUV) is the simplest and most commonly used quantitative method. It measures the intensity of the tracer uptake at a defined time in a specific region. It is dependent on the injected activity of the tracer, uptake time and patient's size (30). There are several methods used to define a region of interest (ROI) depending on the PET software in use, but there is currently no

consensus on which method is the best to define a ROI. It is important to note that the SUV recorded will vary according to the method utilised. Therefore a consistent method should be used within a patient group or study to ensure an accurate use of SUV for treatment monitoring (31).

1.4.6 FDG avidity of FL

A specific subtype of lymphoma is considered to be FDG-avid if there is an increased FDG uptake detected in involved sites of disease identified by conventional imaging. FDG avidity [expressed as the percentage of patients with FDG-avid lymphoma in relation to the total number of patients (32-33)] varies amongst different subtypes of lymphoma with a tendency towards the more aggressive lymphomas being more avid than the indolent lymphomas. However, FL has proven to be highly FDG-avid in contrast to other indolent lymphomas. Several studies investigating the avidity of FL consistently found that 94-100% of cases of FL are FDG-avid (34-35). This percentage is comparable to the results reported for both DLBCL and HL (32-33). The intense avidity of DLBCL has been associated with the high proliferative rate of DLBCL cells (36). Overall, FL has a low proliferative rate, and several clinical studies found no significant correlation between the measured SUVmax and the histological grading of FL (35, 37-38). An alternative hypothesis to explain FDG avidity in FL despite the low proliferation rate is the role of the surrounding microenvironment as a correlation between SUVmax and CD8+, CD68+ and CD34+ positive cells has been reported. There is however a paucity of studies in this area (39).

1.4.7 Current international and national guidelines

The IHP published recommendations regarding the timing and interpretation of post-treatment FDG-PET for DLBCL and HL in 2007 (27). A pre-therapy FDG-PET although deemed not obligatory by the IHP, was encouraged as it could facilitate post therapy PET interpretation for the recognised FDG-avid lymphomas: DLBCL, HL and FL (27). The European Society of Medical Oncology (ESMO) has more recently recommended an additional PET to the conventional staging investigations (CT of the neck, thorax, abdomen and pelvis, and a BMB) in rare cases to confirm localised stage I/II disease (40). The National Comprehensive Cancer Network (NCCN) has indicated that PET may be useful in selected FL cases (41). The guidelines of the Royal College of Radiologists recommend a FDG-PET to confirm staging in patients with early stage FL undergoing radiotherapy (42). In summary, the current available guidelines suggest that there may be a clinical value to a pre-therapy PET in certain circumstances, but overall they are vague and some do not elaborate on what exactly are the selected or rare cases. This is due to an absence of large prospective trials examining the clinical usefulness of a pre-therapy PET in FL, which is reflected by the British Society of Haematology guidelines that do not recommend a FDG-PET for staging of FL until more data via clinical trials becomes available (43).

1.4.8 FDG-PET clinical studies in FL

The sensitivity of FDG-PET in FL ranges from 91 to 97% and the specificity is up to 100% when compared to conventional imaging methods (table 2). An additional 40-54% more nodal lesions and up to 89% more extra-nodal lesions can be detected by FDG-PET in comparison to conventional imaging. The unique ability of FDG-PET to identify disease involved normal-sized LN, as well as extra-nodal (e.g. bone, spleen and muscle)

involvement results in the increased accuracy of FDG-PET to detect areas of disease, which might lead to a change in the stage and subsequently in the management of patients in a variable proportion of cases.

Table 2: Clinical studies evaluating the role of FDG-PET in FL patients

Author (year)	Patient characteristics	% of additional lesions detected by PET	% Avidity	Sensitivity	Specificity	Change in stage (%)	Upstaged (%)	Change in management (%)
Jerusalem (2001) (34)	42 patients (24 FL)	40% more nodal areas	100%	NR	NR	NR	NR	NR
Worher (2006) (38)	62 newly diagnosed FL	NR	NR	98%	94%	NR	NR	NR
Karam (2006) (35)	17 newly diagnosed FL	NR	94%	94%	100%	NR	29%	NR
Bishu (2007) (37)	31 grade 1-2 FL (16 newly diagnosed, 15 relapsed)	NR	NR	95%	88%	NR	NR	NR
Wirth (2008)(44)	42 FL grade 1-3a stage I/II	41 additional sites in 19 patients	97%	NR	NR	NR	40%	45%
Janikova (2008) (45)	82 grade 1-3a FL (62 newly diagnosed, 20 relapsed)	50%: additional sites (nodal:63%; extra-nodal:68%)	NR	NR	NR	NR	22%	18%
Scott (2009) (46)	74 newly diagnosed 'low-grade' NHL (55 FL)	50%	NR	NR	NR	32%	28%	34%
Le Dortz (2010) (47)	45 newly diagnosed grade 1-3a FL (treated with R-CHOP)	51% (nodal) 89% (extra-nodal)	100%	NR	NR	18%	11%	NR

NR: not reported by authors

1.5 Prognostic indices used in patients with FL

The most recent clinical prognostic index specifically designed for FL is the Follicular Lymphoma International Prognostic Index 2 (FLIPI2) published in 2009 (48). This was preceded by the Follicular Lymphoma International Prognostic Index (FLIPI) which had been published in 2004 (49). Previous to the FLIPI the International Prognostic Index (IPI) was used for 'indolent' lymphomas including FL, although it was initially designed for aggressive lymphoma. The IPI was developed in 1993 for the prediction of OS and relapse-free survival (RFS) in patients with aggressive lymphoma (50). It is based on the presence of five prognostic factors (age >60 years, elevated serum LDH, number of extra-nodal sites >1, ECOG performance status (PS) ≥ 2 and Ann Arbor stage III/IV). The IPI was found to be also applicable in patients with 'indolent' lymphoma including FL (51). However, the main flaw of the IPI in FL is that it segregates very few patients to the high-risk group (<15%) and some prognostic factors such as the PS are not as useful in FL, as most patients have a good PS at diagnosis. A more specific prognostic scoring system for FL was needed and this led to the development of the FLIPI (52).

1.5.1 Follicular Lymphoma International Prognostic Index (FLIPI)

The FLIPI was the result of a multicentre retrospective analysis of prognostic factors in 4167 newly diagnosed FL patients between 1985 and 1992 (in the pre-rituximab era) (49). OS was the end-point for the statistical analysis, whereas PFS was not analysed. Five prognostic factors were recognised: age >60 years, Ann Arbor stage III/IV, LDH >ULN, haemoglobin level <12g/dL and number of involved LN regions >4. The FLIPI segregated patients into three different risk groups: low-risk, intermediate-risk and high-risk groups, that are very well balanced in terms of the proportion of patients included in each group (table 3). Although

designed in patients who did not receive rituximab, the FLIPI has also been shown to be predictive of OS in the rituximab era (53).

Table 3: Risk groups, 5-year and 10-year OS according to FLIPI

<i>Risk Group</i>	<i>Number of factors</i>	<i>Patients (%)</i>	<i>5-year OS</i>	<i>10-year OS</i>
Low-risk	0-1	36%	91%	71%
Intermediate-risk	2	37%	78%	51%
High-risk	3-5	27%	52%	35%

1.5.2 Follicular Lymphoma International Prognostic Index 2 (FLIPI2)

A prospective multicentre study was conducted between January 2003 and May 2005 and included 1093 patients with FL in an attempt to prospectively produce a new prognostic index in the era of rituximab. Patients managed expectantly were excluded from this model, as PFS was the chosen statistical end-point for the FLIPI2 trial and its definition differs between patients treated at diagnosis and those managed expectantly. The FLIPI2 includes five prognostic factors: age >60 years, BM involvement, diameter of the largest LN >6cm, raised beta-2-microglobulin (B2M) and haemoglobin <12g/dL. The presence of these factors divides patients into 3 risk groups: low-risk (0 factors), intermediate-risk (1-2 factors), and high-risk (3-5 factors). More than half the patients according to the FLIPI2 fall into the intermediate-risk group (table 4).

Table 4: Risk groups and 5-year PFS according to FLIPI2

<i>Risk Group</i>	<i>Number of factors</i>	<i>Patients (%)</i>	<i>5-year PFS</i>
Low-risk	0	20%	79%
Intermediate-risk	1-2	53%	51%
High-risk	3-5	27%	19%

1.6 Management of patients with FL

1.6.1 Management of patients with limited stage

Most FL patients present in advanced stage, however 10- 20% of patients will present with limited stage at diagnosis (54). The recommended treatment for these patients adopted by international guidelines is involved field radiotherapy (IFRT) (40-41). This is based on the results of several small retrospective studies, which have reported the long-term outcome of limited stage patients treated with radiotherapy, with a 10-year DFS ranging from 41 to 49% and a 10-year OS of 62-79% (table 5) (55-58). Relapse of disease usually occurs outside the irradiated area, and the risk of late relapse (after 10 years) is very low in these patients (58). The potential 'curative' effect of local RT in patients with localised disease has been the basis for the generalised recommendation of RT as the 'standard' in localised stage FL. However an initial watch and wait policy was also shown to be an acceptable option in these patients, especially in patients with stage II disease with involvement of areas difficult to irradiate (59). There has been a paucity of prospective randomized trials in limited stage FL. A randomised prospective trial comparing IFRT *versus* IFRT and chlorambucil treatment for a total of 8 months was performed and found no difference in DFS or OS between both groups (60).

Table 5: Studies on limited stage FL

Centre	Treatment	DFS at 10 years	OS at 10 years
Stanford (57)	35-50 Gy	44%	64%
BNLI (55)	35 Gy	49%	64%
Princess Margaret (58)	20-35 Gy	41%	62%
MD Anderson (61)	30-40 Gy +COP-Bleo/CHOP-Bleo	Time to treatment failure: 72% at 10yrs	80%
Stanford (59)	Watchful waiting	-	85%

1.6.2 Management of patients with advanced stage

Expectant management is recommended as the standard approach for asymptomatic patients lacking any criteria for treatment (40, 42). In contrast, there is currently no consensus on the standard chemotherapy regimen for symptomatic newly diagnosed patients. Treatment for advanced stage symptomatic FL is variable, and the options range from single agent immunotherapy (rituximab), combination treatment (R-CHOP, R-CVP, R-FCM, R-MCP,) and, in patients with recurrent disease, stem cell transplantation.

1.6.3 Expectant management

In 1979 Portlock et al demonstrated retrospectively that the OS in 44 asymptomatic patients diagnosed with advanced stage low grade lymphoma , managed expectantly at diagnosis was not significantly different to the OS of 112 similar patients treated at diagnosis at Stanford University (62). The natural history of 83 asymptomatic patients with advanced stage untreated low grade lymphoma was further described by Horning et al from the same institution. The median time to treatment in this group of patients was 3 years, while spontaneous remission was seen in 23% of patients (63).

Subsequently, there have been three randomised trials in the pre-rituximab era comparing a watchful waiting approach (WW) in asymptomatic advanced stage FL to immediate treatment. The BNLI trial compared chlorambucil with expectant management (9), while the GELF-86 trial compared WW *versus* prednimustine *versus* interferon (64), and the National Cancer Institute trial compared WW *versus* ProMACE-MOPP (65). None of these studies found a difference in the OS of patients treated at diagnosis and those managed expectantly. The median time to treatment in the expectantly managed group ranged between 23 and 36 months, and the median time to second progression and response to treatment was not

affected by deferral of treatment. In the BNLI study 19% of the patients did not require any treatment after 10 years of follow-up.

In the rituximab era, the WW approach has been re-challenged with rituximab treatment in asymptomatic newly diagnosed FL patients. A prospective randomised trial (only published in abstract form) compared WW with rituximab weekly for 4 weeks or rituximab weekly for 4 weeks followed by rituximab maintenance. Treatment with rituximab at diagnosis appears to delay further therapy as the median time to treatment was 33 months in the WW arm, while it was not reached at 4 years in the rituximab arms. However the follow-up was too short to detect any differences in OS (66).

1.6.4 Treatment criteria

The identification of the 'asymptomatic' patients with FL who require treatment at diagnosis and the selection of signs/symptoms that warrant starting treatment are not well established. The currently available treatment criteria mostly used are the Groupe d'Etude des Lymphomes Folliculaires (GELF) (table 6)(67) and the British National Lymphoma Investigation (BNLI) (table 7) (9). Both were retrospectively described by expert opinion and are utilised in trials to define the characteristics of the patients that mandate initiating treatment. As a result , the GELF criteria in particular notably change between different studies (64, 67-68). Most clinicians use probably a combination of GELF and BNLI treatment criteria in clinic when deciding on when to start treatment.

Table 6: GELF criteria

Bulky disease: nodal/extra-nodal $\geq 7\text{cm}$
3 involved nodal areas, each with a LN $\geq 3\text{cm}$
Spleen $\geq 20\text{cm}$
Pleural effusion/ ascites
ECOG PS ≥ 2
B-symptoms
Elevated LDH/ B2M

Table 7: BNLI criteria

Rapidly progressive disease
Life threatening organ involvement
Bone lesions
B symptoms/ pruritus
Cytopenias due to BM involvement (Hb $< 10\text{g/dL}$, WBC $< 1.0 \times 10^9/\text{mL}$, PLT $< 100 \times 10^9/\text{mL}$)

1.6.5 Immuno-chemotherapy

Several trials have demonstrated that the addition of rituximab to chemotherapy improves response rates, PFS and OS. A prospective trial comparing R-CHOP *versus* CHOP in newly diagnosed FL patients showed a better OS at 2 years for the R-CHOP group (95% *versus* 90%). This study involved a second randomisation of responding patients (achieved CR or PR), age < 60 years to HDT/ASCT or interferon α maintenance. No difference in outcome was found between patients that had received initial treatment with RCHOP or CHOP when randomised to HDT/ASCT. Whilst, patients that had received RCHOP and were randomised to interferon α maintenance were found to have a significantly longer duration of response than patients that had received CHOP (69). Marcus *et al* also reported an improved OS in newly diagnosed FL patients treated with R-CVP *versus* CVP, with a 4-year OS of 83% and

77%, respectively (70). Furthermore, the superior OS, response rates and improved disease control in patients receiving rituximab as part of their treatment regimen was confirmed by a meta-analysis of 1,480 FL patients from 7 prospectively randomised trials comparing R-chemotherapy *versus* chemotherapy (5 trials in newly diagnosed and 2 trials in relapsed patients). In consequence, although there is no agreement on the type of chemotherapy to be used, there is general agreement that rituximab should be part of the treatment regimen offered to FL patients (71).

1.6.6 Maintenance therapy with rituximab

In an effort to improve survival in FL patients, maintenance with rituximab and radio-immunotherapy has been explored in several studies in both newly diagnosed and relapsed patients (67, 72-76). The EORTC intergroup study demonstrated an improved PFS in relapsed/resistant FL patients randomised after R-CHOP or CHOP to rituximab maintenance (median PFS: 3.7 years) *versus* observation (median PFS: 1.3 years). The PRIMA trial showed an improved PFS in newly diagnosed patients treated with immuno-chemotherapy who received maintenance with rituximab (PFS at 3 years: 75% and 58% for patients treated with maintenance rituximab and those managed expectantly, respectively) (76). Vidal *et al* demonstrated in a meta-analysis that all groups of patients benefited from an improvement in PFS from maintenance rituximab in comparison with patients that did not receive maintenance, regardless of whether they were previously untreated or had relapsed disease, and whether they had received rituximab or not as part of the induction regimen. In addition, patients with relapsed/refractory disease had an improved OS with rituximab maintenance in comparison with those who did not receive maintenance, whereas it was not possible to demonstrate a benefit in OS in untreated patients (77-78). A recent study presented only in abstract version, however, suggests that there is no difference in time-to-treatment failure

between patients receiving rituximab maintenance and those patients re-treated with rituximab at progression (79).

1.6.7 High-dose therapy with autologous stem cell rescue (HDT/ASCR)

In the pre-rituximab era, 3 prospective randomised trials explored the benefit of HDT/ASCR *versus* conventional chemotherapy in previously untreated FL patients. Both the GLSG and the GOELAMS trial found an improved PFS and EFS, respectively, for patients treated with HDT/ASCT, however in none of the trials an improved OS could be demonstrated (80-83). In contrast, the trial conducted by the GELA did not find any difference in EFS or OS between both groups of patients (HDT/ASCR and patients treated with CHVP + interferon) after a median follow-up of 7.5 years (83). In the rituximab era there is only one trial (conducted by the GITMO group) that randomised patients to R-CHOP treatment *versus* R-HDT/ASCR as first-line treatment. This trial demonstrated an improved PFS in the R-HDT/ASCR arm, but there was no difference in OS. The authors concluded that R-HDT/ASCR might represent an overtreatment for a significant proportion of newly diagnosed patients, and perhaps should be retained for patients with relapse or progression after R-CHOP (84). A systemic review and meta-analysis of HDT/ASCR *versus* conventional chemotherapy in the initial management of FL patients concluded that HDT/ASCR does not improve OS (85).

Several retrospective studies reported a PFS of 28%-48% and an OS of 48%-54% at 10 years in relapsed patients treated with HDT/ASCR in the pre-rituximab era (86-90), but there is only one randomised prospective trial (the CUP trial) that compared HDT/ASCR with conventional chemotherapy in patients with relapsed FL. This trial demonstrated a better PFS and OS for patients treated in the HDT/ASCR arm (91). In contrast, a limited number of studies have explored the benefit of HDT/ASCR in relapsed patients in the rituximab era (92). A recent retrospective analysis by the GELA group of patients included in two previous randomised

trials that had received the same induction regimen analysed the role of rituximab and of HDT/ASCR at relapse. Patients that had received rituximab as part of the salvage treatment had a better OS than patients who had not received rituximab. However, patients that underwent HDT/ASCR after salvage treatment containing rituximab had the best OS and EFS when compared to patients that had received salvage treatment containing rituximab or patients that just had HDT/ASCR alone (93-94).

1.6.8 Type of conditioning regimen in HDT/ASCR

The majority of the prospective randomised studies examining the role of HDT/ASCR in the management of FL have included total-body irradiation (TBI) as part of the conditioning regimen (80-81, 83). However there has been a recent shift towards chemotherapy-based conditioning regimens in patients with FL, mostly due to reports of an increase risk of secondary myelodysplastic syndromes/ acute myelogenous leukemia (sMDS/AML) and other secondary malignancies in patients treated with TBI (82, 95-96). However, several factors other than TBI have been implicated, such as age, inclusion of etoposide in the conditioning regimen, cytogenetic abnormalities prior to transplant, previous radiotherapy exposure, prior alkylating therapy, number of prior lines of treatment and increased interval from diagnosis to HDT/ASCR (97-101). A retrospective analysis by the EBMT with a very long follow-up reported a shorter 5-year OS in patients treated with TBI-containing regimens, in comparison with patients who did not receive TBI. This was related to the higher non-relapse mortality (NRM) and a significantly higher risk of sMDS/AML in the TBI-treated group (89). These retrospective analyses were all performed in the pre-rituximab era but there has been no randomised trial comparing different conditioning regimens to date. Therefore currently there is no consensus on the type of conditioning regimen to be used in HDT/ASCR.

1.6.9 Allogeneic transplant and reduced-intensity conditioning regimens

Allogeneic transplant is a potentially curable treatment in patients with FL. A PFS of up to 43% and an OS of 51% at 5 years has been reported with myeloablative conditioning regimens (102). However, a high treatment-related mortality (TRM) of up to 40% has been reported with myeloablative transplants (103). This has led to a shift to non-myeloablative conditioning regimens or reduced-intensity conditioning (RIC) regimens, which are more feasible in the older patient population diagnosed with FL (104). In 1997 only 10% of allogeneic transplants for patients diagnosed with FL were RIC, whereas this has increased to 80% in 2002 (105). This is due to the lower TRM of 25-31% achieved by this type of conditioning regimens. However, the relapse rate may be higher in patients receiving a RIC than standard myeloablative transplants (105). In addition, a recent study has shown that the TRM in myeloablative transplants has reduced in recent years as supportive measures have improved. The timing of an allogeneic transplant and the best conditioning regimen are highly controversial, as there are no prospective trials comparing myeloablative and RIC conditioning or allogeneic transplant and HDT/ASCR (106).

CHAPTER TWO

FLIPI and FLIPI2 prognostic indices in patients with follicular lymphoma

2.1 Introduction

Prognostic factors should be reproducible, easy to measure and predictive of outcome. Two prognostic indices have been developed specifically for patients with FL: the Follicular Lymphoma International Prognostic Index (FLIPI) and the FLIPI2. The FLIPI, described in 2004, was retrospectively designed in patients diagnosed with FL in the pre-rituximab era (49). It has been found to be predictive of OS and PFS in newly diagnosed FL patients (49, 53, 70), as well as of survival from progression in patients at first relapse (107). On the other hand the FLIPI2, a more recent prognostic index, was described in 2009 based on data prospectively collected in the rituximab era. The FLIPI 2 was found to be predictive of PFS in newly diagnosed FL patients (48).

The aim of this study was to compare the efficacy of the FLIPI and FLIPI2 in discriminating patients with newly diagnosed FL and a distinct outcome in terms of OS and PFS.

2.2 Patients and methods

2.2.1 Patients' characteristics

From 1985 to 2007, 302 patients (160 female/142 male; median age: 55 range: 24-89) were newly diagnosed with grade 1-3a FL in our institution. The FLIPI could be retrospectively assigned in 220 patients and the FLIPI2 in 149 patients. The 122 patients in whom both the FLIPI and FLIPI2 indices were assessable and could be assigned to a specific risk-group according to each index constitute the study population (table 8).

Table 8: Characteristics of the 122 patients in whom a risk group could be assigned according to both FLIPI and FLIPI2

Patients characteristics (n=122)	Patients, n (%)	Missing values
Gender (female)	75 (61%)	0
Age, median (range)	56 years (25-87)	0
Age ≥60 years	52 (43%)	
Stage		0
I	14 (12%)	
II	22 (18%)	
III	21 (17%)	
IV	65 (53%)	
Histological grade		18
Grade 1	53 (51%)	
Grade 2	27 (26%)	
Grade 3	24 (23%)	
Haemoglobin <12g/dL	21 (17%)	0
Lactate dehydrogenase >ULN	22 (18%)	0
Beta-2-microglobulin >ULN	20 (30%)	54
Number of nodal sites >4	42 (39%)	14
Lymph node size ≥6cm	31 (27%)	8
Bone marrow involvement	55 (46%)	2

2.2.2 Definitions of end-points

OS was measured from the date of diagnosis to the date of last follow-up or death. PFS was measured from the time of diagnosis to first treatment in expectantly managed patients, and from the time of diagnosis to relapse or progression in patients treated at diagnosis. A further sub-analysis of the OS and PFS was made in the subgroup of patients treated at diagnosis (n= 75). PFS was analysed from the time of diagnosis to relapse or progression in this subgroup.

2.2.3 Statistical analysis

The analysis of OS and PFS was performed by the Kaplan-Meier method and comparisons were made using the log-rank test. All causes of death were included in the OS analysis. Patients' characteristics were compared with a t-test for continuous variables and a chi-square or Fisher test for categorical variables. All statistical analysis was carried out on STATA.

2.3 Results

2.3.1 Patients' treatment and follow-up

From the study population, 75 patients (61%) received treatment immediately after diagnosis (of which, 25 patients received rituximab containing regimens), whereas the remainder (47 patients) were managed expectantly. After a median follow-up of 7 years (range: 1-23), a further 30 patients subsequently received treatment.

2.3.2 OS and PFS of the study population

The median OS of the study group was 13.5 years, and the 5- and 10-year OS were 74% (95%CI: 65 -81%) and 58% (95%CI: 46-67%), respectively. The median PFS for the study group was 3.4 years and the 5 and 10-year PFS were 39% (95%CI: 30-48) and 21% (95%CI: 13-31%), respectively. There were no significant differences in OS ($p= 0.57$) or PFS ($p=0.85$) between the 122 patients comprising the study population and those patients in whom a risk-group according to one of the prognostic scores could not be assigned (180 patients).

2.3.3 Patients' distribution according to the FLIPI and FLIPI2 scores

The distribution of the patients according to the FLIPI was as follows: 53 patients (42%) were assigned to the low-risk category, 35 patients (29%) to the intermediate category, and 34

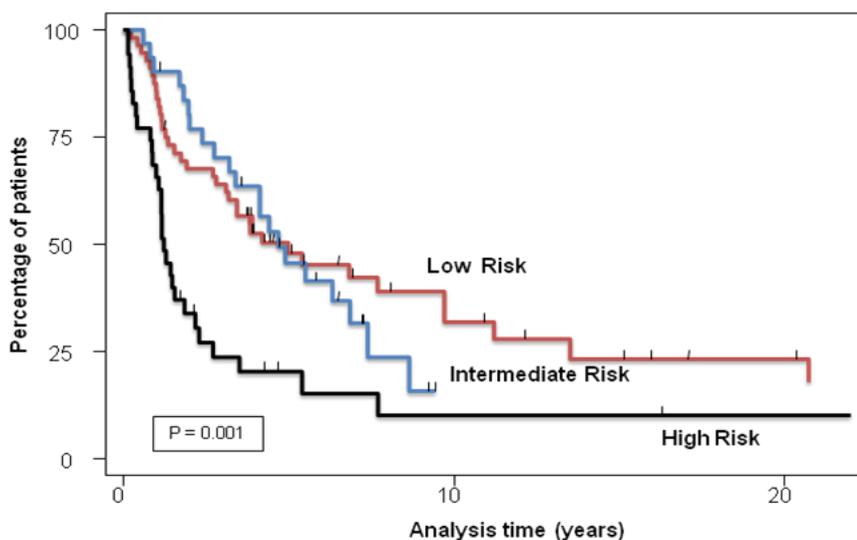
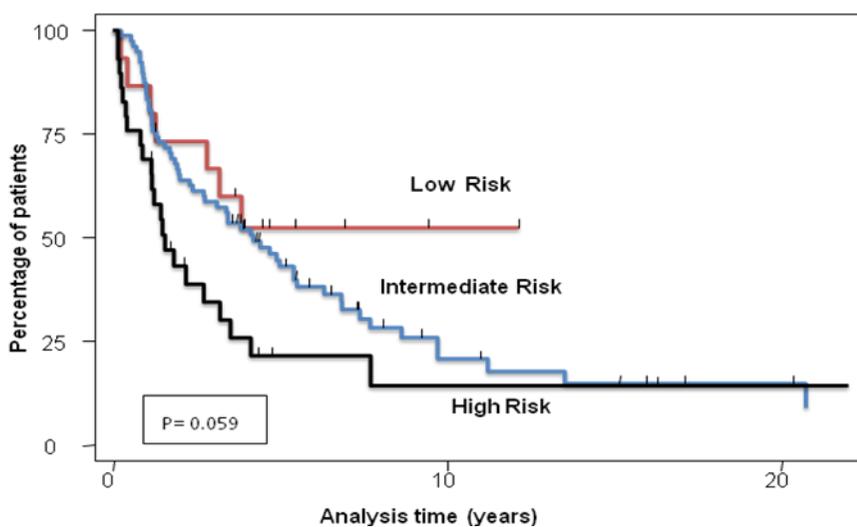
patients (28%) to the high-risk category; whilst according to the FLIPI2 score 15 patients (12%) were included in the low-risk category, 78 patients (66%) in the intermediate-risk category, and 29 (24%) patients in the high-risk category (table 9).

Table 9: Distribution of patients, OS and PFS according to the FLIPI and FLIPI2

FLIPI				FLIPI2			
Risk group (n risk factors)	% of patients	5-year OS	5-year PFS	Risk group (n risk factors)	% of patients	5-year OS	5-year PFS
Low (0-1)	42%	93%	48%	Low (0)	12%	93%	52%
Intermediate (2)	29%	79%	46%	Intermediate (1-2)	66%	85%	43%
High (3-5)	28%	38%	20%	High (3-5)	24%	33%	22%

2.3.4 PFS according to the FLIPI and FLIPI2 indices

The 5-year PFS according to the FLIPI score was 48% (95%CI: 34-60%) for the low-risk category; 46% (95%CI: 27-62%) in the intermediate-risk group, and 20% (95%CI: 10-35) for patients included in the high-risk category ($p=0.001$; figure 4). The 5-year PFS according to the FLIPI2 was 52% (95%CI: 25-74%) in the low-risk category, 43% (95%CI: 32-53%) in the intermediate-risk category, and 22% (95%CI: 10-39%) for the high-risk group ($p=0.059$; figure 5). Thus, the FLIPI score predicted PFS ($p=0.001$) but was not able to discriminate patients with low-risk from those with intermediate-risk ($p=0.6$). There was a trend for the FLIPI2 to predict PFS ($p=0.059$) but it did not segregate the low-risk group from the intermediate-risk group ($p=0.3$).

Figure 4: PFS according to FLIPI risk groups in the study population**Figure 5:** PFS according to FLIPI2 risk groups in the study population

2.3.5 OS according to the FLIPI and FLIPI2 indices

The 10-year OS according to the FLIPI score in the low-risk category was 77% (95%CI: 60-88%), in the intermediate-risk group it was 63% (95%CI: 41-78%), whereas it was 21% (95%CI: 10-40%) in the high-risk category, as shown in figure 6. The 10-year OS according to the FLIPI2 in the low-risk category was 78% (95%CI: 32-95%), 66% (95%CI: 52-77%) in the intermediate category, and 22% (95%CI: 53-46) in patients included in the high-risk group (figure 7). The FLIPI and the FLIPI2 indices predicted OS ($p < 0.001$, both for the FLIPI and the

FLIPI2), but the FLIPI2 did not accurately separate patients with low-risk from those with intermediate-risk in terms of OS ($p=0.3$), whereas the FLIPI score adequately segregated the study population in three distinct risk categories in terms of the OS ($P<0.001$).

Figure 6: OS according to FLIPI risk groups in the study population

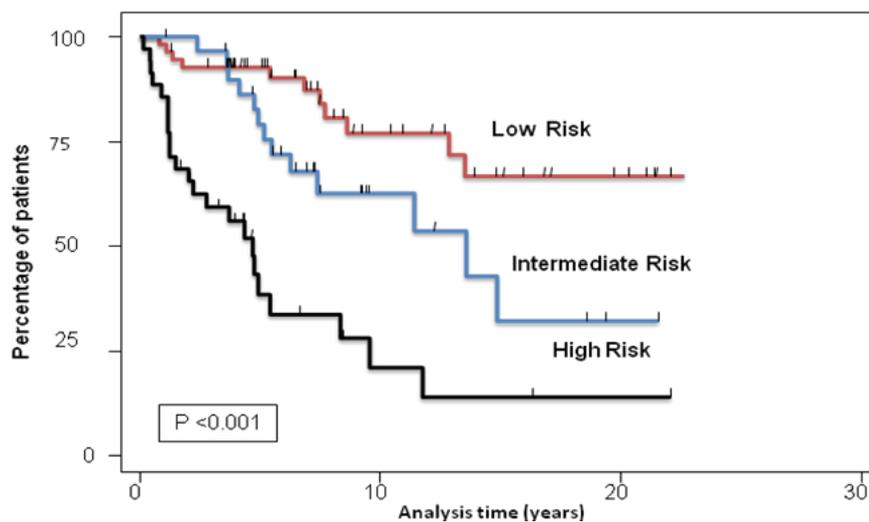
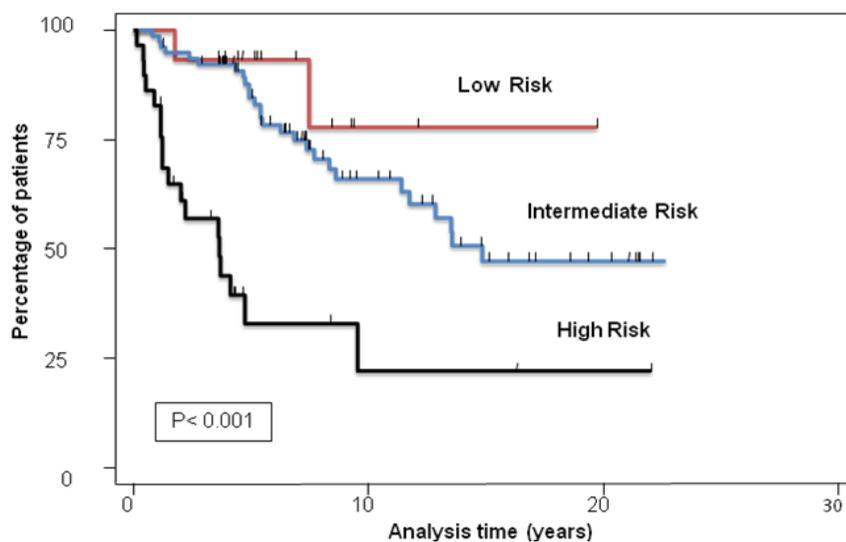


Figure 7: OS according to FLIPI2 risk groups in the study population



2.3.6 Patients treated immediately after diagnosis

A sub-analysis of the group of patients who received treatment straightaway after diagnosis ($n=75$), excluding patients that were initially managed expectantly, was performed. The

distribution of the patients according to the FLIPI score was: 32 patients (43%) in the low-risk category, 18 patients (24%) in the intermediate category, and 25 patients (33%) in the high-risk category; the distribution according to the FLIPI2 was as follows: 11 patients (15%), low-risk; 43 patients (57%), intermediate group; and 21 patients (28%), high-risk category. The FLIPI score predicted PFS ($p=0.002$; figure 8) but was not able to discriminate patients with low-risk from those with intermediate-risk ($p=0.9$). In contrast, the FLIPI2 did not predict PFS ($p=0.25$) in this group of patients (figure 9).

Figure 8: PFS according to FLIPI risk groups in patients treated at diagnosis

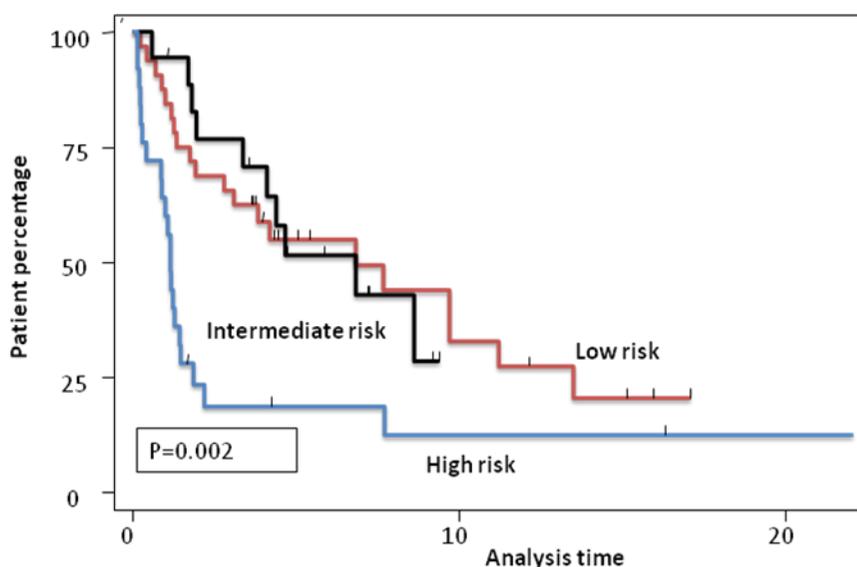
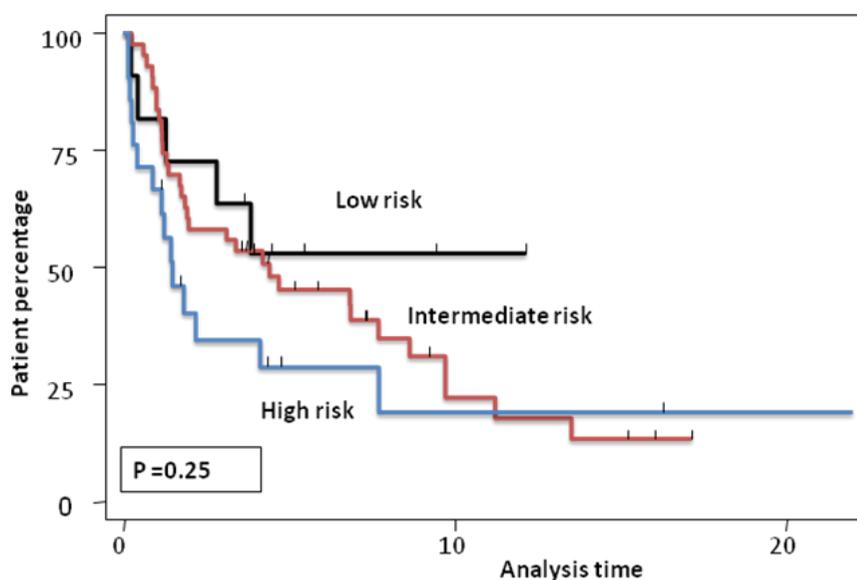


Figure 9: PFS according to FLIPI2 risk groups in patients treated at diagnosis



The FLIPI and the FLIPI2 indices predicted OS ($p < 0.001$ both for the FLIPI and the FLIPI2; figures 10 and 11).

Figure 10: OS according to FLIPI risk groups in patients treated at diagnosis

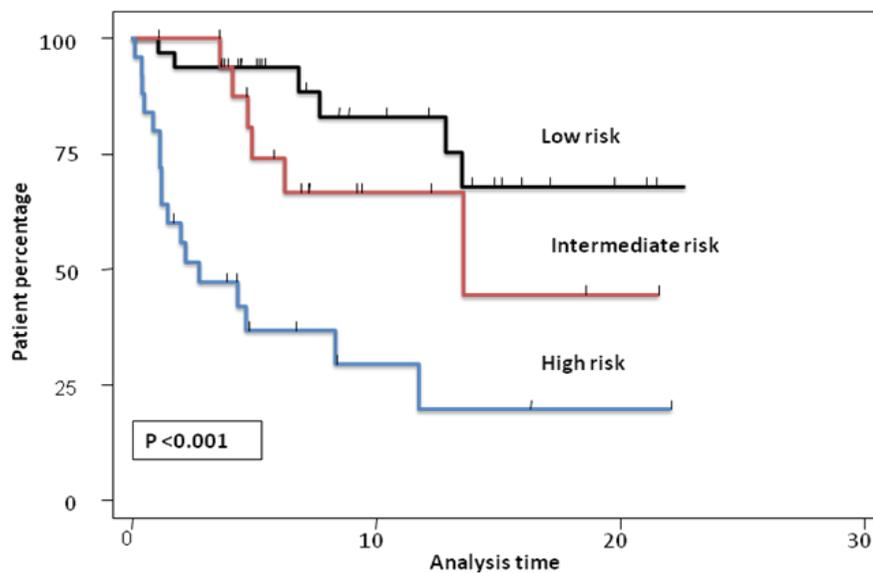
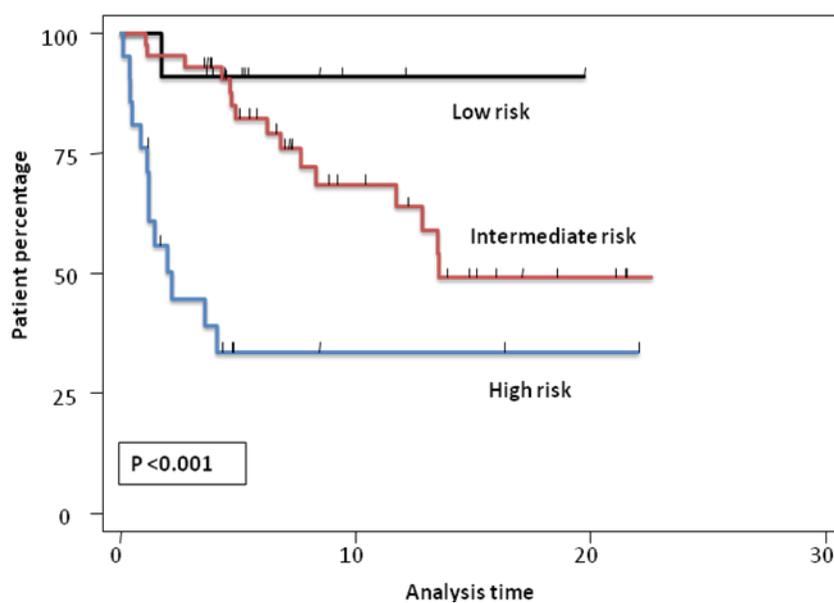


Figure 11: OS according to FLIPI2 risk groups in patients treated at diagnosis



2.4 Discussion

A risk group according to the FLIPI score could be assigned to 220 patients of 302 patients that were assessed (73%), while 149 patients (49%) were assigned a risk-group according to

the FLIPI2 score. The limiting factor with regards to the FLIPI2 assignment in our population was the availability of the B2M results. Of note, the lack of availability of B2M from contributing centres prevented its inclusion in the FLIPI, although it was recognised as an important prognostic factor for patients with FL.

In our study, the FLIPI successfully segregated patients into three risk groups, well-balanced in terms of the proportion of patients in each group and with a distinct OS in both the overall group ($p < 0.001$) and in the group of patients treated at diagnosis ($p < 0.001$). In contrast, according to the FLIPI2 the majority of patients fell into the intermediate-risk category (66%). These findings were consistent in the sub-analysis of the 75 patients treated at diagnosis: in this subgroup 57% of the patients were classified as intermediate-risk. Furthermore, also in the original description of the FLIPI2 reported by Federico *et al*, the majority of patients (53%) fell into the intermediate-risk category (48).

Although the FLIPI2 could separate a high-risk population with a short 5-year OS of 33%, it did not separate the low-risk and the intermediate-risk groups (5-year OS, 93% and 85%, respectively, $p = 0.3$). These findings mirror the results from a similar study that retrospectively assessed the prognostic relevance of FLIPI2 in terms of OS and time to treatment failure in 758 patients, where the FLIPI2 could not differentiate between low-risk and intermediate-risk groups according to OS (108).

With regards to PFS, the FLIPI discriminated a high-risk population with a short 5-year PFS (20%), but it was poor at differentiating the low and intermediate-risk groups (5-year PFS of 48% and 46%, respectively). On the other hand the FLIPI2 tended to separate a high-risk population with a shorter PFS (22%), but was unable also to separate the low-risk from the intermediate-risk group (5-year PFS: 52% *versus* 43%; $p = 0.3$). Interestingly, in our study the FLIPI2 additionally identified a low-risk group of patients that had a plateau in the PFS curve, with a similar PFS at 5 and 10 years. However, there were a very small number of patients in

our study that had a low-risk FLIPI2 score so this suggestion of a plateau in patients with low-risk FLIPI2 needs to be verified with a longer follow-up in a larger group of patients. While both indices are good at defining a high-risk population with a shorter PFS, both are poor at separating the low and the intermediate-risk groups. Of note, the high-risk group includes a relatively small proportion of patients, so most patients with a similar prognosis in terms of PFS are indistinctly grouped together by both indices.

In addition to characteristics related to the patients or to the disease itself, PFS is influenced by a number of factors. A major determinant of PFS is the type of treatment the patients receive, as PFS is a measure of how well a patient does in response to that treatment, whereas OS is a cumulative measure of the effect of multiple treatments a patient receives (109). In our study, the patients received a variety of different treatments, including rituximab in one third of treated patients. Although the FLIPI2 was intended for patients treated in the new era of rituximab, only 62% of the patients actually received rituximab in the study by Federico *et al* (48) and the patients, as in our study, received different treatments regimens. In addition, PFS is dependent on the timing of progression. The definition of time of progression, in a disease such as FL in which patients can be managed expectantly at progression, may differ between institutions and is liable to bias, contrasting with OS measurement in which the date of death is known and not disputable (109). Due to the above reasons, the replication of a prognostic score with PFS as an end-point may be difficult between different studies and not practical when patients have not received a similar treatment.

2.5 Conclusions

In summary, in our study both indices are good at defining a high-risk population with a short OS and a short PFS, but do not accurately segregate the rest, resulting in a relatively large

group of patients with a similar prognosis. According to our results, the FLIPI2 does not appear to be superior to FLIPI for risk stratification. Finally, the PFS may not be an appropriate end-point for patients treated heterogeneously.

CHAPTER THREE

The role of FDG-PET/CT in the staging and prognosis of patients with follicular lymphoma

3.1 Introduction

Staging according to the Ann Arbor classification describes the extent of the disease and is a cornerstone in the management of patients with FL (8), as the prognosis and type of treatment offered to patients depends on the stage at presentation. Patients with localised disease (I-II) can be offered radiation therapy, which is considered curative in some cases, whereas asymptomatic patients with advanced stage (III-IV) can be managed expectantly until they become symptomatic requiring institution of treatment.

Currently the FLIPI is the most commonly used prognostic index in FL. It is based on a score defined by the presence of five prognostic factors. This score allows classifying patients into three risk groups (49). Two of the prognostic markers included in the FLIPI are dependent on imaging: the stage and the number of involved nodal areas.

The imaging method of choice in patients with FL recommended by several international guidelines is still a contrast enhanced CT (CeCT), as there is a lack of consensus on the additional role of ¹⁸F-fluoro-deoxyglucose positron-emission tomography (FDG-PET) in the staging of FL (40-41). In addition, most studies to date have investigated the role of staging FDG-PET or FDG-PET/CT using a visual interpretation (34-35), but there is a paucity of studies examining the value of additional semi-quantitative analysis (33). The aim of this study was to investigate the role of FDG-PET/CT in the staging of FL and its consequent impact on the prognostic score (FLIPI) and on the management of patients in comparison with staging by CeCT. An additional objective was to examine the usefulness of semi-quantitative measurement to identify BM involvement in patients with FL by FDG-PET/CT.

3.2 Patients and methods

3.2.1 Patients' characteristics

Between January 2008 and July 2011, 41 patients (median age: 64 years, range: 30-87) diagnosed with FL (grade 1-3a) were included in this study. All patients had a FDG-PET/CT, a CeCT and a unilateral BM aspirate and trephine (BMB) as part of the staging investigations. Seventy-five percent of the patients had their staging FDG-PET/CT and CeCT on the same day (range: 0–75). Thirty patients had not been treated previously while 11 had received prior treatment and had their staging investigations at relapse. The last treatment for patients included at relapse was administered at least 3 months (range: 3-54 months) prior to the staging investigations. The main characteristics of the patients are shown in table 10.

Table 10: Patients' characteristics

	<i>Patients, n (%)</i>
Age, median (range)	64 years (30-87)
Gender (female)	22 (51%)
LDH >ULN	8 (20%)
Haemoglobin <12mg/dL	11 (26%)
LN >6cm	5 (12%)
Histological grade	
Grade 1	7 (16%)
Grade 2	23 (55%)
Grade 3a	8 (18%)
Unknown	3 (7%)
Management at diagnosis	
Immediate treatment	21 (51%)
Expectant management	20 (49%)

3.2.2 FDG-PET/CT acquisition

All the FDG-PET/CT images were acquired on a Phillips Gemini TF LSO 64 slice scanner at one site. Images utilised in the visual and semi-quantitative analysis were acquired in 3D and

reconstructed using OSEM (ordered subset expectation maximization; 33 subsets, three iterations, no filters). All patients fasted for 6 hours and the median uptake time was 60 minutes. Half body imaging was acquired in 10 or 11 bed positions from skull base to thighs. The low dose (LD) CT scan for attenuation correction and anatomical localization was performed at 120Kvp and 60mAs (table 11). Additional views of the head and neck were acquired at the end to minimize effects of patient movement. Diagnostic CTs were performed either on the PET/CT scanner with 120 kVp, 99mAs (maximum-dose modulation used) and a pitch of 0.83 or on a Siemens CT scanner using 120kVp and mAs modulation with "Caredose" (which optimally reduces dose), pitch at 1.5mm.

Table 11: FDG-PET acquisition parameters

<i>FDG-PET/CT</i>	
Uptake period: median (range)	60 min (53-78)
Fasting glucose: median (range)	5.2 mmol (4.0-7.6)
Injected activity: median (range)	349 MBq (249-399)

3.2.3 Imaging analysis

FDG-PET/CT images were interpreted both visually and by semi-quantitative assessment.

Lymph node regions were defined as per the FLIPI. The number of involved nodal areas was recorded by both modalities, FDG-PET/CT and CeCT. A positive lesion was defined by visual analysis as an area of increased uptake, higher than the mediastinal blood pool (MBP), unrelated to physiological sites and consistent with a defined anatomical site on CT. Spleen and bone involvement were defined by an increased uptake higher than the liver.

Semi-quantitative analysis involved measuring the maximum SUV (SUVmax) at all involved regions. The SUV was calculated according to the formula $SUV = \text{tissue activity (kBq/ml)} / \text{activity injected (MBq/kg)}$. The SUVmax was calculated using the single maximum pixel value

within a defined ROI and it was measured in all lesions. In addition, the SUVmax was measured in the bone regions usually involved by disease: the sternum, both iliac blades and the T12 vertebra (as a representation of the central skeleton). The average maximum SUV (SUVav) was calculated for these 4 sites and compared to the BMB result. The single highest SUVmax in the four sites as well as the ratios SUVav/MBP and SUVav/liver were also compared to the BMB result.

3.2.4 Statistical analysis

The BMB was considered the gold-standard to define BM involvement so patients were classified as having BM involvement or not according to it. A normal distribution for both populations (patients with and without BM involvement) was assumed. The two populations did not have equal variance, as shown by Levene's test for equal variances; therefore a t-test for unequal variances (the Welch test) was performed, using SPSS. The t-tests were performed for the SUVav, the highest BM SUVmax, SUVav/MBP and SUVav/liver. Optimal SUVmax, SUVav, SUVav/MBP and SUVav/liver cut-offs were defined by Receiver Operating Characteristic (ROC) curves.

3.3 Results

3.3.1 Staging and management

Thirty-seven patients (90%) had increased FDG uptake in a pathological site on FDG-PET/CT. The four patients that had no pathological uptake on FDG-PET/CT had no pathological lesions on CeCT or disease on BMB. FDG-PET/CT identified more involved nodal lesions (178) in comparison to CeCT (154). When taking into account the results of the BMB in combination with the CeCT, there was a change in stage in 29% of the patients, with 10% (4 patients) being up-staged by FDG-PET/CT (table 12). Two patients would be up-staged from stage I on

CeCT + BMB to stage III and IV as a result of the findings on the FDG-PET/CT, and another 2 patients from stage II on CeCT + BMB to stage III and IV, respectively, after FDG-PET/CT. All four patients would have been up-staged to an advanced stage from localised stage, which would have led to a change in management based on FDG-PET/CT. The rest of the patients would have been down-staged by FDG-PET/CT, as 10 patients had BM involvement on BMB, not identified visually on FDG-PET/CT.

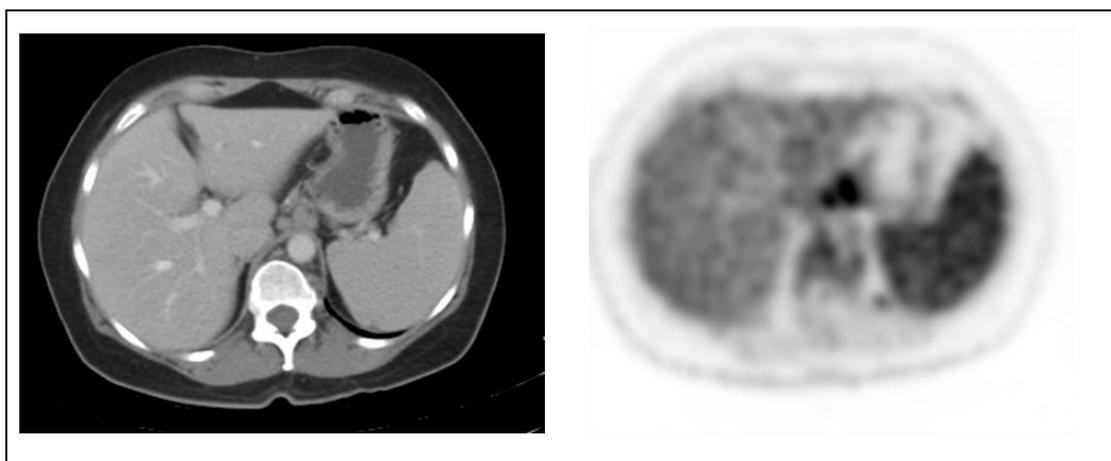
Table 12: Staging according to imaging modalities

Stage	CeCT	CeCT + BMB	FDG-PET/CT
0	3 (7%)	3 (7%)	3 (7%)
I	6 (14%)	5 (12%)	3 (7%)
II	5 (12%)	4 (9%)	3 (7%)
III	21 (51%)	11 (26%)	20 (48%)
IV	6 (14%)	18 (44%)	12 (29%)

Ten patients had an increased tracer uptake in the spleen on FDG-PET/CT and thus were identified as having splenic involvement. Seven patients had a diffuse increase in uptake higher than the liver uptake, while 3 patients had focal lesions with increased uptake (2 with single lesions and 1 with multiple lesions). The mean SUVmax for involved spleens was 4.0 (range: 3.1-6.9) while it was 2.2 (range: 1.1-3.0) for uninvolved spleens. Four patients had an enlarged spleen on CeCT, but did not have an increased uptake on FDG-PET/CT (although the enlargement of these spleens could also be demonstrated on the low-dose component of the CT) (table 13, figure 12). One patient had focal liver lesions on CeCT, identified by an increased uptake on FDG-PET/CT, while another patient had an enlarged liver on CeCT, but no increased uptake detected on FDG-PET/CT.

Table 13: Spleen involvement by FDG-PET/CT *versus* CeCT

	<i>FDG-PET+VE</i>	<i>FDG-PET -VE</i>
CeCT +VE	4	4
CeCT -VE	6	27

Figure 12: Spleen and liver involvement seen on FDG-PET but not on CeCT

3.3.2 Prognostic score: FLIPI

The use of a FDG-PET/CT resulted in a change in the FLIPI score in 20% of the patients, with 2 patients moving from the low-risk group to the intermediate-risk, while 10% of the patients moved from the intermediate-risk group to the high-risk. In this group of patients, staging by FDG-PET/CT would result in more than 50% of the patients being included in the high-risk category of the FLIPI (table 14).

Table 14: FLIPI score according to FDG-PET/CT *versus* CeCT+ BMB

<i>FLIPI risk groups</i>	<i>CeCT + BMB</i>	<i>FDG-PET/CT</i>
Low-risk (0-1)	15 (37%)	12 (29%)
Intermediate-risk (2)	7 (17%)	6 (15%)
High-risk (3-5)	19 (46%)	23 (56%)

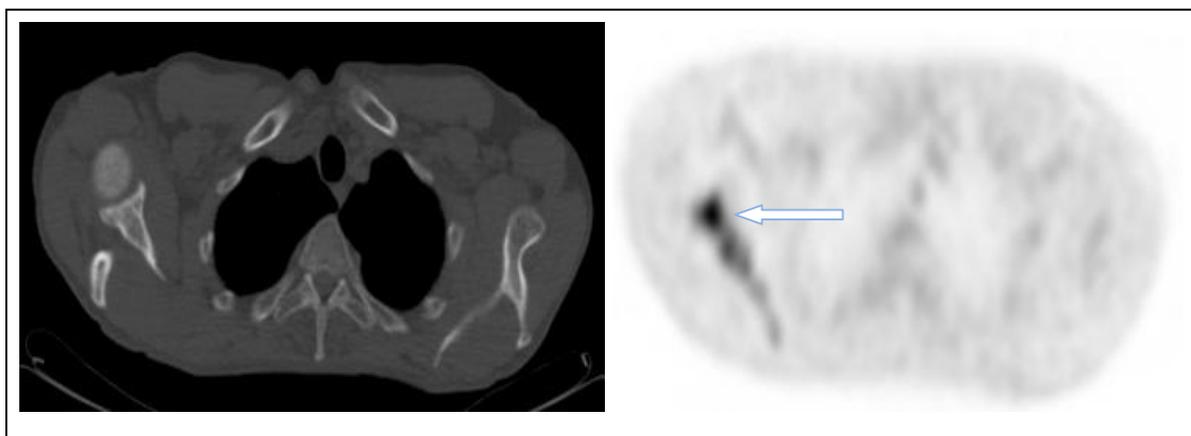
3.3.3 Bone marrow involvement

BMB identified BM involvement in 16 patients (37%), of which 9 patients had $\leq 10\%$ infiltration of the BM. On visual analysis of the FDG-PET/CT, 7 patients had BM involvement (focal lesions in 6, of which 4 patients had multifocal bone lesions). Two patients deemed to have possible bone involvement on visual analysis of the FDG-PET/CT had a negative BMB (table 15, figure 13). Visual analysis had thus a sensitivity and specificity of 31% and 92%, respectively.

Table 15: Bone marrow involvement detected by FDG-PET/CT *versus* BMB

	<i>BMB +VE</i>	<i>BMB -VE</i>
FDG-PET/CT +VE on visual analysis (n patients)	5	2
FDG-PET/CT -VE on visual analysis (n patients)	11	23

Figure 13: Bone involvement in scapulae detected by FDG-PET but not by CeCT



All semi-quantitative measurements were significantly higher in patients with a positive BMB in comparison with those with a negative BMB (table 16). The sensitivity and the specificity for $SUV_{max} \geq 2.5$ were 56% and 84%; those of $SUV_{av} \geq 2$ were 63% and 96%, and the sensitivity and specificity of $SUV_{av}/liver \geq 0.75$ were 81% and 80% respectively. The ratio $SUV_{av}/MBP \geq 1$ had the best sensitivity of 88% with a specificity of 80%.

Table 16: Accuracy of methods utilised in the assessment of BM involvement

<i>Methods for assessment of BM involvement</i>	<i>BMB +VE</i>	<i>BMB-VE</i>	<i>p-value</i>
SUVmax	2.8	2.0	0.02
SUVav	2.3	1.6	0.004
SUVav/liver	1.0	0.6	<0.001
SUVav/MBP	1.4	0.9	<0.001

3.4 Discussion

The addition of FDG-PET to CT has led to a powerful combination of both metabolic and structural imaging. Following the introduction of CT in the early 1980 era the criticism directed at CT was the complete reliance on the anatomical detection of enlargement of Lymph nodes and organs. Thus structurally normal LN and organs involved with disease were no longer detectable when relying only on CeCT. The possible under-staging of patients as a result was recognised as an accepted flaw when compared to the morbidity of laparotomy and lymphangiography.

It is now recognised that FL is an FDG-avid lymphoma. This is corroborated in our series, as 90% of the patients had an FDG-avid disease (32). Several studies have now demonstrated that FDG-PET identifies additional areas of both nodal and extra-nodal disease in comparison to standard imaging modalities in patients with FL (34-35, 37, 45-47). Le Dotz and colleagues showed that this was as much as 51% for nodal disease and 89% for the detection of additional extra-nodal lesions (47). In our series FDG-PET/CT identified more involved LN regions than CeCT leading to a change in the stage in around one third of patients.

Furthermore, FDG-PET has been found to have a sensitivity of 80-100% in the detection of splenic involvement, in comparison with the sensitivity of 33-94% of CT (depending on the size criteria used by the investigator) (110). In line with this, in our series 6 patients with

normal size spleen on CeCT were found to have increased uptake on FDG-PET/CT, compatible with splenic involvement.

The critical issue is whether up-staging the patients using FDG-PET/CT would result in a change in the management of these patients. As the management of patients with stage III or IV does not differ, only patients with localised disease (I-II) up-staged to an advanced stage by FDG-PET/CT would have their management modified, as they would not be treated with local radiotherapy. In a study by Writh *et al* only early stage FL patients were included which led to a 45% change in the management of patients (44). In our retrospective analysis, 4 patients were up-staged by FDG-PET/CT. Two of these patients had focal bone lesions on FDG-PET/CT not apparent on CeCT or BMB, while the other 2 patients had additional nodal regions involved both above and below the diaphragm, not seen on conventional imaging. If FDG-PET/CT had not been done, these patients would have been treated ineffectively with local radiotherapy. These data suggest that FDG-PET/CT staging provides more accurate information than conventional imaging whilst there is little difference in the amount of radiation a patient is exposed to if a low-dose CT protocol is used with PET.

The FLIPI, the current prognostic index for FL, is not used to determine the management of FL patients, however it is utilised to predict prognosis, as well as in clinical trials to define and compare different patient populations. In the current study FDG-PET/CT identified significantly more LN regions than CeCT, which led to a change in the FLIPI score in 20% of patients, with most patients being up-graded to a higher risk category. Whether the patients that appear to have a higher FLIPI score by FDG-PET/CT indeed have a worse prognosis, is difficult to ascertain with the current available data. Larger number of patients and longer follow-up would be necessary to answer this question. Nevertheless, a potential change in the FLIPI score by FDG-PET/CT should be taken into account when comparing different study populations.

BMB is an essential part of the staging of patients with FL, as 40-60% of them have BM involvement at presentation (8). However, given the patchy infiltration of BM in patients with FL, BMB -an unpleasant investigation- can miss disease not present in the biopsy area. Several studies evaluating the role of FDG-PET/CT in assessing BM involvement have relied on visual analysis, which has proved inadequate in FL. This is reflected in our experience as in our patient group the visual analysis of BM involvement had a high specificity but a sensitivity of only 31%. FDG-PET is unique in its ability to provide quantitative assessment, in comparison to other anatomical imaging modalities such as CT and MRI. This additional characteristic is now gaining recognition in DLBCL, as SUVmax is being utilised to successfully assist visual interpretation of interim FDG-PET. In the present series, all the parameters that were assessed in the semi-quantitative analysis (SUVmax, SUVav, SUVav/MBP and SUVav/liver) were significantly different between patients with a positive and a negative BMB. Although we found that a cut-off of 2 for SUVav has a sensitivity of only 63% and a specificity of 97%, we found that a ratio of SUVav/MBP ≥ 1 increased the sensitivity to identify BM involvement to 88% while maintaining a high specificity of 80%. Absolute SUV measurements are affected by variations in blood sugar, duration of FDG uptake period and FDG injected activity. The use of ratios will minimize this error, as another measurement is utilised as a comparator to counter these effects. If these results are confirmed in further prospective studies, they may help to select patients who might benefit from having a BMB because of likely involvement, with the potential to consider avoiding BMB in the rest.

3.5 Conclusions

FDG-PET/CT identifies more involved LN and extra-nodal regions than conventional CeCT. This results in up-staging of patients which can in turn result in alternative management plans. In addition, the FLIPI score of patients staged by FDG-PET/CT differs from that in

patients staged by CeCT. This needs to be taken into account when comparing the population of patients and the results from different centres utilizing a mixture of imaging techniques.

Semi-quantitative measurement utilizing SUVmax has the potential to increase the specificity and sensitivity of FDG-PET/CT in identifying patients with involved BM.

CHAPTER FOUR

Criteria for treatment and prognostic indices in patients with follicular lymphoma managed expectantly

4.1 Introduction

Three major prospective randomised trials have demonstrated that asymptomatic patients with FL managed expectantly at diagnosis have a similar survival to that of patients treated immediately after diagnosis. The median time to treatment for the patients managed expectantly in these studies was around 3 years and 5-19% of them never required any treatment (9, 64-65). These studies constitute the basis to consider a watch and wait (WW) approach as the current standard for asymptomatic patients with advanced stage FL.

However, expectant management in asymptomatic advanced FL patients has recently been challenged with the advent of rituximab, a very effective, relatively non-toxic treatment.

Thus, Ardeschna *et al* recently reported a superior PFS in patients treated initially with rituximab in comparison to patients managed expectantly, although the follow-up was too short to show any significant differences in OS (66).

Nevertheless, as there is not a curative treatment for advanced FL, expectant management remains the standard approach in asymptomatic patients. However, the 'right time' to start treatment is arbitrary, and differs between institutions. There are two main sets of criteria that are generally used to guide decisions on when to start treatment: the Groupe d'Etude des Lymphomes Folliculaires (GELF) and the British National Lymphoma Investigation (BNLI). However, both are the result of expert opinion and neither of these criteria is based on prospective clinical trials.

On the other hand, patients managed expectantly have been excluded from the design of prognostic indices for newly diagnosed FL: only treated patients were retrospectively included in the design of the FLIPI (49), while patients managed expectantly were excluded from the prospective analysis of FLIPI2 (48, 50).

Against this background, the objectives of this study were: 1) to assess the actual application of the GELA and BNLI criteria in the routine practice and their potential prognostic value, and

2) to analyse the predictive value of FLIPI and FLIPI2 to guide initiation of treatment in this population.

4.2 Patients and methods

4.2.1 Patients' characteristics

Three hundred and two patients [median age: 55 years, range (29-85)] newly diagnosed with FL presented to our institution between 1985 and 2007. One hundred and ninety-seven patients were treated at diagnosis, while 105 patients were followed-up expectantly and constitute the study population. Patients' characteristics are shown in table 17.

4.2.2 Definition of end-points

Patients that had not received any treatment (chemotherapy or radiotherapy) within 3 months of diagnosis were considered to be on expectant management. OS was measured from the date of diagnosis to death from any cause. Time-to-treatment (TT) was measured from the time of diagnosis to the time of initiation of first treatment.

4.2.3 Statistical analysis

Survival analysis and duration of remission was performed by the Kaplan-Meier method and comparisons were made using the log-rank test. All causes of death were included in the OS analysis. Continuous variables between two different groups were tested by t-test. All statistical analysis was carried out on STATA.

Table 17: Characteristics at diagnosis of 105 patients managed expectantly

	<i>Patients, n (%)</i>
Gender (female)	52 (50%)
Age ≥60 years	41 (39%)
Stage	
I	9 (9%)
II	19 (18%)
III	23 (22%)
IV	53 (51%)
Unknown	1
Histological grade	
Grade 1	52 (50%)
Grade 2	32 (30%)
Grade 3	15 (13%)
Unknown	6 (7%)
Haemoglobin <12g/dL	20 (21%)
Unknown	9
Lactate dehydrogenase >ULN	3 (4%)
Unknown	24
Number of nodal sites >4	27 (35%)
Unknown	27
Beta-2-microglobulin >ULN	5 (20%)
Unknown	80
Bone marrow involvement	45 (45%)
Unknown	4
Lymph node size ≥6cm	10 (9%)
Unknown	16

4.3 Results

4.3.1 Patients' treatment and follow-up

After a median follow-up of 90 months (range: 14-264), 33 patients (31%) have never required any treatment, whilst 72 patients have required treatment (31, single agent alkylating; 13, anthracycline-containing regimens; 24, a rituximab-containing regimen; and 4 patients, other treatments). There were no significant differences in age, gender, histological grade, number of involved LN regions or FLIPI score between patients that never required

treatment and patients that eventually were treated. However, the group of patients that never required any treatment were less likely to have a LN mass ≥ 6 cm ($p=0.02$).

4.3.2 Prognostic indices

The FLIPI and FLIPI2 score could be retrospectively assigned in 71 patients and 56 of the 105 patients managed expectantly, respectively. The distribution of the patients in risk groups according to the FLIPI and FLIPI2 were not significantly different in patients managed expectantly in comparison to patients treated at diagnosis ($p=0.89$ and $p=0.22$, respectively) (table 18).

Table 18: Distribution of patients according to FLIPI, FLIPI2 and treatment at diagnosis

<i>FLIPI</i>			<i>FLIPI2</i>		
<i>Risk group</i>	<i>W&W</i>	<i>Treated</i>	<i>Risk group</i>	<i>W&W</i>	<i>Treated</i>
Low-risk (%)	32 (45%)	67 (45%)	Low-risk (%)	4 (7%)	12 (13%)
Intermediate-risk (%)	21 (30%)	39 (26%)	Intermediate-risk (%)	43 (77%)	59 (63%)
High-risk (%)	18 (25%)	43 (29%)	High-risk (%)	9 (16%)	22 (24%)

4.3.3 Treatment criteria

A total of 12 patients were found to have at least one BNLI criteria, while 20 patients had at least one GELF criteria (4 patients had more than one criterion) (table 19). Twenty-four of 105 patients managed expectantly (23%) had at least one GELF or BNLI criteria for treatment. None of the patients evaluated had symptomatic compression of a vital organ, or life threatening organ involvement.

Table 19: GELF and BNLI criteria for treatment

<i>GELF/BNLI criteria</i>	<i>Patients, n (%)</i>	<i>GELF/BNLI criteria</i>	<i>Patients, n (%)</i>
B-symptoms	6 (7%)	Spleen ≥ 20 cm	1 (1%)
Unknown	19	Unknown	11
Lymph node mass ≥ 7cm	3 (3%)	Pruritus	2 (2%)
Unknown	11	Unknown	19
Pleural effusion/ascitis	4 (4%)	Bone involvement	1 (1%)
Unknown	11	Unknown	8
3 nodal areas, with LN ≥ 3	1 (1%)	Kidney/liver	1 (1%)
Unknown	25	Unknown	11
ECOG ≥ 2	3 (3%)	Cytopenia	1 (1%)
Unknown	29	Unknown	10
LDH \geqULN	3 (4%)	B2M \geqULN	5 (5%)
Unknown	25	Unknown	81

4.3.4 Time-to-treatment

The median TT for the 72 patients who eventually required treatment was 31 months (range: 5-248). Both the FLIPI and the FLIPI2 predicted TT ($p=0.02$, $p=0.04$ respectively) and identified a high-risk population with a shorter TT. Patients with a high-risk FLIPI score and a high-risk FLIPI2 score were treated at a median of 2.6 years and 2.7 years, respectively, in comparison to a median of 5 years and 3.2 years for low-risk patients as per FLIPI and FLIPI2, respectively (figures 14 and 15). In contrast, there were no significant differences in TT between the low-risk and intermediate-risk groups according to the FLIPI and FLIPI2. The median TT in the intermediate-risk group was 4.9 years and 3.8 years according to the FLIPI and FLIPI2, respectively.

Figure 14: TT according to FLIPI risk groups

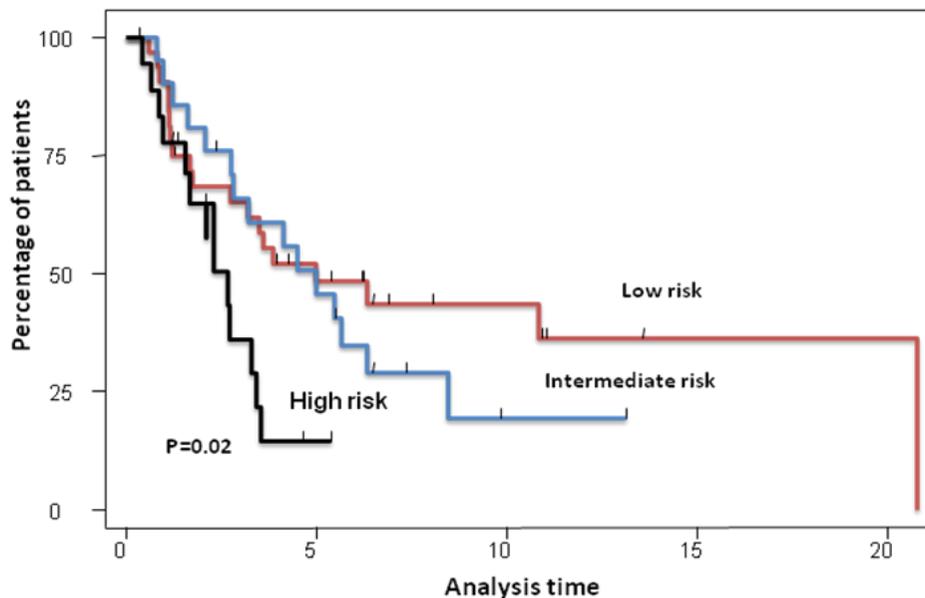
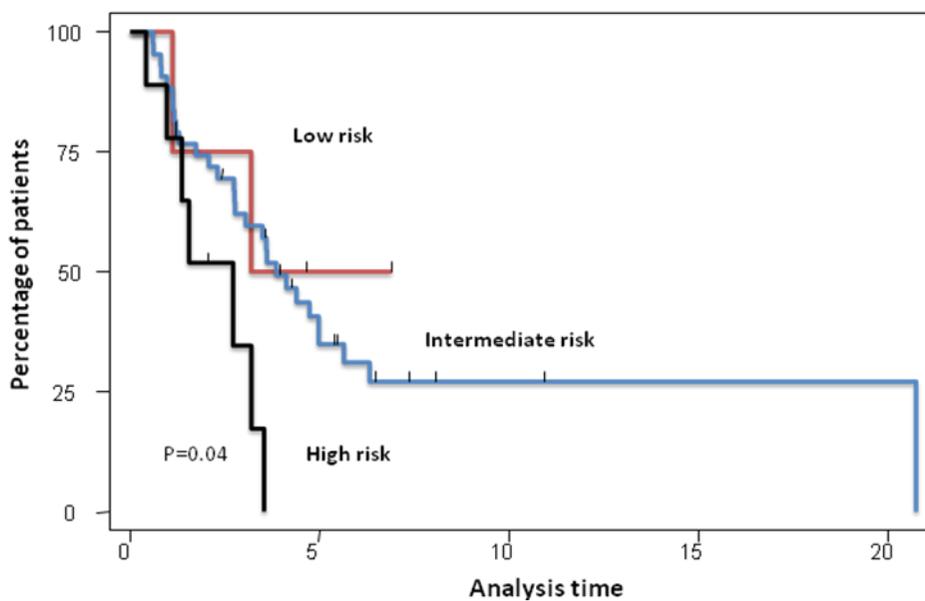


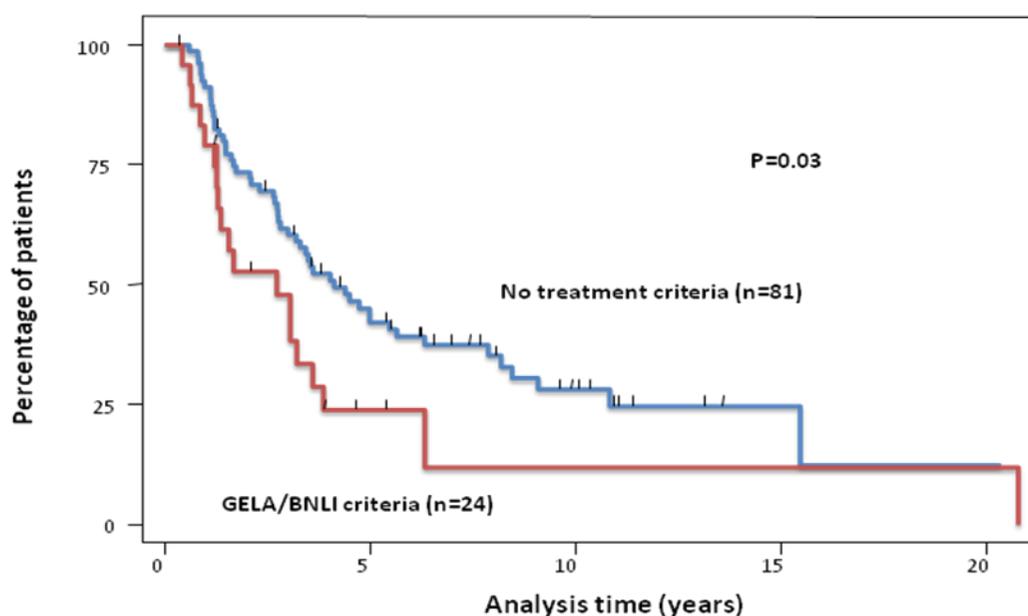
Figure 15: TT according to FLIPI2 risk groups



There was a trend towards a shorter TT for patients with at least one GELF criteria versus those with none (median TT: 32 months *versus* 49 months, $p=0.06$). Likewise, patients that had at least one BNLI criteria had a trend towards a shorter median TT than the rest (19

months *versus* 43 months, $p=0.09$). The TT was significantly shorter in the 24 patients with at least one GELF or BNLI criteria than in patients with no treatment criteria at all ($p=0.03$) (figure 16).

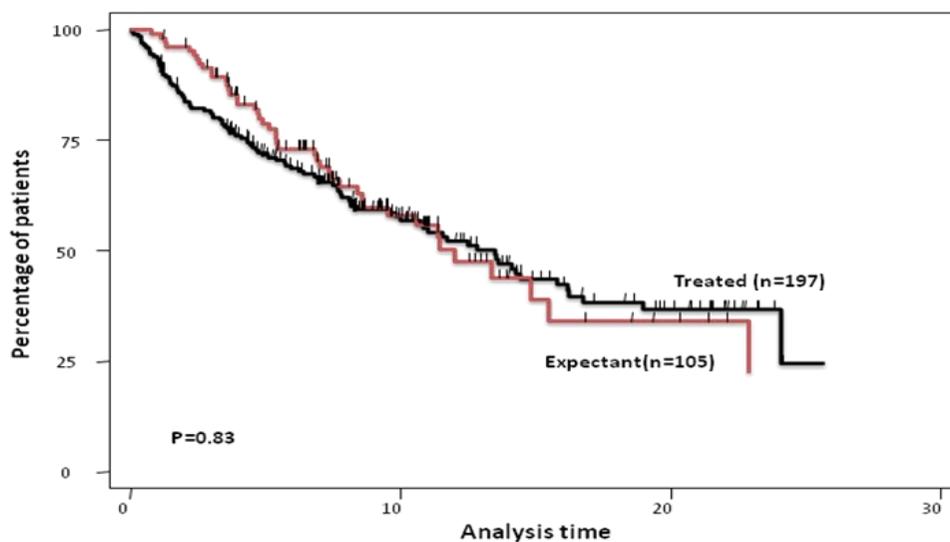
Figure 16: TT in patients with ≥ 1 GELF/BNLI treatment criteria in comparison with patients with no criteria for treatment



4.3.5 Overall survival

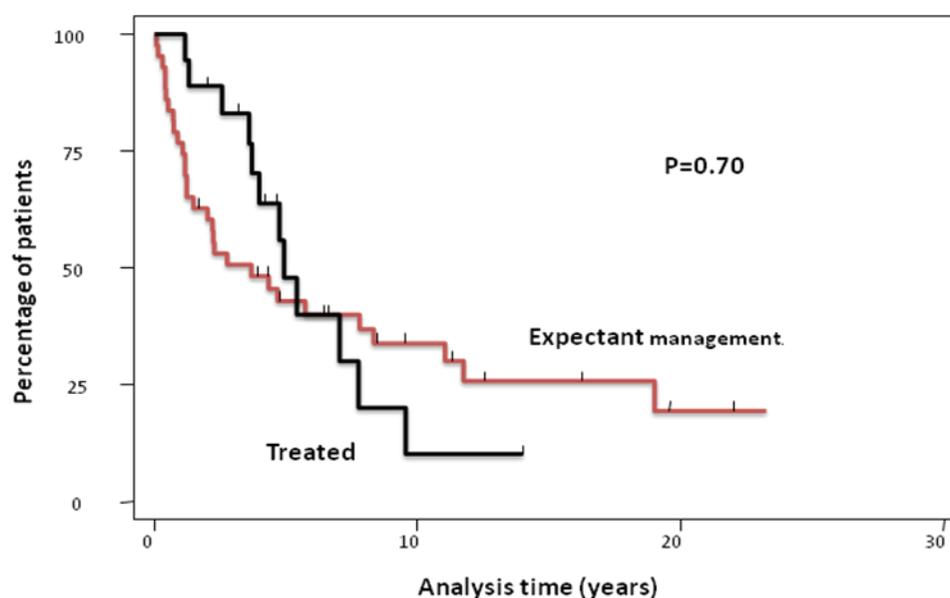
Thirty-eight patients have died, including 6 patients that had not required any treatment for FL. The causes of death were related to lymphoma in 19 patients, other malignancies in 3 patients and other causes in 16 patients. The cause of death was unrelated to lymphoma in the 6 patients that died without having required treatment for FL. The 5-year, 10-year and 20-year OS of the patients managed expectantly were 77% (95%CI: 68-85%), 58% (95%CI: 46-68%) and 34% (95%CI: 19-50%), respectively. The OS of the study group was not significantly different from that of the group of patients treated at diagnosis ($p=0.83$) (figure 17).

Figure 17: OS of patients managed expectantly *versus* those treated immediately after diagnosis



Having one or more treatment criteria according to GELF or BNLI had no impact on OS ($p=0.9$ and $p=0.6$, respectively). In contrast, the FLIPI and FLIPI2 predicted OS ($p=0.001$ for both) and both indices identified a high-risk population with a shorter OS. However, the OS of patients with high-risk FLIPI managed expectantly was not significantly different from the OS in patients with high-risk FLIPI treated at diagnosis ($p=0.7$) (figure 18).

Figure 18: OS of patients with high-risk FLIPI according to whether they were managed expectantly or treated at diagnosis



4.4 Discussion

The FLIPI score is one of the factors (in addition to age, stage and the histological grade) that was found to influence the initiation of therapy by physicians in the National Lymphocare Study (54). In contrast, in our study the percentage of patients in each risk group according to the FLIPI and the FLIPI2 prognostic indices did not differ significantly between patients managed expectantly and those patients treated at diagnosis ($p=0.89$, $p=0.22$ for FLIPI and FLIPI2, respectively), suggesting that neither the FLIPI nor the FLIPI2 were taken into account to decide initiation of treatment. Of note, almost a quarter of the patients managed expectantly had a high-risk score according to the FLIPI. Both the FLIPI and the FLIPI2 indices identified patients managed expectantly with a high-risk score that have a shorter OS than patients with a low- or intermediate-risk score, and were more likely to receive treatment earlier. Thus, in our study patients with high-risk FLIPI were treated at a median of 2.6 years in comparison to a median of 5 years for low-risk patients. The current results suggest that the FLIPI should not be used to guide treatment decisions at diagnosis in terms of the indication for treatment, as the OS of patients in the high-risk category did not differ between patients managed expectantly and those treated immediately. However, it might be used to identify a high-risk cohort of patients that may need to be followed up more closely as they are likely to need treatment earlier. The number of patients managed expectantly in whom the FLIPI2 score could be assigned in our series is too small to be able to draw similar conclusions for this index.

Despite being managed expectantly, a number of patients in our series were found to have one or more treatment criteria according to the GELF or BNLI criteria. Although the TT was shorter in patients having GELF or BNLI criteria, this did not reach statistical significance, probably due to the small number of patients included. When combining the patients that had at least one GELF or one BNLI criteria, the difference in the median TT between those

patients with at least one criterion and those without criteria for treatment reached statistical significance. When analysing if there was an effect on OS, having a treatment criteria did not affect the OS of patients. It is important to convey this message to patients, as being 'symptomatic' might mean requiring treatment, but in itself may not imply a worse prognosis in the long term.

4.5 Conclusions

This long follow-up study confirms that around one third of the patients with asymptomatic FL do not require treatment for a prolonged time and adds further support to an expectant management approach in asymptomatic patients. Neither the FLIPI nor the FLIPI2 are able to identify a population in whom initial management results in a better prognosis. The FLIPI does identify a high-risk population more likely to be treated earlier than the remainder and with a shorter OS, but the OS of these patients does not differ significantly from that in patients treated immediately at diagnosis.

CHAPTER FIVE

The outcome of patients with follicular lymphoma in the rituximab era treated with HDT/ASCR according to the high-dose regimen received

5.1 Introduction

A major advancement in the treatment of patients with FL has been the development of the chimeric anti-CD20 MoAb rituximab. Rituximab has become an essential component of FL treatment, either in combination with chemotherapy or as maintenance (69, 74, 111). In recognition of the importance and prevalence of rituximab treatment, many authors now distinguish a pre-rituximab from a rituximab era in the management of patients with FL. In contrast, HDT/ASCR has been utilised in both newly diagnosed and relapsed FL patients to prolong remission duration since the late 1980 era (80, 82, 91). The high-dose regimen received as the conditioning regimen can be either chemotherapy-based or total-body irradiation (TBI)-based. There have been concerns about an increased NRM in TBI-treated patients due to a higher incidence of sMDS/AML in comparison with patients treated with chemotherapy-based conditioning regimens (89). This has led to a shift to chemotherapy-based conditioning regimens in most centres, including Barts. However, there has never been a randomised trial comparing the different conditioning regimens (chemotherapy-based *versus* TBI-based) in FL patients, that would add further evidence of the higher risk of sMDS/AML associated with TBI by controlling other factors. Of note, most available studies examining the role of HDT/ASCR in patients with FL have been performed in the pre-rituximab era, so there is limited available data regarding the influence of prior rituximab on the survival of patients with FL after HDT/ASCR.

Against this background, the aim of this Lymphoma Working Party (LWP)-EBMT study was to assess the outcome of patients with FL having HDT/ASCR according to the high-dose regimen received and according to whether they had received previous treatment with rituximab.

5.2 Patients and methods

5.2.1 Patients

Between 1995 and 2007, 7910 adult patients with FL had HDT/ASCR and were reported to the EBMT registry. Patients were included in the current study if they had received TBI or the chemotherapy-only regimen BEAM (BCNU, etoposide, cytarabine and melphalan) as conditioning regimens. Patients with histological transformation at the time of HDT/ASCR were excluded as well as those undergoing a second transplant. A complete data set was available for 2233 FL patients, which constitutes the study group.

5.2.2 Response definitions

Complete response (CR) was defined as the complete disappearance of disease and related symptoms. A very good partial response (VGPR) was defined as a reduction in tumour burden of at least 90%. Partial response (PR) was defined as a reduction in the burden of disease by at least 50% or more. According to EBMT definitions, relapse is the occurrence of new sites of disease, or the re-occurrence of disease or systemic symptoms after having achieved a CR which lasted for 3 months or more. Progressive disease (PD) is the occurrence or reoccurrence of new disease sites or symptoms if CR lasted less than 3 months. Progression also describes any worsening of the disease status in patients previously assessed as not in CR. Stable disease (SD) is defined as achieving less than a PR but not fulfilling criteria for progressive disease. Primary refractory disease is defined by a lack of response to treatment and progression of the disease. For the purpose of this analysis, primary refractory, SD, relapsed and progressive disease were grouped together as active disease. First complete response (CR1), first partial response (PR1) and first very good partial response (VGPR1) were grouped together as first remission (remission 1). Subsequent CR or PR were grouped together as subsequent remission (remission>1).

5.2.3 Outcome measures

OS was measured from the time of HDT/ASCR to death from any cause. EFS was measured from the time of HDT/ASCR to progression, reoccurrence of the disease or death from any cause. NRM was defined as death from any cause without progression.

5.2.4 Statistical analysis

Patient characteristics were compared with a t-test for continuous variables and a chi-square or Fisher test for categorical variables. OS and EFS were determined using the Kaplan-Meier method, and curves were compared by the log-rank test. The relevance of prognostic factors was validated by multivariate analysis using the Cox model. Incidence of relapse (IR) and NRM were calculated by cumulative incidence curves to account for competing risks and compared by Gray test. The relevance of prognostic factors was validated by multivariate analysis utilising the Fine and Gray model.

5.3 Results

5.3.1 Patients' characteristics

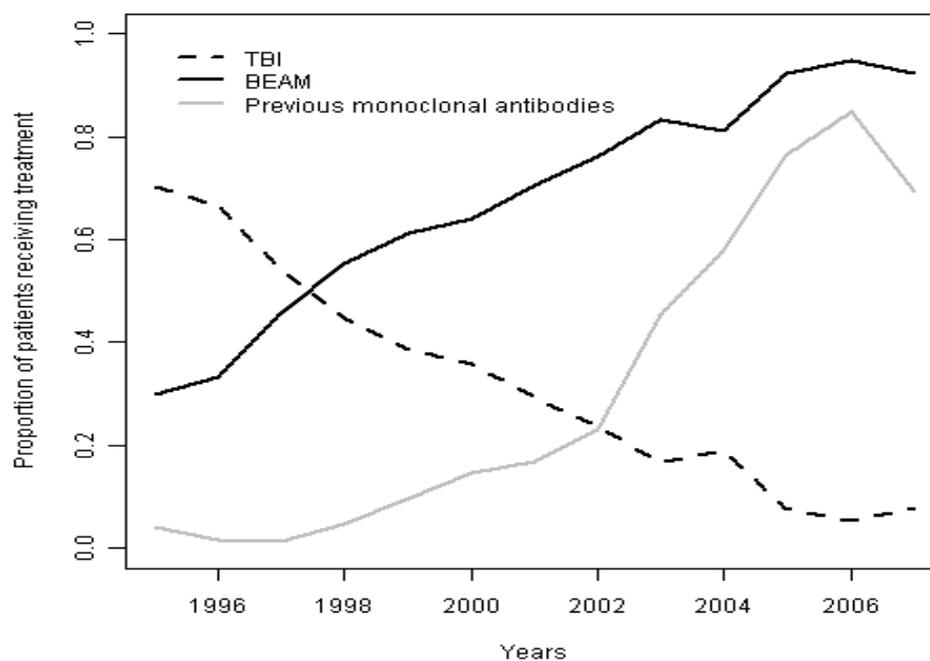
Six hundred and eighty patients received a TBI-containing regimen and 1553 patients received BEAM. The main characteristics of the patients according to the regimen received are described in table 20. A total of 1098 patients (49%) were in first remission, 619 (28%) received HDT in remission >1 whereas 398 patients (18%) had active disease at the time of HDT. The response at the time of HDT/ASCR was unknown in 118 cases (5%). Seven hundred and thirteen patients (32%) had been treated with MoAb before HDT/ASCR (confirmed as rituximab in 665). Two thousand one hundred and seven patients (94%) received peripheral blood as the source of stem cells, whereas 66 patients (3%) received BM. The number of

patients receiving BEAM conditioning regimen and MoAb prior to HDT/ASCR progressively increased per study year, while the number of patients treated with TBI declined (figure 19).

Table 20: Patients' characteristics according to the conditioning regimen received

<i>Patients characteristics</i>	<i>BEAM (n=1553)</i>	<i>TBI (n=680)</i>	<i>p-value</i>
Age median (range)	49 years (18 -72)	47 years (21-66)	< 0.01
Gender (female)	46%	43%	0.2
Time from diagnosis to HDT/ASCR (median)	30 months	18 months	<0.001
Time from diagnosis to HDT/ASCR <1 year	21%	39%	<0.001
Disease status			<0.01
Remission 1	44%	60%	
Remission>1	32%	18%	
Active disease	17%	19%	
MoAb prior to HDT/ASCR	37%	21%	<0.01
Peripheral blood stem cells (PBSC)	98%	93%	<0.01

Figure 19: Patterns of use of TBI, BEAM and MoAb during the study period



5.3.2 Non-relapse mortality (NRM)

The 5-year NRM for the whole group was 7% (95% CI: 6-8%, figure 20). On univariate analysis there were no significant differences between patients treated with TBI and patients treated with BEAM conditioning regimen (figure 21).

Figure 20: NRM and IR for the whole group

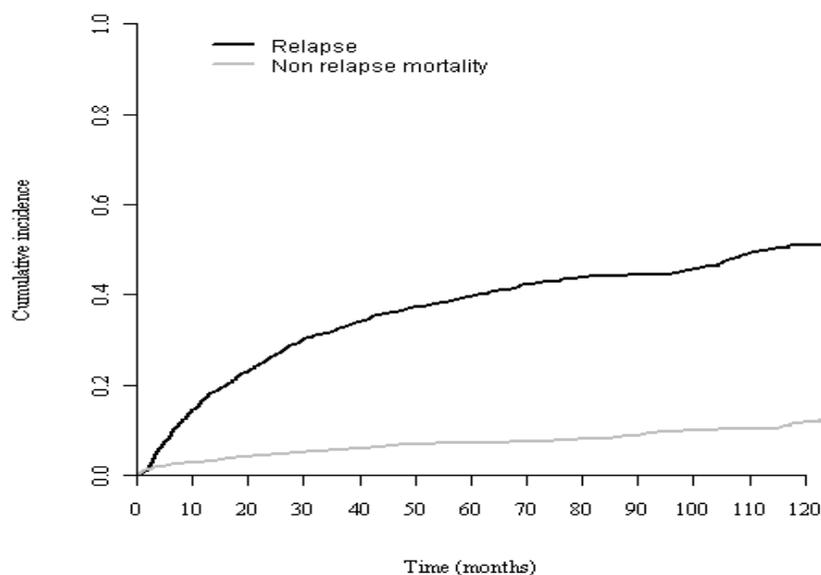
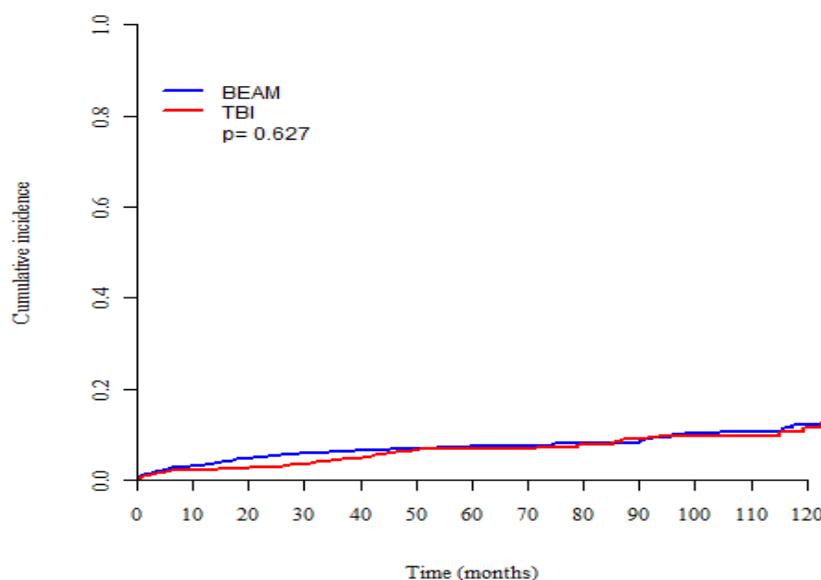


Figure 21: NRM according to the conditioning regimen



On multivariate analysis for NRM, increasing age, time from diagnosis to HDT/ASCR >1 year, male gender, no previous MoAb treatment, and BM as the source of stem cells were all predictors of an increased NRM (table 21).

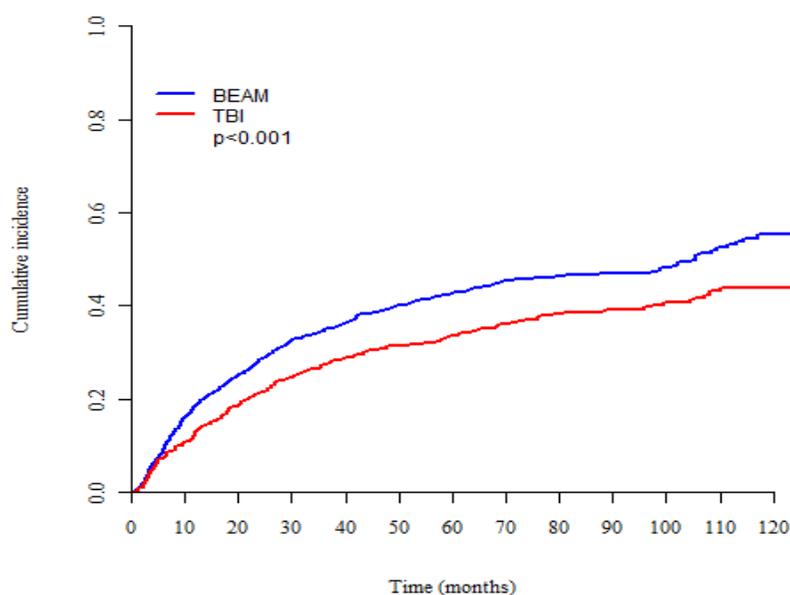
Table 21: Multivariate analysis for NRM

<i>Factors associated with increased NRM</i>	<i>HR (95% CI)</i>	<i>p-value</i>
Age (for one year increase)	1.0 (1.0-1.04)	0.04
Time from diagnosis to HDT/ASCR (>1 year versus <1 year)	1.6 (1.04-2.5)	0.03
Male versus female	1.4 (1.1-2)	0.02
No MoAb versus previous MoAb	1.7 (1.2-2.5)	0.005
BM versus PBSC	2.0 (1.2-3.6)	0.007

5.3.3 Incidence of relapse (IR)

After a median follow-up of 60 months, the IR was 39% (95% CI: 37-42%; figure 20). The 5-year IR was significantly better in patients treated with TBI (33%, 95% CI: 29-37%) compared to patients treated with BEAM conditioning (42%, 95% CI: 39-45%; figure 22).

Figure 22: IR according to the conditioning regimen



On multivariate analysis of IR, patients treated with BEAM conditioning were more likely to relapse than patients treated with TBI conditioning, as well as patients treated with active disease in comparison with those treated in first or subsequent remission (table 22).

Table 22: Multivariate analysis for IR

Factors associated with increased IR	HR (95% CI)	p-value
BEAM versus TBI	1.4 (1.2-1.7)	<0.001
Disease status at ASCT (specified category versus remission1)		
Active disease	1.5 (1.3-1.9)	<0.001
Remission>1	1.0 (0.8-1.3)	0.8
Unknown	1.0 (0.7-1.5)	0.8

5.3.4 Event-free survival (EFS)

The median EFS for the whole group was 72 months, with a 5-year EFS of 53% (95% CI: 51-55%). On univariate analysis patients who had BEAM conditioning had significantly shorter EFS than patients who received TBI (5-year EFS: 50% versus 60% respectively) (figure 23). In addition, patients that had received previous MoAb treatment had a longer EFS than patients that did not (5-year EFS: 56% versus 52%) respectively (figure 24).

Figure 23: EFS according to the conditioning regimen

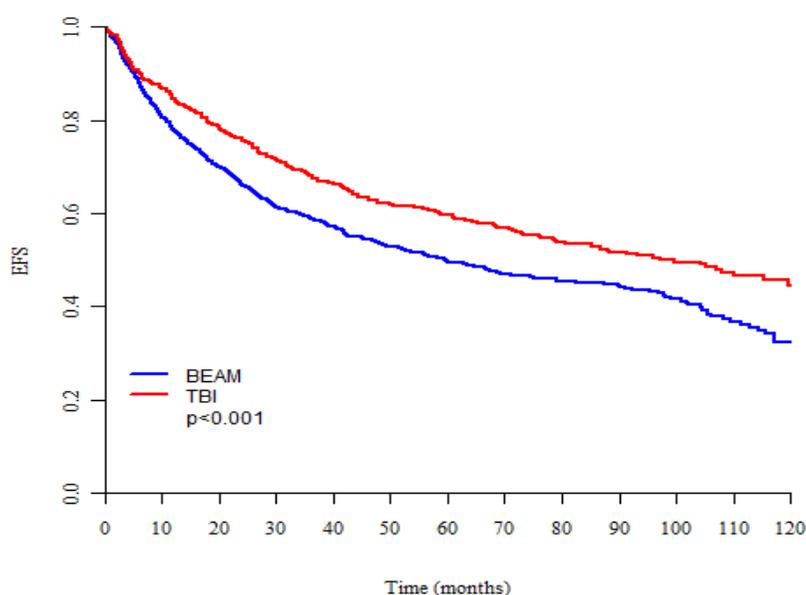
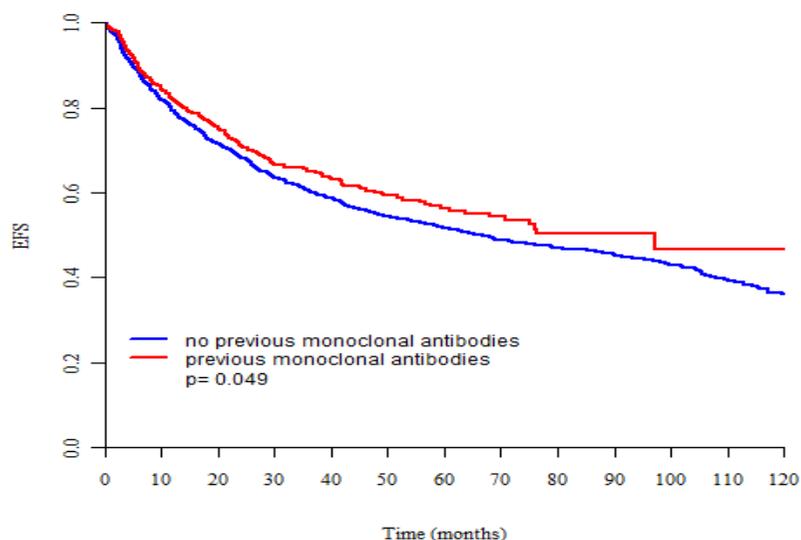


Figure 24: EFS according to previous MoAb treatment

On multivariate analysis the following factors retained significance and predicted a shorter EFS: BEAM conditioning, increasing age, no previous MoAb treatment and BM as source of stem cells (table 23).

Table 23: Multivariate analysis for EFS

<i>Factors associated with a shorter EFS</i>	<i>HR (95% CI)</i>	<i>p-value</i>
BEAM versus TBI	1.4 (1.2-1.7)	<0.001
Age (for 1 year increase)	1.0 (1.0-1.01)	0.04
No MoAb versus previous MoAb	1.2 (1.1-1.4)	0.009
BM versus PBSC	1.3 (1.0-1.7)	0.04

5.3.5 Overall survival (OS)

A total of 536 deaths occurred in the whole group (167 in patients receiving TBI and 369 in the BEAM group). Two hundred and seventy-seven patients died due to relapse or progression of disease, 37 patients due to secondary malignancies, 128 patients due to HDT/ASCR related causes, 32 patients due to other causes unrelated to HDT/ASCR, whereas the cause of death was unknown or missing in 62 patients. The median OS for the study

population was 146 months. The 5-year OS was 75% (95% CI: 73-76%) for the whole group.

On univariate analysis BEAM conditioning regimen was associated with a shorter OS than TBI conditioning regimen (5year OS: 74% *versus* 78% respectively) (figure 25). In addition, patients that had received previous MoAb treatment had a longer OS than patients that did not received prior MoAb (5 year OS: 80% *versus* 74%, respectively) (figure 26).

Figure 25: OS according to the conditioning regimen

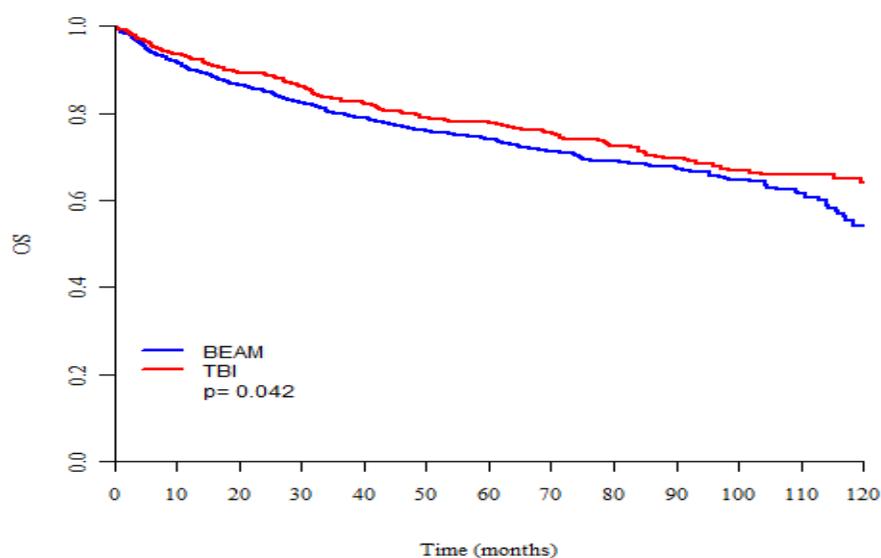
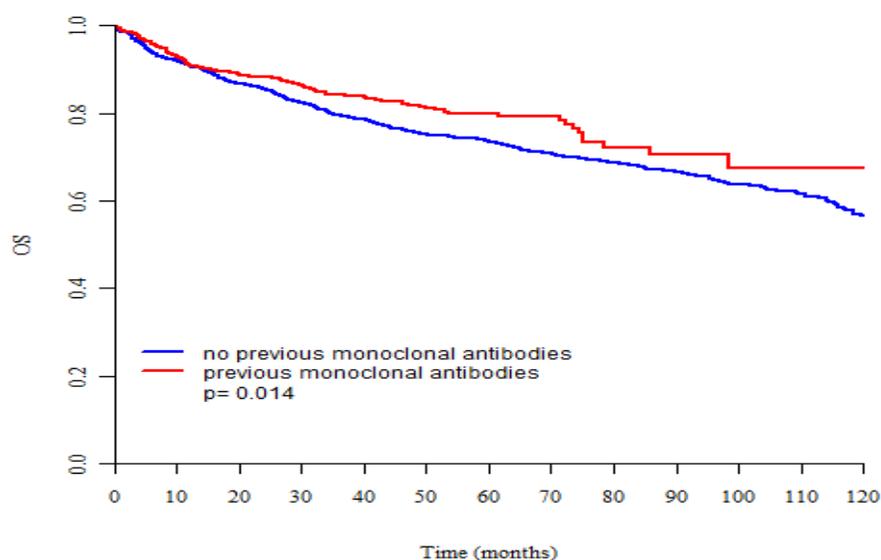


Figure 26: OS according to previous MoAb treatment



On multivariate analysis (table 24), patients treated with BEAM conditioning tended to have a shorter OS than patients treated with a TBI-containing conditioning regimen ($p=0.06$). In addition, the following factors predicted a shorter OS: increasing age, no previous MoAb treatment, time from diagnosis to ASCT >1 year and BM as the source of stem cells.

Table 24: Multivariate analysis for OS

<i>Factors associated with a shorter OS</i>	<i>HR (95% CI)</i>	<i>p-value</i>
BEAM versus TBI	1.2 (1.0-1.4)	0.06
Disease status at BMB (specified category versus remission1)		0.001
Active disease	1.4 (1.1-1.8)	
Remission >1	0.9 (0.7-1.2)	
Unknown	1.2 (0.8-1.8)	
Age (per 1 year increase)	1.02 (1.01-1.03)	<0.001
No MoAb versus previous MoAb	1.4 (1.1-1.7)	0.003
Time from diagnosis to ASCT (>1 year versus <1 year)	1.3 (1.02-1.6)	0.03
BM versus PBSC	1.7 (1.2-2.3)	<0.001

5.4 Discussion

Several randomised trials have demonstrated an improved PFS and, importantly, an improved OS in patients diagnosed with FL treated with rituximab in combination with chemotherapy in comparison with those treated only with chemotherapy (67, 69-70). Hence, since its approval by the FDA in 1997, there has been a steady increase in the use of rituximab in the treatment of patients with FL prior to progressing to HDT/ASCR. This is corroborated in our study, which clearly shows a steady increase in the use of rituximab since 1997. In contrast with DLBCL, the data on the effect of prior rituximab treatment on the outcome of patients diagnosed with FL undergoing an HDT/ASCR is still limited. In the CORAL study patients who had received prior rituximab treatment had a worse EFS following salvage chemotherapy and HDT/ASCR than patients that had not received prior rituximab treatment, suggesting that patients relapsing after rituximab treatment may have developed a more

resistant disease that is less likely to respond to salvage with HDT/ASCR. In the study by Ladetto *et al* comparing the outcome of patients treated with HDT and rituximab (R-HDT) followed by ASCR and that of patients treated with R-CHOP, there was no difference in the OS between both arms. In this study, patients that had relapsed after treatment with R-CHOP had a good salvage rate with R-HDT and a very good EFS of 68% at 3 years, suggesting that patients who relapse after a rituximab-containing regimen are still salvageable with HDT, contrasting with the situation in patients with DLBCL (112). Another study by Sebban *et al* reported that patients that had rituximab as part of the salvage treatment prior to HDT/ASCR had a better outcome in terms of 5-year EFS and survival after relapse (SAR) (67% and 93%, respectively) in comparison with a 5-year EFS and SAR of 46% and 63% for patients who did not receive rituximab as part of the salvage treatment (93). Our study is consistent with the positive outcome of patients treated with rituximab prior to HDT/ASCR in FL. In the current study patients that had received rituximab prior to HDT/ASCR had a significantly better outcome in terms of EFS and OS (at 5 years: 56% and 80%, respectively) than patients that were rituximab naïve at the time of HDT (5-year EFS and OS: 52% and 74%, respectively). This result is reassuring in FL patients, as it confirms that HDT/ASCRT remains an excellent salvage option in patients previously treated with immuno-chemotherapy.

Another important question in the treatment of patients with FL is the type of conditioning regimen to be utilised in HDT/ASCR. TBI-based conditioning regimens were reasonably incorporated into HDT based on the known sensitivity of FL to radiation (113). The type of conditioning regimen had a significant impact on the IR in our study. Patients receiving a TBI regimen were less likely to relapse and had a better EFS on multivariate analysis in comparison to patients treated with the BEAM conditioning regimen. The reduced IR in our study is consistent with the results of a previous EBMT study in the pre-rituximab era that also concluded that patients receiving TBI condition were less likely to relapse than those

who received a non-TBI-based conditioning regimen (89). A previous study also suggested an improved failure-free survival following HDT/ASCT patients receiving a TBI-based conditioning regimen (96). However, the increased risk of development of sMDS/AML in patients treated with TBI conditioning was felt to outweigh the benefit of an improved IR in both of the above studies. In the study by Montoto *et al*, the increased NRM in the TBI treated group, contributed to a reduced OS in patients treated with TBI in comparison with those who did not receive TBI. In contrast, in our study both groups of patients had a similar NRM (7% at 5 years), which led to a lack of differences in the OS of both groups of patients on multivariate analysis.

A well known side-effect of HDT/ASCR is the development of sMDS/AML or other malignancies in the long-term [a risk between 5 and 20% has been reported for sMDS/AML and around 10% at 10 years for other second malignancies (100)]. Several studies have shown an association of TBI with an increase in the incidence of sMDS/AML after HDT/ASCR (82, 89, 96, 100) in comparison to chemotherapy-based regimens. Given the evidence of an increased risk of sMDS/AML after HDT/ASCR, there has been a considerable decrease in the use of TBI as part of the conditioning regimen. This is reflected in our study which shows a steady decline in the use of TBI conditioning in Europe since 1997. Several factors other than TBI have also been implicated in the risk of secondary malignancies, such as age, inclusion of etoposide in the conditioning regimen, cytogenetic abnormalities prior to transplant, previous radiotherapy exposure, prior alkylating therapy, number of prior lines of treatment and increased interval from diagnosis to HDT/ASCR (97, 99, 101, 114). One of the flaws of the present study, inherent to its retrospective/registry-based nature, is the difficulty to ascertain accurately the number of secondary malignancies that may have occurred in patients still alive in both groups. This additional information will require further follow-up of the participating centres. A recent Cochrane review including prospective studies comparing

HDT/ASCR (all had utilised TBI as part of the conditioning) and chemotherapy and immuno-chemotherapy regimens in untreated FL patients found no significant difference in the occurrence of secondary malignancies between both arms. However, as the follow-up in all the studies included in the Cochrane review was shorter than 10 years this conclusion needs to be taken with caution (115).

The OS of patients in this study (75% at 5 years) is encouraging, as it compares well with the previous EBMT study (albeit with a very different population) which reported an OS of 64% at 5 years. This suggests a continued improvement in the survival of patients undergoing HDT/ASCR, which might partially be due to the inclusion in the current study of patients previously treated with rituximab, which seems to have a positive effect on outcome as reported by the GELA group. In addition, the improved supportive care given to patients has most likely impacted on the outcome of patients receiving HDT/ASCR in the rituximab era.

5.5 Conclusions

The outcome of FL patients after HDT/ASCR appears to be improving in the current rituximab era. This is possibly due to the introduction of more effective novel treatments and better supportive care offered to these patients. Patients treated with TBI conditioning are less likely to relapse and may have a better outcome than patients treated with chemotherapy-based conditioning regimens. However, these results will need to be confirmed on a longer follow-up, before final conclusions can be drawn.

CHAPTER SIX

The clinical course of patients diagnosed with follicular lymphoma in the rituximab era

6.1 Introduction

Several studies published in the last decade have reported for the first time an improvement in the OS of patients diagnosed with FL in more recent years. This improvement has been attributed to several factors including treatment with rituximab, HDT/ASCR and improved supportive care of patients (12, 14). However, despite these significant improvements, FL remains an incurable disease. Given the lack of a curative treatment there is no consensus on the best treatment to be utilised at diagnosis or relapse. However, there is generalised agreement that the treatment offered should contain rituximab, due to the better outcomes achieved with rituximab-containing regimens (69-70, 75), which has led to the term 'rituximab era' to be coined.

Johnson *et al* described in 1995 the pattern of relapse in patients with FL in the pre-rituximab era (10). Since then, a decrease in the rate of response, in addition to a gradual decrease in the length of response duration, an increase in the relapse rates and a shortened survival with each subsequent relapse has been considered the characteristic clinical course in patients with FL. However, the treatment of FL has undergone major changes since this pivotal study was published with the introduction of MoAb treatment. In addition, HDT/ASCR has a more widespread use as salvage treatment and the advent of RIC regimens has broadened the population of patients eligible for an allogeneic transplant. A further study examining the pattern of survival of FL patients in the current rituximab era has not been reported.

Against this background we hypothesised that these therapeutic options have resulted in a change in the clinical course of patients with FL, so that the response rates, response durations and relapse rates do not decrease with each subsequent relapse. To test this hypothesis we analysed the duration of remission, response rates, and relapse rates in addition to the OS and PFS of patients diagnosed with FL in the rituximab era.

6.2 Patients and methods

6.2.1 Patients' characteristics

For the purpose of this study, we arbitrarily defined the rituximab era as starting in 1997, as this is when an anti-CD20 MoAb was first used in our institution to treat patients with FL (in the setting of a clinical trial). Hence, between 1997 and 2007, 177 patients (median age: 56 years, range 25-89) were diagnosed with grade 1-3a FL and constitute the study population. Ninety-six patients received rituximab at some point as part of their treatment (77 patients received rituximab as part of one treatment line, 16 patients as part of 2 separate treatments and 3 patients as part of 3 different treatments), while 81 patients have never received rituximab. Patients' characteristics at diagnosis are shown in table 25. Amongst patients who received rituximab 34/96 (35%) had histological transformation during the course of their disease (and 29 patients had received rituximab treatment at the event of transformation), contrasting with 15 of 81 rituximab-naïve patients (18%) ($p=0.016$). In addition, a higher proportion of patients who received rituximab during the course of the disease had been diagnosed with advanced stage (stage III-IV: 78% in contrast with 56% in rituximab naïve patients, $p<0.001$).

Table 25: Patients' characteristics

	<i>Rituximab naïve (n=81)</i>	<i>Treated with rituximab (n=96)</i>	<i>p-value</i>
Age, median (range)	57 years (28–89)	55 years (25-87)	0.85
Gender (female)	34 (42%)	52 (54%)	0.16
Stage			<0.001
I	23 (29%)	4 (4%)	
II	12 (15%)	17 (18%)	
III	13 (16%)	20 (21%)	
IV	32 (40%)	55 (57%)	
unknown	1	0	
Grade			0.86
1	38 (48%)	41 (45%)	
2	26 (33%)	29 (31%)	
3a	15 (19%)	22 (24%)	
Unknown	2	4	
FLIPI			0.54
1	29 (50%)	27 (41%)	
2	15 (26%)	18 (27%)	
3	14 (24%)	21 (32%)	
unknown	23	30	
Initial management			0.54
Expectant	40 (49%)	43 (45%)	
Treated	41 (51%)	53 (55%)	

6.2.2 Definitions of response

Response was categorised according to the standard international criteria published in 1999 (116). Response rate (RR) included CR/CRu and PR. Best response was defined as the first optimum response (CR, CRu or PR) a patient had achieved irrespective of the number of treatments required to achieve that response.

6.2.3 Definitions of study end-points

OS was measured from the date of diagnosis to the date of last follow-up or death. Duration of best response was measured from the best response achieved to relapse (in patients achieving CR/CRu) or progression (in patients achieving PR).

6.2.4 Statistical analysis

Survival analysis and duration of remission was performed by the Kaplan-Meier method and Cox-regression test was used to test for significant associations where appropriate. For continuous data t-test was utilised and for categorical data the chi-square test was used. All statistical analysis was carried out on STATA.

6.3 Results

6.3.1 Patients' follow-up and management

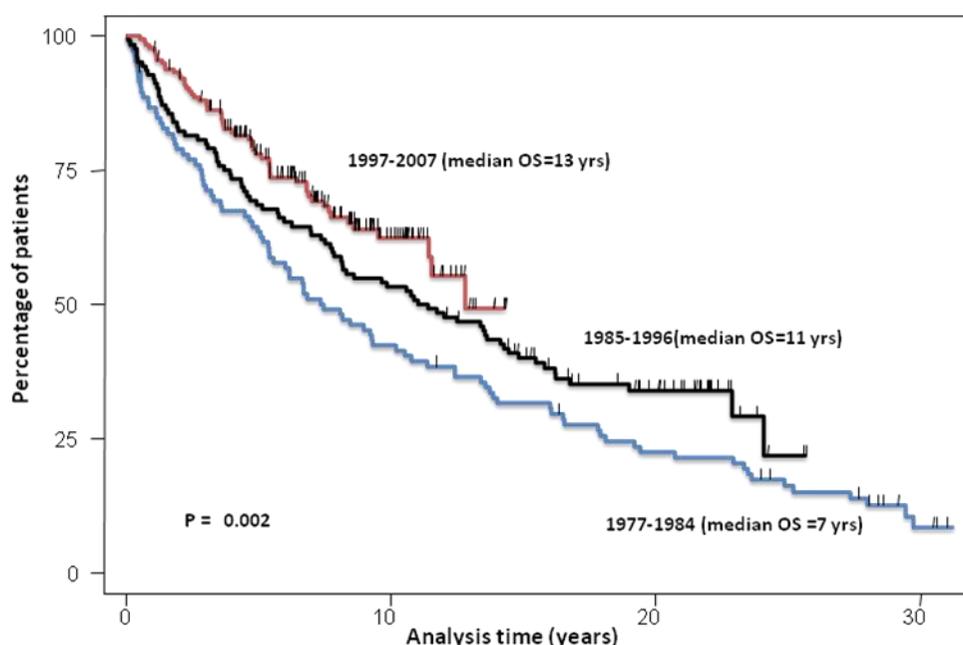
The median follow-up was 7.3 years (range: 1.2-14). There were no significant differences in the median follow-up of patients treated with rituximab (7.2 years) and that of rituximab-naïve patients (7.8 years). One hundred and fifty patients received treatment at some point during the follow-up, while 27 patients never received any treatment, and were managed expectantly (median follow-up: 6.5 years, range: 1.2-13.1). In the rituximab-treated group 42 patients received rituximab as part of the first treatment (R-CHOP: 24 patients; R-chlorambucil: 13 patients and single agent rituximab, 5 patients), while 43 patients received rituximab as part of the second line treatment and 33 at third or subsequent treatments. In the rituximab-naïve group, 16 patients received CHOP, 13 patients received localised RT, 20 patients received monotherapy with chlorambucil or cyclophosphamide, 4 patients received a fludarabine-based regimen, and one patient was treated for lymphoblastic transformation with the UKALL12 protocol. Six patients had HDT/ASCR in the first event, 15 in the 2nd event

and only 1 patient had HDT/ASCR in the 3rd event. Two patients had an allograft (in the 2nd and 3rd event).

6.3.2 Overall survival and progression-free survival

The OS of patients with FL diagnosed and treated at St Bartholomew's Hospital has significantly improved between 1977 and 2007: the median OS for patients diagnosed in the rituximab era (1997-2007) is 13 years, compared to 11 years for 124 patients diagnosed between 1985 and 1996 and 7 years for patients diagnosed from 1977 to 1984 (n=105) (figure 27).

Figure 27: OS of patients diagnosed with FL from 1977 to 2007 at St Bartholomew's Hospital



The 5-year and 10-year OS for the 177 patients diagnosed in the rituximab era was 78% (95%CI: 71 – 83%) and 61% (95%CI: 52-69%) respectively, while the PFS for the whole group was 38% (95%CI: 31- 46%) and 23% (95%CI: 16-32%) at 5 and 10 years, respectively (figure 28). The survival of the 27 patients who never needed any treatment was significantly superior to that of the remainder (p=0.03). Amongst the 150 patients that received

treatment for FL during follow-up, there was a trend for a better OS for patients in the rituximab-treated group than for rituximab-naïve patients: 5-year and 10-year OS for the rituximab-treated group were 80% and 62% respectively, while in the rituximab-naïve group the 5-year and 10-year OS were 60% and 47%, respectively ($p=0.06$) (figure 29).

Figure 28: OS and PFS of the study population

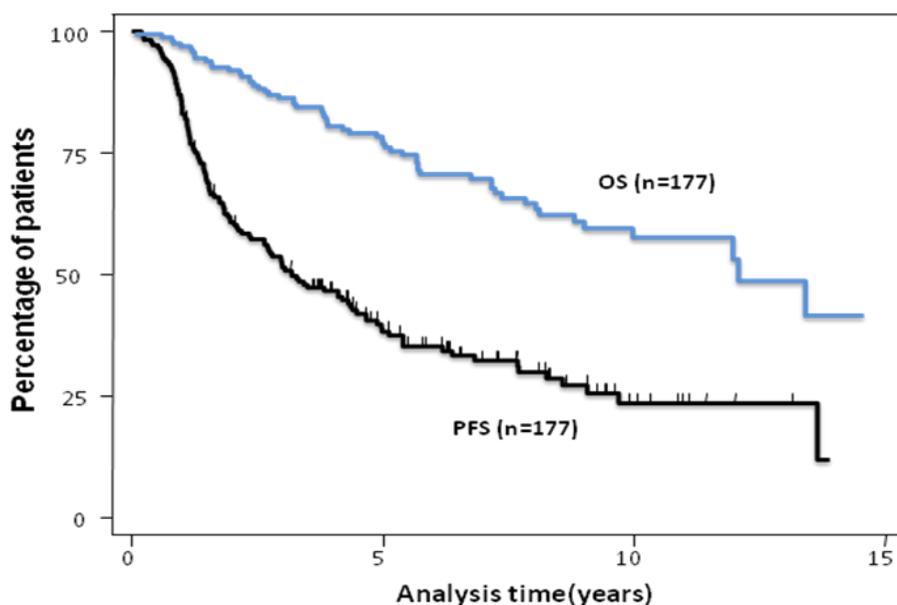
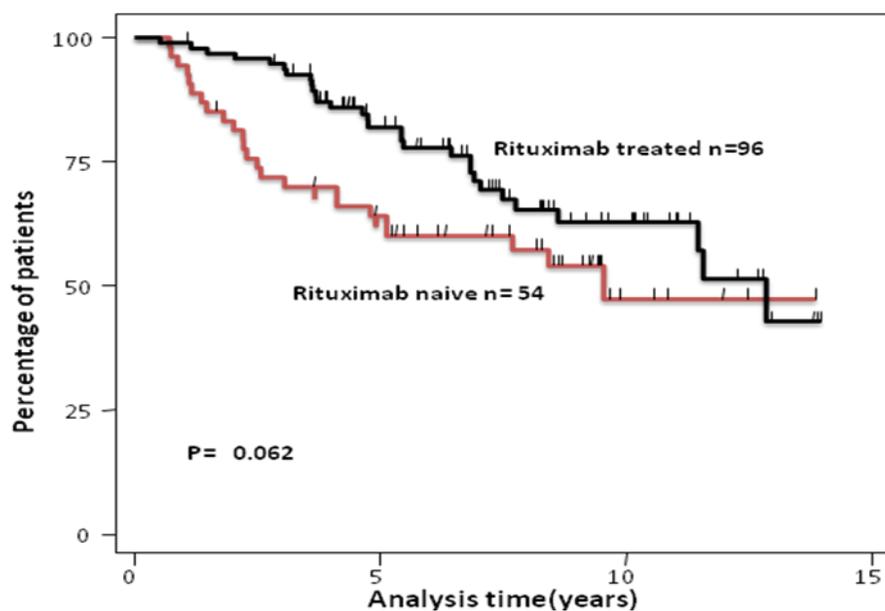


Figure 29: OS of treated patients according to whether they received rituximab or not



6.3.3 Response rates and duration of remissions

The RR decreased by only 10% with each relapse, whereas the duration of the remissions did not decrease with subsequent treatments. The median duration of first response (from best response) was 2.8 years whilst the median second response duration was 3.5 years and the median duration of the 3rd remission has not been reached (figure 30, table 26). A similar pattern was observed when analysing only the rituximab-treated group (n=96). In this subgroup the median duration of the first remission was 2.4 years, while the median duration of second response was 4.5 years, and the median duration of the third remission was not reached.

Figure 30: Duration of remissions from best responses for the study group

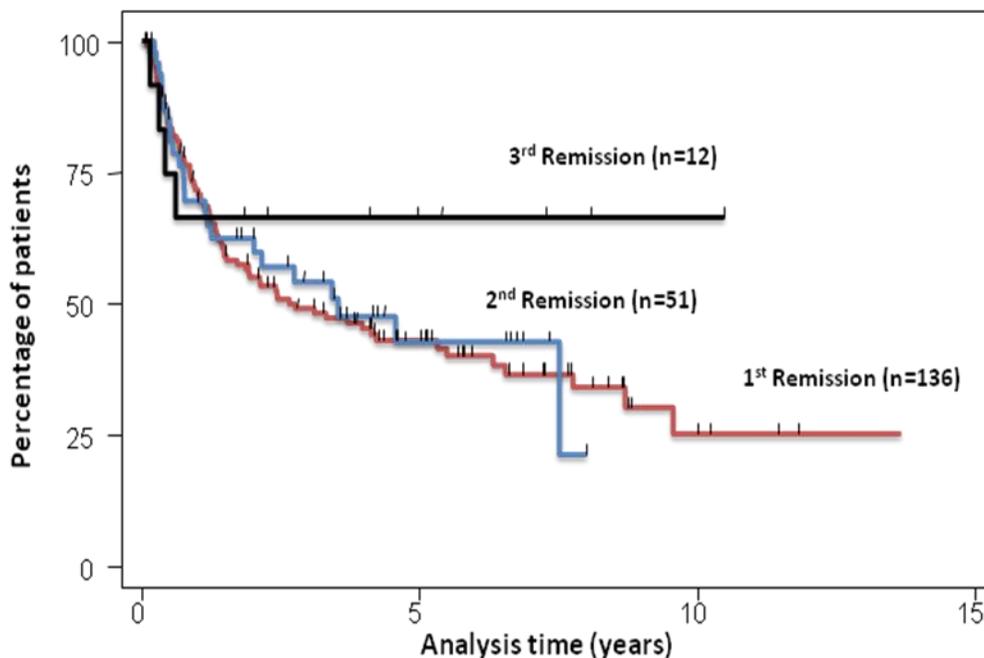
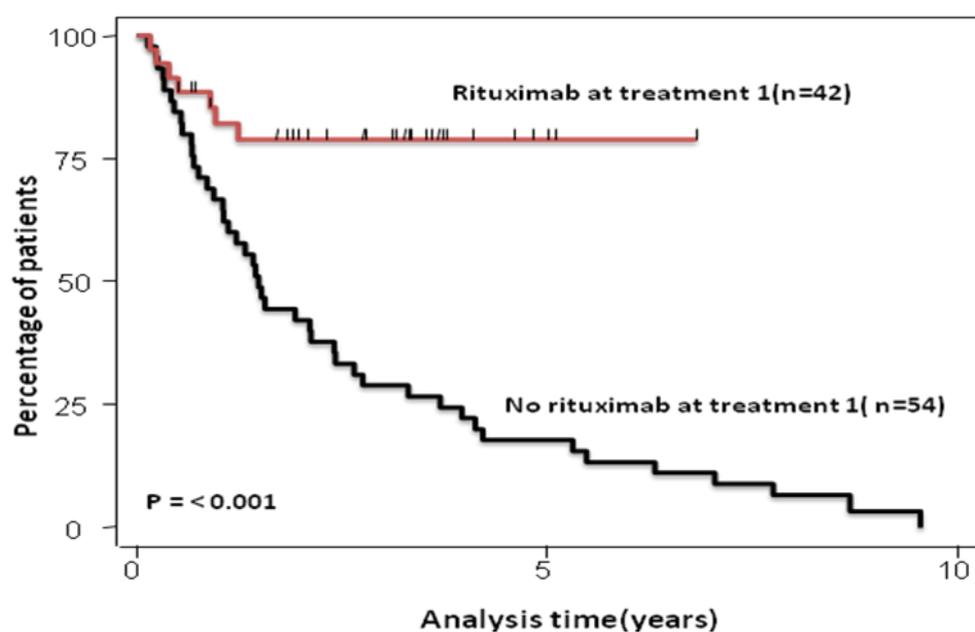


Table 26: Response rate, duration of remission, relapse rate and survival for each event

	<i>Number of patients treated</i>	<i>Patients treated with rituximab</i>	<i>Response rate</i>	<i>Median duration of best response</i>	<i>Relapse rate</i>	<i>Median survival from best response</i>
At presentation	150	52 (34%)	91% (136/150)	2.8 years	57% (78/136)	12 years
1st relapse	61	46 (75%)	83% (51/61)	3.5 years	47% (24/51)	10 years
2nd relapse	16	11 (68%)	75% (12/16)	Not reached	33% (4/12)	Not reached

6.3.4 Timing of treatment with rituximab

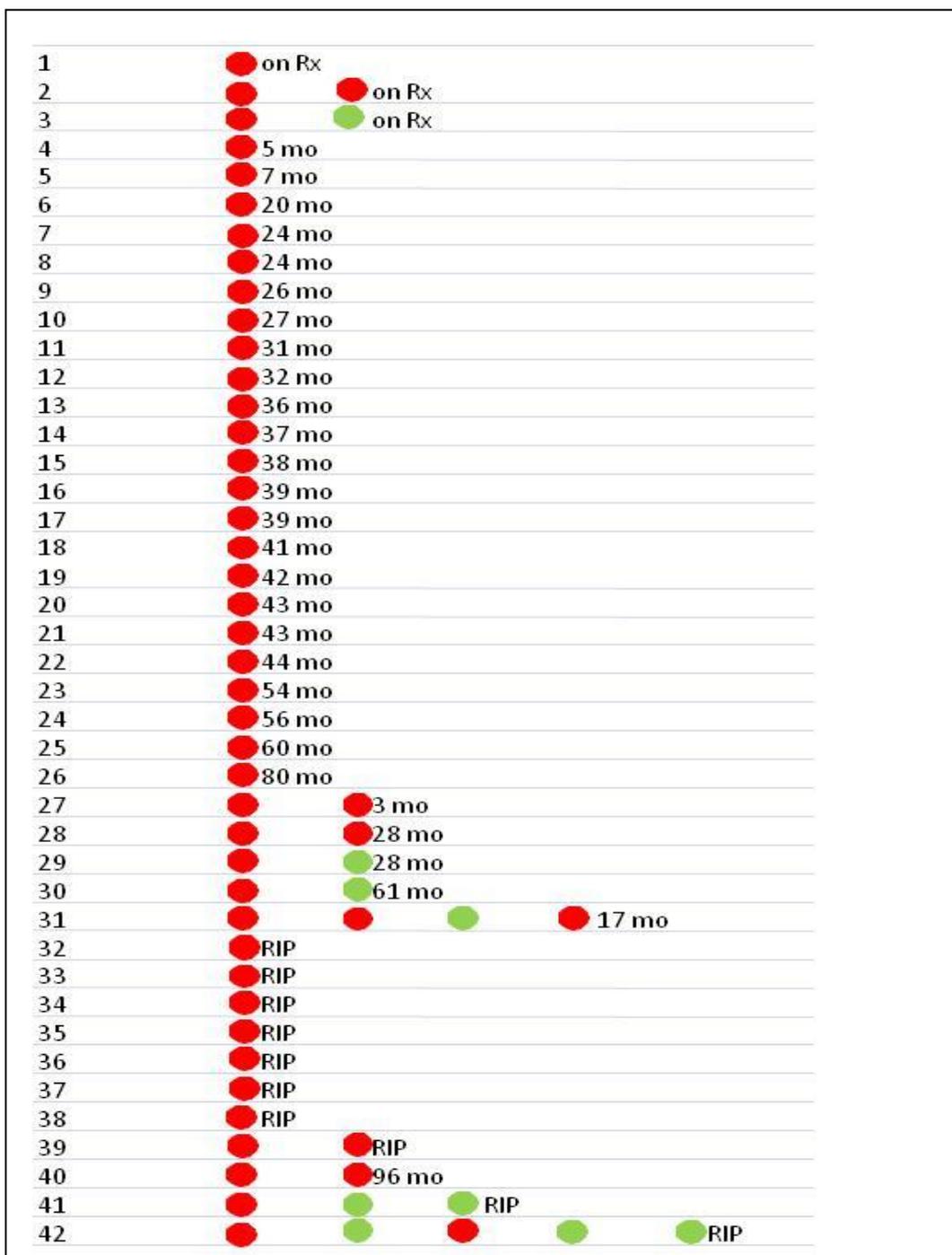
The outcome of patients who received rituximab was also analysed. Amongst them, 42 received rituximab as part of the first-line therapy, while 54 patients had received rituximab treatment as part of subsequent treatments. The median follow-up for the 42 patients who had rituximab as part of their initial treatment was 5 years, while it was 9 years for the remaining 54 patients. The median duration of response from first treatment for patients who had rituximab as part of their initial therapy was not reached whereas it was 1.5 years for patients that received rituximab not as part of the first treatment but subsequently ($p < 0.001$) (figure 31).

Figure 31: Duration of first remission according to the timing of treatment with rituximab

The duration of responses from the last treatment administered in the 42 patients that received rituximab as part of the first treatment is shown in figure 32, whereas the duration of responses for patients who had rituximab at a subsequent event is shown in figure 33.

There were no significant differences in OS between patients that received rituximab as part of the first treatment and those who receive it as part of the second or subsequent treatments (figure 34).

Figure 32: Duration of remissions from last treatment in patients that received MoAb as part of their 1st treatment



● MoAb treatment
● Treatment not containing MoAb
 RIP: Dead
 Rx: on current treatment

Figure 33: Duration of remissions from last treatment in patients that had not received MoAb as part of the 1st treatment but at subsequent treatments

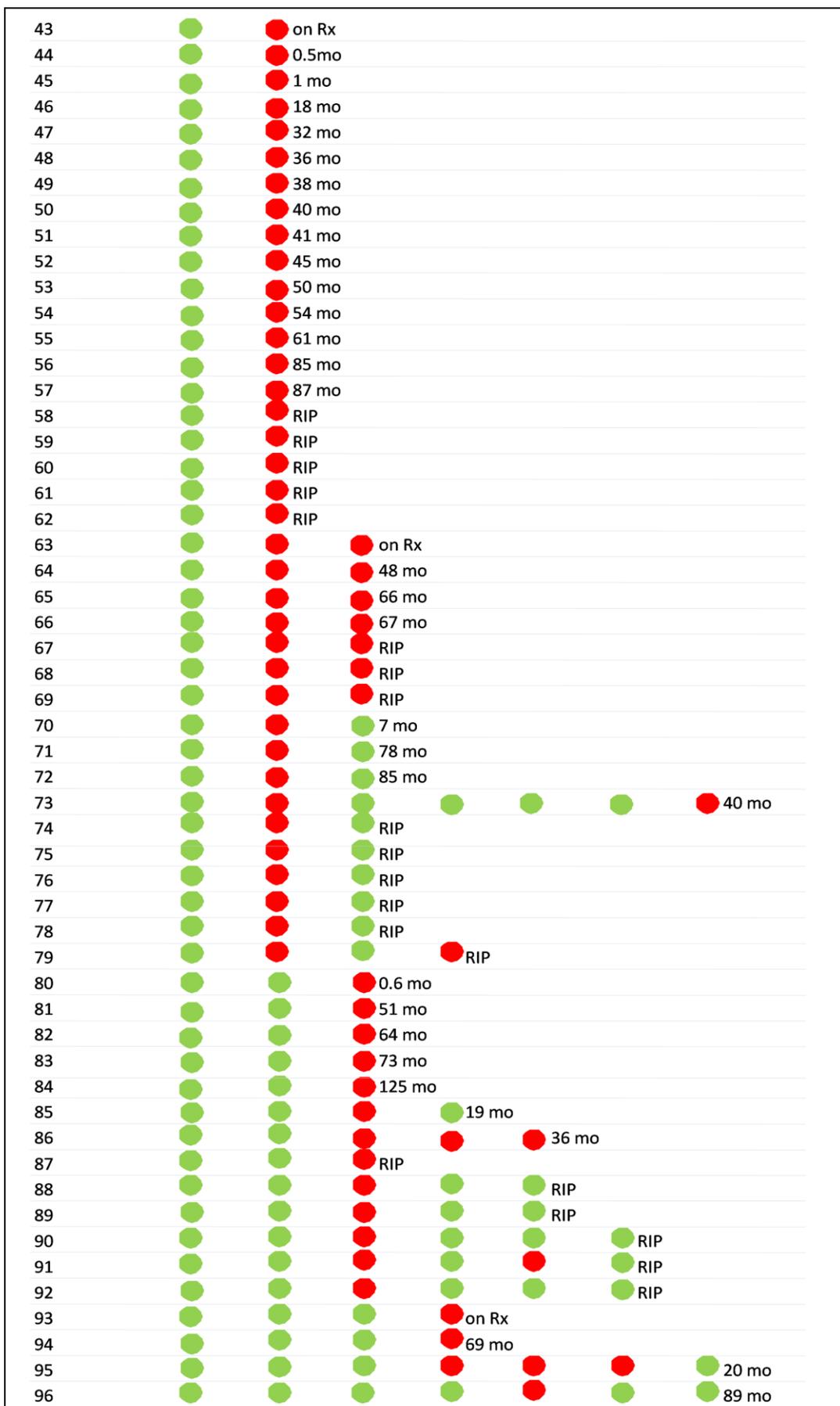
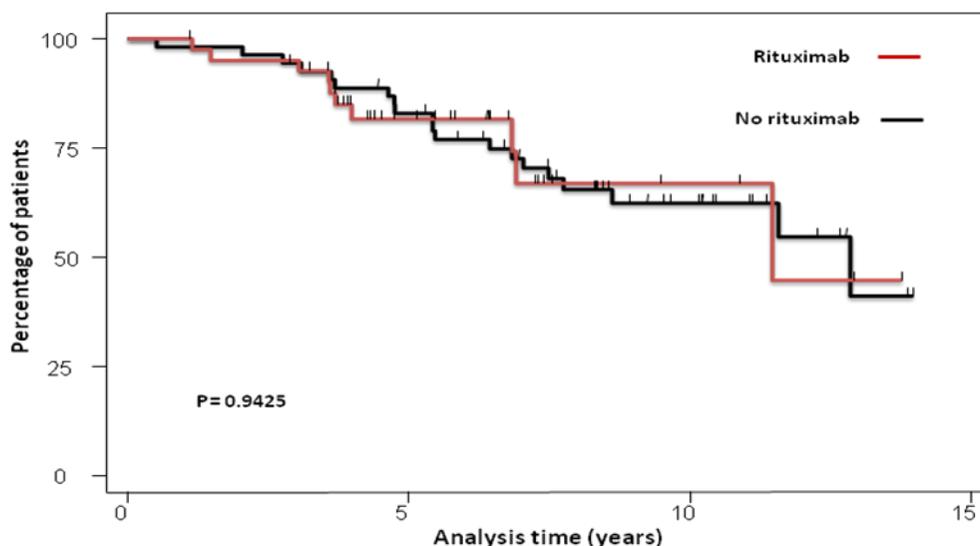


Figure 34: OS according to the timing of treatment with rituximab

6.4 Discussion

Horning *et al* published a seminal paper in 1993 demonstrating that between 1970 and 1993 there had not been a significant improvement in the OS of patients diagnosed with FL according to the year of diagnosis at Stanford University, despite the better response rates achieved by combination chemotherapy (11). The first study to demonstrate an improved OS in FL patients was reported by Swenson *et al* in 2005, showing that the outcome of patients diagnosed in more recent years (1990-1999) was significantly better with an improved OS in comparison with that of patients diagnosed in previous years. Furthermore, two subsequent studies from M.D. Anderson Cancer Center and SWOG confirmed these results, and partly attributed the improved OS to the use of MoAb in the treatment of patients diagnosed in the late 90's, in contrast to only-chemotherapy treatment utilised in the previous decades (13-14). In line with the results reported by the above studies, the current one demonstrates that there has also been a significant improvement in the survival of patients with FL diagnosed at Barts in the 1990s, with a median OS improving from 7 to 13 years. In our study a progressive decline was seen in the response rates of patients with each subsequent treatment. This is

similar to the results previously reported by Johnson *et al* analysing the clinical course of patients diagnosed with FL in our centre in the pre-rituximab era (10). In contrast, for those patients that did achieve a response, a progressive shortening of the duration of subsequent responses was not detected in the current study. On the contrary, the second response duration was longer than the first, and the median was not reached in patients achieving a third remission. This improved response duration is reflected in the relapse rate which also declined with each successive response. On sub-analysis of the rituximab-treated group (n=96) a similar pattern of improving response duration and relapse rate was also seen with each subsequent event of treatment.

This improvement in the response duration can be attributed to both the introduction of rituximab and of HDT/ASCR. HDT/ASCR was introduced to practice in 1987 at St.

Bartholomew's hospital as consolidation of the response following a salvage regimen at first relapse and in patients that had required more than one line of treatment to achieve a first response. Monoclonal antibodies against CD20 were first used to treat patients diagnosed with FL at Barts in 1997 as part of clinical trials. Subsequently rituximab treatment became more accessible in 2005 when NICE approved rituximab for relapsed/refractory patients and in 2006 when it was approved as first-line treatment. As a result, in the present study a larger proportion of patients received rituximab treatment in the second event compared to the first event: 75% *versus* 34%. In addition, a larger number of patients underwent HDT/ASCR in second event than at first event. It is thus likely that the use at relapse of these very effective treatments has contributed to the prolonged response durations achieved in the second event. When analysing only the rituximab-treated group of patients, the response duration of patients that had received rituximab as part of their initial therapy was significantly longer than that of patients that received rituximab as part of subsequent treatments. However, the OS of both groups was similar, indicating that receiving rituximab as part of subsequent

treatments might have a similar effect in terms of survival than receiving it as part of the first treatment. This is consistent with the results reported by the prospective randomised trial EORTC 20981, which investigated the effect of maintenance with rituximab on PFS and OS in patients treated with second line R-CHOP or CHOP. After a median follow-up of six years there were no significant differences in the OS of patients that had received maintenance compared to those patients that did not. This was attributed by the authors to the high successful salvage rate with rituximab-chemotherapy at relapse in patients who had not received maintenance (117). Unfortunately, in our study the number of patients that received rituximab both as part of the first and subsequent treatment is very small. In addition, the follow-up of patients included in the PRIMA trial is still too short to have any indication on the effect of further treatments on response duration in patients relapsing after immuno-chemotherapy and maintenance rituximab (76). As immuno-chemotherapy followed by maintenance rituximab has become the current standard for first-line therapy, the proportion of rituximab-naïve patients will progressively decline and consequently, subsequent remission durations may eventually become similar in duration to the first one, rather than being longer. Nonetheless, for the time being there is still a significant group of patients who have not received rituximab as part of the initial therapy or did not receive maintenance with rituximab after first remission, especially in the United Kingdom where maintenance with rituximab following first-line treatment was not approved until 2011.

6.5 Conclusions

The clinical course of patients with FL has changed considerably in the rituximab era. The typical pattern of sequentially declining response durations and an increased likelihood of relapse following each treatment is no longer the course that one should expect in patients with FL. This change in response duration certainly needs to be taken into account when

planning and interpreting the results of future clinical trials in FL, in addition to when planning the clinical care of patients with FL.

CHAPTER SEVEN

Discussion

FL is a heterogeneous incurable malignancy. The presentation and behaviour of the disease varies amongst patients: a proportion of them will never require treatment, and remain asymptomatic, while some patients' disease will progress rapidly and may transform to a more aggressive form of lymphoma. As a result, the management options are also diverse ranging from expectant management to an allogeneic transplant, with the same patient being a candidate for these opposed modalities of treatment at some point. A typical patient will receive several courses of treatment during the course of their disease. The lack of improvement in the OS of patients diagnosed with FL over decades, despite the improved response rates achieved by more intensive chemotherapy regimens (11) became one of the paradigms of this disease. However, recently an improvement in the OS of patients diagnosed with FL in more recent years has been demonstrated, as a result of better supportive care and the introduction of newer treatment options (12-14). In addition to newer treatment options, better staging and prognostication tools are essential to contribute to improve the outcome of any malignancy. In this regard, a new imaging modality, FDG-PET/CT, has proven of great value in the staging and assessment of response to treatment in patients with lymphoma. Furthermore, a new prognostic index designed specifically for FL, the FLIPI2, has been developed in this current era. This thesis has examined how the introduction of such advances has impacted on the management and on the clinical course of patients with FL.

Given the prolonged OS patients with FL are expected to achieve in the current era, PFS is deemed a reasonable end-point of studies, as it is considered more achievable and realistic. In line with this, a new clinical prognostic index, the FLIPI2, was designed in the rituximab era with PFS as the primary end-point (48). The efficacy of the FLIPI and FLIPI2 indices in predicting both OS and PFS in our population of FL patients diagnosed at Barts were compared. The FLIPI2 clearly groups together a large segment of the population into the

intermediate-risk group: in our study 66% of patients belonged to the intermediate-risk category. This is a consequence of the reduction in the number of required risk factors in the low-risk category: from 0-1 in the FLIPI to none in the FLIPI2 (48). As around 80% of FL patients present with advanced stage, and more than half have BM involvement, over 50% of the patients are included in the intermediate or high-risk category according to the FLIPI2. This unequal distribution is clear in the original description of the FLIPI2 by Federico *et al* where 57% of the patients were assigned an intermediate-risk score (48). As a result of the unbalanced distribution, a smaller number of patients are allocated to the high and low-risk categories. This defeats the purpose of a prognostic score in identifying distinct risk groups of patients that might warrant different management, and contrasts with the balanced distribution according to the FLIPI, both in the original description (low, intermediate and high-risk: 36%, 37% and 27%, respectively) and in our series (42%, 29%, 28%). In our study, the FLIPI2 could identify a high-risk population but failed to differentiate the low-risk and the intermediate-risk groups according to OS, which might be related to the fact that the FLIPI2 was not designed with OS as the main end-point. In addition, both prognostic scores failed to differentiate the low-risk and the intermediate-risk groups according to PFS, possibly due to the different treatment regimens these patients had received. Indeed, PFS is a reflection not only of the 'aggressiveness' of the underlying disease, but also of the efficacy of the treatment administered. Patients included in the study by Federico *et al* had a heterogeneous treatment, with 60% of the patients receiving rituximab treatment. It might not be possible to reproduce the results of the FLIPI2 with PFS as the end-point in a different group of patients treated with different chemotherapy regimens. In contrast, OS as an end-point is a reflection of all the treatment lines a patient has had and is less subject to bias than PFS (109, 118). As the definition and the documentation of the time of progression is liable to

bias, and may differ between studies if PFS is to be used as an end-point, patients to be compared should have received a similar treatment.

Prognostic factors should be useful to predict the outcome of patients, in addition to possibly guide treatment (119), especially in a disease as FL where the choices at diagnosis include expectant management. However, in FL the FLIPI is not used to guide treatment, but it has been utilised to define study populations in clinical trials (120). In our study we demonstrated that the proportion of patients in each risk group according to either the FLIPI or FLIPI2 were similar in patients that have been treated at diagnosis and patients that have been managed expectantly. In addition, there was no difference in the OS of high-risk FLIPI patients managed expectantly in comparison with that of high-risk FLIPI patients treated at diagnosis. As in other institutions in the United Kingdom, the FLIPI score is not used at Barts to decide initiation of treatment of patients with FL. These results enforce this view. However, if the prognostic indices are not useful for guiding treatment, what other tools are available to make such decisions? Traditionally two sets of treatment criteria have been used to identify patients at diagnosis in whom expectant management is not advisable: the GELF and the BNLI criteria. These were selected from the experience of patients being treated on clinical trials (9, 64), but have never been validated in a prospective manner and they differ slightly between different clinical trials. One hundred and five patients were managed expectantly at diagnosis in our study. Surprisingly, 24 patients had at least one GELF or BNLI criteria for treatment, and were still managed expectantly. Although in our study the patients that had at least one criterion were treated earlier than patients that had none, the OS did not differ between both groups. It is undeniable, that, given, the retrospective nature of this study, there are significant missing data. Nevertheless, this study demonstrates that although the presence of symptomatic disease would lead to earlier treatment, it does not necessarily affect the OS of patients in the long term. Therefore, although both the FLIPI and the FLIPI2

can segregate a high-risk population in terms of survival and time-to-treatment, a high-risk FLIPI should not be utilised as an indication to start treatment. On the other hand, although the presence of treatment criteria appears to influence the time-to-treatment, the presence of symptoms does not appear to affect the survival of patients diagnosed with FL, emphasising the differences between reasons to initiate therapy and predictors of outcome. The introduction of CT in the early 1980 era caused a revolution in the staging of lymphoma and led to several investigations such as lymphangiography and laparotomy becoming obsolete. In addition, it also led to the first modification of the Ann Arbor classification for staging of lymphoma in 1989 (18). PET technology has been available since the 1950's, however it was the successful introduction of FDG as a PET tracer and subsequently the development of PET/CT technology which has put this technique in the forefront of research in lymphoma (25). Its unique ability to combine both anatomical and metabolic data has proven advantageous in detecting disease not apparent on CT imaging in lymphoma (27). In our institution the effective dose of radiation resulting from the low-dose CT component of FDG-PET/CT is 4.5 mSv, and for the FDG-PET is 8 mSv (121), with a total of 12.5 mSv compared to an effective dose of radiation of 15 mSv for an staging CeCT. FDG-PET has been reported to identify up to a 51% increase in nodal disease and 89% increase in extra-nodal disease compared to standard imaging in FL patients (47). However, an increase in the identification of disease does not always lead to a change in the stage. A number of studies have shown that 11-40% of patients diagnosed with FL staged by FDG-PET/CT had a more advanced stage in comparison to standard staging methods (35, 44, 46). The wide range is likely to be due to different study populations, with FDG-PET results less likely to impact on the management in those populations with more advanced disease. Nevertheless, a change in stage does not always translate into a change in the management in FL, either, as other factors, such as the location of the disease and the presence of symptoms will also affect the

management decision. In our study, FDG-PET/CT identified more LN regions than CeCT as well as identifying two patients with bone involvement not apparent on CeCT. This resulted in a change of stage from limited to advanced stage and a potential change in the management in these patients. The effect of detecting additional involved LN regions by FDG-PET/CT on the FLIPI score has not been previously described. In our study the detection of additional LN regions led to a change in the FLIPI score in 20% of patients and, as a result, more than 50% of patients were included in the high-risk category. Whether the patients identified by FDG-PET/CT staging as having a high-risk FLIPI have the same poor prognosis as patients recognised as high-risk by CeCT is unknown. To answer this question would require larger number of patients with a longer follow-up. However, it is important to acknowledge the effect that staging by FDG-PET/CT has on the FLIPI score, especially when it is used to compare different study populations. A meta-analysis published in 2004 examined the role of FDG-PET/CT in the assessment of BM involvement in both HL and NHL and showed that FDG-PET/CT had a high specificity for the detection of a negative BM but the sensitivity, especially for NHL, was disappointing (43%). As a result the authors concluded that FDG-PET/CT could complement the BMB, but cannot replace it in the management of lymphoma (122). Most clinical studies agree that there is a significant rate of false negative FDG-PET/CT in patients with a positive BMB in 'low-grade' NHL and a few studies including FL patients reported a low sensitivity of 39% (34). The inadequacy of visual assessment of BM involvement is reflected in our experience, as in our patients the visual analysis of BM involvement had a high specificity but a sensitivity of only 31%. This is most likely related to the focal involvement of the BM in FL, making it difficult to be visualized on FDG-PET/CT, especially in patients with < 10% infiltration on the BMB, in contrast to the diffuse infiltration in aggressive lymphoma (33). We investigated the additional value of semi-quantitative measurements in the detection of BM involvement. Although absolute SUV measurements (SUVmax and SUVav) increase the

sensitivity of FDG-PET/CT for the detection of BM involvement, the best accuracy was reached by the use of ratios. Ratios minimise the error associated with absolute SUV measurements, as this decreases the bias encountered in SUV measurement by variations in blood sugar, uptake period and FDG injected activity that may affect an absolute measurement. Utilising $SUV_{av}/MBP \geq 1$ increased the sensitivity of FDG-PET/CT for detection of BM involvement to 88%, while maintaining a high specificity of 80%. Increasing the sensitivity of FDG-PET/CT in the detection of BM involvement may spare patients less likely to have BM involvement having a BMB. Nevertheless, it is still essential to perform a BMB in patients likely to have BM involvement to ascertain the histology of the disease, in particular to rule out the presence of 'high-grade' lymphoma. Our results demonstrate that staging by FDG-PET/CT leads to a change in the stage and in the management of a proportion of patients with FL. The use of SUV to increase the accuracy of FDG-PET/CT is feasible and the radiation exposure is not increased by FDG-PET/CT. Therefore, FDG-PET/CT should be the next logical step in the progress of imaging in FL. In line with this, if the results reported by Trotman *et al* on the strong predictive value of the response assessed by FDG-PET/CT in patients receiving first-line immuno-chemotherapy for FL are confirmed in further studies, this will make a baseline FDG-PET/CT mandatory (123).

A recent meta-analysis and a Cochrane review of HDT/ASCR treatment in FL have concluded that there is no evidence to support a role for HDT/ASCR in first remission in FL. In contrast, an improved PFS and possible OS benefit for patients in second or subsequent remissions undergoing HDT/ASCR has made of this an attractive treatment option in this setting (85, 115). However, some questions, such as the optimal conditioning regimen remain open. In addition, as immuno-chemotherapy has become the standard treatment for FL patients, more patients will have been exposed to MoAb treatment before being offered HDT/ASCR as a treatment option. There is a paucity of studies examining the effect of previous rituximab

treatment on the outcome of patients with FL undergoing HDT/ASCR. Given the concerns on the potential adverse effect of prior rituximab in the outcome after HDT/ASCR –as had been described for patients with DLBCL (112)- the LWP-EBMT designed a retrospective study to analyse the impact of prior rituximab in patients having HDT/ASCR for FL. An additional objective was to study the effect of TBI-containing conditioning regimens in the prognosis of patients with FL. In our study we found a significantly improved EFS and OS in patients that had been previously treated with rituximab in comparison to patients that were rituximab naïve. This result is reassuring in FL patients, as it confirms the effectiveness of HDT/ASCR even in patients previously treated with immuno-chemotherapy. Along the same lines, a retrospective analysis by the GELA demonstrated that patients previously treated with rituximab and undergoing HDT/ASCR had the best outcome in comparison with patients treated only with rituximab or with HDT/ASCR at second line (93). Nevertheless, further studies will need to evaluate the outcome of patients relapsing after having received maintenance rituximab. As patients treated on the PRIMA trial relapse (76), more data will emerge with regards to the effect of long-term MoAb exposure on the response to salvage treatments including HDT/ASCR. In our study in the rituximab era, patients treated with TBI conditioning regimen were less likely to relapse, and had an improved EFS on multivariate analysis, in comparison with those who received BEAM conditioning regimen, although there were no differences in OS. This is in contrast to a previous EBMT study in the pre-rituximab era that attributed the reduced OS in patients treated with TBI compared to chemotherapy regimens to an increased NRM, which was related to a significantly higher risk of sMDS/AML in patients treated with TBI (89). In our study, after a median follow-up of 5 years, patients treated with TBI or BEAM had a similar NRM, leading to a lack of differences in the OS between both groups of patients on multivariate analysis. The analysis of the incidence of secondary malignancies was not an end-point of our study, which would require a different

design, and therefore complete data is not available to be able to draw any meaningful conclusions on this aspect. A further study specifically addressing the risk of secondary malignancies according to different conditioning regimens should be carried out in the rituximab era. A study by Tarella *et al* reported an increased risk of solid tumours in patients who received rituximab as part of HDT/ASCR; despite this, patients treated with rituximab still had a better outcome in terms of OS than patients who did not receive R-HDT/ASCR. Furthermore, in this study patients treated with TBI were not found to have an increased risk of sMDS/AML, although only a very small proportion of patients had received TBI (124). In summary, our study demonstrates that HDT/ASCR remains an excellent treatment option for patients with relapsed FL in the rituximab era. For the time being, there is some suggestion from our study, that TBI results in a better outcome than BEAM. However, the current follow up is not long enough to draw a more decisive conclusion, so this remains an open question.

There is no doubt that MoAb, particularly rituximab, has had a crucial impact on the treatment and outcome of patients with FL. Several prospective trials have demonstrated significant improved response rates, PFS and OS in patients treated with rituximab (69-70, 75, 125-127). This has resulted in the current consensus that treatment for FL should include rituximab. In fact, in recognition of the importance and prevalence of rituximab treatment, many authors now distinguish a pre-rituximab from a rituximab era in the management of patients with FL. On the other hand, better supportive care and better salvage options including HDT/ASCR have contributed to an improved OS of patients diagnosed with FL in comparison to previous eras. This has changed the previous paradigm stating that no improvement in OS in patients with FL could be observed over a prolonged time, in spite of better responses. Our results confirm such paradigm shift, as patients diagnosed at Barts between 1997 and 2007 had a median OS of 13 years in comparison to a median of 11 years

and 7 years for patients diagnosed in 1985-1996 and 1977-1984, respectively. Another accepted paradigm in FL is that all patients will eventually relapse following treatment, with a sequential reduction in the response duration and a shorter survival with each subsequent treatment. This notion is based primarily on a publication by Johnson *et al* in 1995 including patients treated at Barts between 1968 and 1987. Patients treated in that era had a response duration that decreased to 13 months after the second episode of treatment in comparison to 30 months at presentation. The median OS from best response also decreased from 9.6 years at presentation to 4.9 years after the second episode of treatment (10). In our study examining the relapse pattern of FL patients in the rituximab era, we found considerably different results. Although the response rates were reduced with sequential relapses, the response duration following the second episode of treatment did not decrease in comparison to the response duration after the first episode of treatment. The median OS also did not seem to decline in the same proportion it did in Johnson *et al* study: the OS at presentation of FL in the rituximab era was 12 years, whereas it was still significantly long at 10 years after first relapse. The changes in the relapse pattern of patients are due to the improved treatment options at relapse currently available, in contrast to the situation 20 years ago. Patients in our study were more likely to have received MoAb treatment and/or had HDT/ASCR in the second episode of treatment. The successful salvage of relapsing patients by these treatment options has compensated for any possible acquired resistance they may have developed following the first treatment. In this sense, Davis *et al* explored the effect of re-treatment with rituximab in FL patients and reported that the second response duration patients experienced did not differ from the first, and in some cases it was even longer (128). In our study 45 patients received MoAb as part of their first treatment and, after a median follow-up of 5 years, only 11 patients had required re-treatment, of which 7 patients had been re-treated with a MoAb. Further data on the remission duration of patients re-treated

with rituximab is lacking and is a limitation of our study. It should be noted that not many patients included in our study had received maintenance with rituximab. Following the PRIMA trial reporting an improved PFS in patient receiving maintenance rituximab after first-line treatment with immuno-chemotherapy, this has become the standard recommended treatment for advanced stage FL patients requiring treatment (43). As more patients receive rituximab as the initial therapy, the sequential response durations may either become similar to the first one or start to decrease as resistance to treatment develops, rather than being longer than the first response, as in the current study. We found no differences in the OS between patients that had received MoAb as part of their first treatment and patients that had received MoAb in subsequent treatments. This is probably due to the successful salvage of rituximab-naïve patients with MoAb and HDT/ASCR. A similar effect was demonstrated in the EORTC trial that detected no OS advantage between patients that received maintenance rituximab at relapse and those patients who did not receive maintenance but received rituximab as part of the salvage therapy of the subsequent recurrence. Thus, the timing of MoAb treatment does not seem to make a difference in the OS of patients. In conclusion, MoAb has not only had a huge impact on the prognosis of patients with FL but it has also significantly altered the clinical course and the pattern of recurrences in these patients.

To sum up, whereas the new FLIPI2 score needs to demonstrate that it can add a significant advantage in the management of patients with FL, there is growing evidence that FDG-PET/CT imaging contributes to better disease detection at the time of staging, with a subsequent effect on the FLIPI score which should be recognised. Furthermore, the added value of quantitative measurements such as SUV should be further explored as they increase the sensitivity of FDG-PET/CT. With regards to the treatment of patients with FL, the advent of MoAb and the use of HDT/ASCR have changed the landscape to the point that previously

held beliefs in the management of patients with FL are no longer acceptable and should be re-evaluated in the current rituximab era.

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Statement of work undertaken

This work was undertaken while I was a Clinical Research Fellow at the Centre for Haemato-
at the Barts Cancer Institute, Queen Mary University of London.

This work was supervised by Dr Silvia Montoto and Prof John Gribben

Chapter two, four and six: Patients' clinical data was retrieved from the clinical notes and
electronic records by me. Mrs Janet Matthews, Database Manager at the Centre for
Haemato-oncology, verified the data from the database and performed the statistical
analysis, under my guidance.

Chapter three: I identified and retrieved patients' clinical data from the clinical notes and
electronic records. Dr Teresa Szyszko, Nuclear Medicine and Radiology Consultant at St.
Bartholomew's Hospital interpreted the imaging data. Mrs Amy Mc Dowell, Medical Physicist
at St. Bartholomew's Hospital performed the statistical analysis, under my guidance.

Chapter five: I presented a proposal for the study to the 'low-grade' sub-committee of the
EBMT-Lymphoma Working Party, identifying the inclusion criteria and the aims of the study
with the assistance of Dr Montoto and Dr Peter Dreger, Consultant Haematologist and chair
of the EBMT-Lymphoma Working Party. Mrs Arianne Boumendil provided the clinical data of
the patients' from the EBMT database and performed the statistical analysis.

Publications

The work described in this thesis has been presented in the following abstracts and presentations to date.

FDG-PET/CT staging in follicular lymphoma: effect on FLIPI score

British Journal of Haematology, volume 153, supplement 1, April 2011

El-Najjar I, Szyszko TA, Avril N, Gribben J, Montoto S

Follicular lymphoma staging with co-registered 18F-FDG PET and low dose CT – do we need contrast enhanced CT?

Nuclear Medicine Communications, volume 32 , supplement 5, May 2011

Szyszko TA, El-Najjar I, Moore E, Goddard I, Vinnicombe S, Montoto S, Avril N

Neither the FLIPI nor the FLIPI2 accurately segregates low-risk from intermediate-risk follicular lymphoma patients in terms of progression-free survival.

Blood (ASH Annual Meeting Abstracts) 118: Abstract 2663, December 2011

El-Najjar I, Matthews J, Gribben J, Montoto S

The role of total body irradiation (TBI) in the high-dose regimen of patients with follicular lymphoma (FL) treated with autologous stem cell transplant (ASCT) in the rituximab era. A retrospective study of the EBMT Lymphoma Working Party.

Blood (ASH Annual Meeting Abstracts) 118: Abstract 502 (oral presentation) December 2011

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