**Risk of secondary hematological malignancies in patients with follicular lymphoma: an analysis of 1028 patient treated in the rituximab era**

<table>
<thead>
<tr>
<th>Journal:</th>
<th>British Journal of Haematology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID:</td>
<td>BJH-2019-00602.R1</td>
</tr>
<tr>
<td>Manuscript Type:</td>
<td>Ordinary Papers</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>08-May-2019</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Prusila, Roosa; University of Oulu and Oulu University Hospital, Medical Research Center and Cancer and Translational Research Unit Sorigue, Marc; ICO-Hospital Germans Trias i Pujol, Institut de Recerca Josep Carreras, Department of Hematology Jauhiainen, Jyrki; University of Eastern Finland, Department of Applied Physics Mercadal, Santiago; ICO-Hospital Duran i Reynals, Hospital de Llobregat, Department of Hematology Postila, Aleksi; University of Oulu and Oulu University Hospital, Medical Research Center and Cancer and Translational Research Unit Salmi, Petteri; University of Oulu and Oulu University Hospital, Medical Research Center and Cancer and Translational Research Unit Tanhua, Taru; University of Oulu and Oulu University Hospital, Medical Research Center and Cancer and Translational Research Unit Tikkanen, Susanna; University of Oulu and Oulu University Hospital, Medical Research Center and Cancer and Translational Research Unit Kakko, Sakari; Oulu University Hospital, Department of Hematology Kuutunen, Hanne; University of Oulu and Oulu University Hospital, Medical Research Center and Cancer and Translational Research Unit Pollari, Marjukka; Tampere University Hospital, Department of Oncology Nystrand, Ilja; Turku University Hospital, Department of Oncology Kuusisto, Milla; University of Oulu and Oulu University Hospital, Medical Research Center and Cancer and Translational Research Unit; Siunsote, Hospital District of North Carelia Vasala, Kaija; Central Finland Central Hospital, Department of Oncology and Radiotherapy Jantunen, Esa; Kuopio University Hospital, Department of Medicine; Siunsote, Hospital District of North Carelia; University of Eastern Finland, Institute of Clinical Medicine, Faculty of Health Medicine Korkelia, Eija; Turku University Hospital, Department of Oncology Karihtala, Peeter; University of Oulu and Oulu University Hospital, Medical Research Center and Cancer and Translational Research Unit Sancho, Juan; ICO-Hospital Germans Trias i Pujol, Institut de Recerca Josep Carreras, Department of Hematology Turpeenniemi-Hujanen, Taina; University of Oulu and Oulu University Hospital, Medical Research Center and Cancer and Translational Research Unit Kuittinen, Outi; University of Oulu and Oulu University Hospital, Medical Research Center and Cancer and Translational Research Unit; University of Eastern Finland, Institute of Clinical Medicine, Faculty of Health Medicine; Kuopio University Hospital, Department of Oncology</td>
</tr>
<tr>
<td>Key Words:</td>
<td>follicular lymphoma, LATE EFFECTS OF THERAPY, secondary hematological malignancies, secondary leukemia, treatment related</td>
</tr>
</tbody>
</table>
Risk of secondary haematological malignancies in patients with follicular lymphoma: an analysis of 1028 patients treated in the rituximab era

Running title: Secondary malignancies in patients with follicular lymphoma

Ms Roosa E.I. Prusila¹, Dr Marc Sorigue², Mr Jyrki Jauhiainen³, Dr Santiago Mercadal⁴, Mr Aleksi Postila¹, Mr Petteri Salmi¹, Dr Taru Tanhua¹, Dr Susanna Tikkanen¹, Dr Sakari Kakko⁵, Dr Hanne Kuitunen², Dr Marjukka Pollari⁶, Dr Ilja Nystrand⁷, Dr Milla E.L. Kuusisto¹,⁸, Dr Kaija Vasala⁹, Prof Esa Jantunen⁹,¹⁰,¹¹, Dr Eija Korkeila⁷, Dr Peeter Karihtala¹, Dr Juan-Manuel Sancho², Prof Taina Turpeenniemi-Hujanen¹ and Prof Outi Kuittinen¹,¹¹,¹²

¹Medical Research Centre and Cancer and Translational Research Unit, University of Oulu and Oulu University Hospital, Oulu, Finland
²Department of Haematology, ICO-Hospital Germans Trias i Pujol, Institut de Recerca Josep Carreras, Badalona, Spain
³Department of Applied Physics, University of Eastern Finland, Kuopio, Finland
⁴Department of Haematology, ICO-Hospital Duran i Reynals, Hospitalet de Llobregat, Barcelona, Spain
⁵Department of Haematology, Oulu University Hospital, Oulu, Finland
⁶Department of Oncology, Tampere University Hospital, Tampere, Finland
⁷Department of Oncology, Turku University Hospital, Turku, Finland
⁸Siunsote – Hospital District of North Carelia, Joensuu, Finland
⁹Department of Oncology and Radiotherapy, Central Finland Central Hospital, Jyväskylä, Finland
¹⁰Department of Medicine, Kuopio University Hospital, Kuopio, Finland
¹¹Institute of Clinical Medicine, Faculty of Health Medicine, University of Eastern Finland, Kuopio, Finland
¹²Department of Oncology, Kuopio University Hospital, Kuopio, Finland

Corresponding author:
Roosa Enni Inkeri Prusila
Oulu University Hospital
Syöpätautien tutkimuslaboratorio
Kajaanintie 50
90220 Oulu
FINLAND

Tel: +358 8315 55212
Fax: +358 8315 4544
Email: roosa.prusila@student.oulu.fi

Received 27 March 2019, accepted 21 May 2019
SUMMARY

Follicular lymphoma (FL) is the most common indolent lymphoma. Currently there are many comparable treatment options available for FL. When selecting the most optimal therapy it is important to consider possible late effects of the treatment as well as survival. Secondary haematological malignancy (SHM) is a severe late effect of treatments, but the incidence of SHMs is still largely unknown. The goal of the present study was to determine the incidence of SHMs and how therapeutic decisions interfere with this risk. The study included 1028 FL patients with a median follow-up time of 5.6 years. The 5-year risk of SHM was 1.1% and the risk was associated with multiple lines of treatment (p=0.016). The 5-year risk of SHM was 0.5% after the first-line treatment and 1.6% after the second-line. The standardized incidence ratio (SIR) was 6.2 (95% confidence interval 3.4–10.5) for SHM overall. This retrospective study found the risk of SHM was low after first-line treatment in FL patients from the rituximab era. However, the risk of SHM increases with multiple lines of treatment. Therapeutic approaches should aim to achieve as long a remission as possible with first-line treatment, thereby postponing the added risk of SHM.

KEY WORDS

follicular lymphoma, late effects of therapy, secondary haematological malignancies, secondary leukaemia, treatment related cancer

INTRODUCTION

Follicular lymphoma (FL) is the most common indolent lymphoma worldwide. It is a chemosensitive disease and most patients respond to initial treatment. However, relapses are common and many patients need to be re-treated (Izutsu, 2014). The introduction of the CD20
antibody rituximab into the therapeutic armamentarium has resulted in major improvements in the prognosis of indolent non-Hodgkin lymphomas (NHL) over the last two decades (Kahl & Yang, 2016, Friedberg, 2008).

Secondary malignancies are important causes of morbidity and mortality in patients with indolent lymphomas (Friedberg, 2008). In FL patients treated in the rituximab era, the cumulative incidence of other malignancies is 2.9% in 10 years, being the third most common cause of death after lymphoma-related mortality and treatment-related mortality (Sarkozy et al, 2019).

Myelodysplastic syndrome (MDS) and acute myeloid leukaemia (AML) are important subsets of secondary malignancies. MDS is a group of haematopoietic stem cell disorders, characterized by ineffective haematopoiesis that leads to cytopenia, and carries a major risk of transforming into AML (Gill et al, 2016). The incidence of therapy-related MDS/AML has increased in recent years, with 10–20% of MDS cases being therapy-related (Candelaria & Duenas-Gonzalez, 2015, Yang et al, 2015). The incidence of MDS in the European background population is 4.15/100 000/year and is higher in males and in elderly people (Neukirchen et al, 2011).

With the improvement in overall survival (OS), FL patients have potentially more time to develop secondary malignancies. The MDS and AML risk among patients with different lymphomas is still, however, largely unknown (Friedberg, 2008). Moreover, the data is often extracted from registry data, where it is still usually not possible to study the impact of different treatment options on these risks. Because there are several different treatment options available for FL, this data would be important in order to optimize the therapy-associated risk/benefit ratio. In this large retrospective analysis, we collected data from hospital records of patients with FL to find out the incidence of MDS/AML and other secondary haematological malignancies (SHM) in patients
treated during the rituximab era. We also studied whether SHM incidence is related to certain
types of treatment.

METHODS AND MATERIALS

This is a retrospective registry study. The principles of the Declaration of Helsinki were followed
throughout the study. The study was approved by the Regional Ethics Committee for the Northern
Ostrobothnia Hospital District.

Clinical data was collected from the hospital records of all patients with FL diagnosed between
1997 and 2016. Data was collected from four university hospitals and three central hospitals in
Finland and from two hospitals in Spain. In total, 1045 patients were identified; 344 from Spain
and 701 from Finland. Information collected included age, stage and treatment details;, details
regarding possible relapses and SHMs were also evaluated. After excluding patients with
composite lymphoma or patients with first-line watchful waiting who had a diagnosis of diffuse
large B-cell lymphoma at first relapse, we had 1028 evaluable patients.

When comparing the risk of SHM per first-line treatment, the treatments were grouped as follows:
anthracycline-containing therapy, bendamustine-containing therapy, radiation therapy, other
chemotherapy (fludarabine-containing, chlorambucil, cyclophosphamide-vincristine-prednisone
and other chemotherapy) and others (watch and wait, surgical removal, single-agent rituximab
and no treatment).

Details of other lymphoid malignancies after FL were investigated but these were not included in
the concept of SHM. Haematological malignancy was considered as secondary if the diagnosis was
made after one year from the first day of treatment for FL.

Statistical analysis
Survival estimates were calculated with Kaplan-Meier analyses and the statistical significance between different variables was determined using the log-rank test. P values under 0.05 were considered statistically significant. Cox regression analysis was used in multivariate analyses.

Disease-specific survival (DSS) was calculated from the date of diagnosis to the date of lymphoma-related death or the last follow-up. Deaths caused by secondary malignancies were not included in DSS. Overall survival (OS) was calculated from the date of diagnosis to the date of death from any cause or the last follow-up. Progression-free survival (PFS) was calculated from the date of diagnosis to the date of first relapse or death from any cause. Analyses were performed with the Statistical Package for the Social Sciences, version 23 (IBM SPSS, Chicago, IL, USA). Time to SHM was calculated from the first day of first-line treatment to the diagnosis of SHM. The risk of SHM in second-line treatment was calculated from the date of first relapse to SHM or last follow-up. Cox regression analysis was performed with time-dependent variables when studying the risk of SHM to eliminate the influence of longer follow-up time among patients who had received multiple lines of treatment. This analysis was performed with PROC PHREG in SAS® Enterprise Guide 7.1 (SAS Institute, Cary, NC, USA).

The person-years at risk were computed by ten-year age groups, sex and five-year time intervals starting from two months after the first treatment date to the date of death or last follow-up. The time intervals started from 1980. Eighty-three patients were excluded for not having received any treatment or due to missing information about the treatment date. Thirty-six patients were excluded due to missing the date of last follow-up or death. The expected number of incidents for each group was computed by multiplying the specific person-years at risk by the corresponding number of incidents per person-years and summing up the results. The person-years, gender and time-interval-specific incidents were obtained from The Finnish Cancer Registry (https://Tilastot.syoparekisteri.fi/syovat. data from 2017-09-29, version 2018-05-02-001).
For Peer Review

confidence intervals (CI) were computed as Wald confidence intervals. Absolute excess risk was computed as the difference between the observed and the expected cases divided by the person-years in the study and multiplied by one thousand. The computations were done in R using the popEpi package (https://rdrr.io/cran/popEpi/).

RESULTS

Patient demographics

Patient demographics are presented in Table I. The median follow-up time was 67.0 months (0–226). The median age at the time of FL diagnosis was 60.0 years (17–100) and 51.5% of the patients were female. The prognosis of FL is presented in Fig 1. In all patients, the 1-year PFS was 89.5% and the 5-year PFS was 52.7%. In all patients, the 1-year DSS was 97.2% and the 5-year DSS was 90.4%. The 1-year and 5-year OS was 96.7% and 84.2%, respectively.

Secondary haematological malignancies

In all these patients, a total of 14 SHMs were diagnosed after the lymphoma diagnosis. The risk of SHM is presented in Fig 2A. The SHMs included five MDS, four AML and one each case of: acute lymphoblastic leukaemia, acute promyelocytic leukaemia, large granular lymphocytic leukaemia, chronic myeloid leukaemia and multiple myeloma. A single case of chronic lymphocytic leukaemia (CLL) was also diagnosed two months after the lymphoma diagnosis, but that was not counted as a SHM. In the whole patient group, at a median time of 62.5 months (25-143), seven patients were diagnosed with SHM. The median time to the diagnosis of MDS (n=3) and AML (n=3) after first line treatment was 55 months (41–93) and 51 months (25–137), respectively. The 5-year risk of SHM was 1.1% and the estimated 10-year risk was 2.7%. The standardized incidence ratio (SIR) was 6.2
(95% CI: 3.4–10.5) for SHM overall, 22.0 (95% CI: 7.1–51.3) for MDS and 11.9 (95% CI: 3.2–30.3) for AML.

Follow-up data after the diagnosis of SHM was available for 12 patients, with a median follow-up of 3.5 months (0–76). During the follow-up, eight of the patients (66.7%) with SHM had died at a median of five months (0–69) and three of these patients died within one month from the diagnosis of SHM.

Patient and treatment characteristics associated with SHM risk

Table II presents correlations between different clinical factors and the risk of SHM. The median age of the patients with later SHM at the time of the lymphoma diagnosis was 57.5 years (32–69). A correlation between a high FL International Prognostic Index (FLIPI) and SHM was found (p=0.044). Also, B-symptoms at the time of diagnosis were associated with a higher risk of SHM (p=0.008). The number of treatment lines received was also correlated with higher risk (p=0.016).

In multivariate analysis including FLIPI, B-symptoms and the number of treatment lines, three or more lines of treatment was an independent risk factor for the risk of SHM with an odds ratio of 5.0 (95% CI: 1.2–20.7, p=0.026).

The risk of SHM based on the number of treatment lines received is presented in Fig 2B. The 5-year risk of SHM was only 0.5% after the first-line treatment and 1.6% after the second-line treatment. In patients treated with one line of treatment, the SIR for SHM was 2.45 (95% CI: 0.8–7.6). In patients treated with two lines of treatment, the SIR was 6.9 (95% CI: 2.6–18.3), and for three or more lines of treatment it was 15.9 (95% CI: 7.6–33.4). When a time-dependent variable was added to the analysis, association between the number of treatment lines and SHM incidence was retained. When compared to patients treated with one line of treatment, the risk for SHM was higher in patients with two lines of treatment (95% CI: 1.1–86.7, p=0.043) and those with
three or more lines of treatment (95% CI: 1.5–115.8, p=0.022). This indicates that the higher risk of SHM is associated with higher number of treatment lines and not to longer follow-up among patients with multiple lines of treatment.

The 5-year risk of SHM in patients treated with chemotherapy was 1.2% and in patients treated without chemotherapy it was 0.1%. The estimated 10-year risks were 3.3% and 0.1%, respectively. However, there was no statistically significant difference in the risk of SHM between these groups (p=0.329), probably due to the low number of patients treated without chemotherapy. In patients treated with anthracycline-containing therapy, the 5-year risk was 1.3% and the estimated 10-year risk was 3.7%. The risk of MDS was increased if anthracycline-containing therapy (p<0.001) or other chemotherapy (p<0.001) was used in first-line treatment. Also, anthracycline-containing therapy (p=0.003) and other chemotherapy (p=0.009) in first-line was associated with higher risk of AML. There were no SHMs in patients who received bendamustine in first-line treatment (n=63).

The risk of SHM did not correlate with gender (p=0.172), elevated lactate dehydrogenase level (LDH) (p=0.221) or haemoglobin level (p=0.283) at the time of diagnosis. There was no statistically significant difference in the risk of SHM between patients treated with or without rituximab (p=0.302) or in patients treated with or without stem cell transplantation (SCT) (p=0.269). In patients treated with SCT, the 5-year risk of SHM was 3.7% and the estimated 10-year risk was 9.0%.

**DISCUSSION**

In the present study, we found a low incidence of SHM after first-line immunochemotherapy in FL. The 5-year risk of SHM was only 0.5% after the first-line treatment. However, the risk increased to
1.6% at five years after second-line therapy. The estimated 10-year risks were 1.3% and 5.5%, respectively.

The reported incidence of SHMs varies. In an Italian study of 563 NHL patients, 12 (2.1%) developed secondary MDS/AML. The median time from diagnosis of NHL to SHM was 25 months (6–168) (Sacchi et al, 2008). In 771 FL patients from the pre-rituximab era, the risk ratio (RR) for leukaemia was 10.4 (95% CI: 4.5–20.5) (Mudie et al, 2006). In 871 Hodgkin lymphoma (HL) patients with a median follow-up of 12 years, the SIR was 5.8 (95% CI: 2.3–12.0) for secondary leukaemia (Petrakova et al, 2018).

SHMs are major causes of morbidity and mortality in patients with FL. SHMs are more aggressive than corresponding de novo neoplasms due to their more complex karyotype. In therapy-related myeloid neoplasms, the 5-year survival rate is less than 10% (Fianchi et al, 2018). In a study of 3938 patients with MDS, the median survival was 16 months in patients with a previous cancer history and 23 months for those without (De Roos et al, 2007).

When choosing therapy, disease status, patient comorbidities and preferences are all considered. For symptomatic patients, immunochemotherapy is usually the choice of treatment (Izutsu, 2014). Bendamustine is an old drug that has been rediscovered in the treatment of lymphoid malignancies. Improved PFS and fewer toxic effects have been reported with rituximab plus bendamustine when compared to R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone) (Rummel et al, 2013). However, bendamustine is an alkylating agent and the risk of secondary malignancies has caused concern (Cheson & Rummel, 2009). The use of rituximab as a single agent has recently been included in frontline therapy and, in some countries, it has taken the place of immunochemotherapy as a less toxic regimen (Reagan & Friedberg, 2015).
SHMs are associated with the use of alkylating agents, topoisomerase inhibitors and radiation therapy. In a previous study of 149 NHL patients that had received bendamustine at some point in their treatment, the cumulative incidence rate of MDS/AML was reported to be 6.2% with a median follow-up of 8.9 years (Martin et al, 2017). The median time to MDS/AML after NHL diagnosis was 89 months. These patients had been treated with multiple lines of treatment and the median number of prior therapies before bendamustine was three. No clear association between bendamustine dosage and secondary MDS/AML was found (Martin et al, 2017). In our study, the use of bendamustine was not found to be associated with a higher risk of SHM although the number of such patients was limited.

A study of 531 FL patients treated with R-CHOP or CHOP combined with radioimmunotherapy (RIT) has been recently published (Shadman et al, 2018). After a 10-year follow-up, five patients (1.8%) treated with R-CHOP and 13 patients (4.9%) treated with CHOP-RIT had developed MDS or AML. Of these patients, 12 died due to secondary MDS or AML (Shadman et al, 2018). In another study of NHL patients treated with CHOP, the RR for leukaemia was 14.2 (95% CI: 6.8–26.2) and it was 19.2 (95% CI: 9.6–34.3) in patients treated with chlorambucil (Mudie et al, 2006). We also observed a connection between the use of anthracyclines and the risk of MDS or AML in our study.

Fludarabine-based treatment is less common nowadays in the treatment of FL. In 119 patients treated with FCM (fludarabine, cyclophosphamide, mitoxantrone), three patients (2.3%) developed secondary MDS/AML. Diagnoses were made 7, 8 and 9.5 years after FCM. The median follow-up with these patients was 12.5 years (1.6–15) (Magnano et al, 2017). In a series of 176 patients with CLL and indolent lymphoma treated with a fludarabine combination, the overall rate of MDS/AML has been shown to be as high as 10.8% and the median time to diagnosis was
42 months (Carney et al, 2010). The present study included too few patients treated with fludarabine to draw any conclusions.

The latency for developing secondary MDS/AML varies between two and ten years while secondary solid tumours have a longer latency period, exceeding seven years (Damlaj et al, 2019).

It has been noted that leukaemia related to alkylating agents peaks around five years after therapy and epipodophyllotoxin-related AML peaks two to three years after therapy (Koontz et al, 2013).

Among 5798 HL patients treated with chemotherapy, it was shown that the RR for leukaemia peaked between five and nine years after the first treatment, with few cases after 14 years (Swerdlow et al, 2011). Similar results in a cohort of 2-456 NHL patients treated in the pre-rituximab era have been presented, with the leukaemia risk being elevated for the first ten years but then reduced, and no leukaemia was diagnosed after 15 years (Mudie et al, 2006). However, studies with a follow-up time of less than ten years probably underestimate the risk of secondary MDS/AML (Reiss et al, 2015) and the median follow-up time in our present study was 5.6 years.

The present study also found an increase in risk between five and ten years of follow-up, although the 10-year risk is only an estimation, due to the small number of patients available at the 10-year follow-up time.

The results of FL treatment improved vastly after the introduction of rituximab and nowadays the median OS is approaching 20 years (Tan et al, 2013). In our cohort, the 5-year DSS was 91.6%.

Relapses are typical for FL patients and some patients receive many different lines of treatment.

Several studies have recognized that the number of chemotherapy cycles correlates strongly with the risk of secondary leukaemia (Koontz et al, 2013, Swerdlow et al, 2011). The findings in our study are in line with these studies in the rituximab era, since the risk of SHM was higher in patients with multiple lines of treatment.
SCT has been considered to be a major risk factor for SHM. A 5-year risk of secondary MDS or AML of 4 to 14.5% was reported in 834 NHL patients treated with autologous SCT (Kollmannsberger et al, 1998). An excessive 50- to 100-fold risk of AML in NHL patients has been connected with the use of total body irradiation, total nodal irradiation and hemi-body irradiation (Kollmannsberger et al, 1998). In our study, we were not able to demonstrate any increased risk of SHM with SCT compared to conventional salvage therapy. This discrepancy may be explained by the fact that patients were treated with many previous treatment lines and whole-body irradiation during the high-dose therapy in other studies. In the present study, most of the patients received only chemotherapy during their high-dose therapy.

It can also be discussed whether patients with lymphoma have a higher risk of haematological malignancies at the time of diagnosis, before any treatments, as risk factors for lymphoma and leukaemia are overlapping. It has been noted that NHL patients with certain autoimmune diseases have, in fact, a 1.5- to 2-fold risk of secondary AML/MDS. Also, certain infections, both before and after diagnosis, have been connected with a higher risk, such as upper respiratory tract infections, sinusitis, pneumonia, urinary tract infections, prostatitis and gastroenteritis (Lam et al, 2016).

These findings suggest that immune dysfunction may play a role in the development of SHM.

There was an indication that the risk of SHM was not increased in patients receiving active follow-up or immunotherapy only, although the number of these patients was small.

In our study, secondary lymphatic neoplasms were excluded from SHMs because no clonal analyses were performed from these cases and, thus, it was not possible to reliably separate false primary diagnosis or transformations from secondary lymphomas. By doing this, we also ruled out the actual secondary lymphomas, which may have led to an underestimation of real SHM numbers.
The current study found a low risk of SHMs after first-line treatment. The risk of SHM was higher with multiple lines of treatment, which is in line with other studies (Koontz et al 2013). As expected in patients treated with chemotherapy, the risk of SHM was higher than those treated with surveillance or immunotherapy alone.

The strength of our study is the considerable sample size and the detailed information on therapies that patients have received. However, the follow-up time is limited, so the true incidence may be underestimated. We found that we were able to reliably estimate the risk of SHM after different first-line treatment options, but later the true effect of individual therapies is hard to evaluate because patients are treated with many different modalities, and receive multiple lines of treatment. The use of supportive treatment should also be considered. Lymphoma treatments also predispose patients to many different late effects in addition to SHMs. When choosing treatment, all possible late-effects should be considered.

Nowadays, the prognosis of FL is excellent. As many treatment options are available, it is important to find methods with optimal risk/benefit ratios and to also take long-term adverse events into account. In this study on FL patients from the rituximab era, the risk of SHM was low after the first-line treatment. However, the risk increases with multiple lines of treatment. Therefore, it could be useful to strive for as long a remission as possible in the first-line treatment, meaning the second-line treatment with an increased risk of SHM would be postponed. With low-risk patients, treating with immunotherapy only could also be a useful option. Further studies with a longer follow-up are needed to identify the late effects of modern treatments used in FL to maximize quality of life after treatments.

ACKNOWLEDGEMENTS
RP, MS, SM, AP, PS, TT, ST, SK, MP, IN, MK, KV, EJ, EK and PK participated in data collection. RP, JJ and OK performed the data analysis. RP wrote the manuscript with support from all the authors. J-M, OK and TT-H supervised the study. All the authors accepted the final version of the manuscript. The authors would like to thank M.Sc. Paula Pesonen for statistical help. This study was funded by the Finnish Haematology Association, the Blood Disease Research Foundation and The Väisänens Fund in TERTTU-foundation.
REFERENCES


Table I. Patient demographics.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>1028</td>
<td></td>
</tr>
<tr>
<td>Follow-up, years; median (range)</td>
<td>5.6 (0-18.8)</td>
<td></td>
</tr>
<tr>
<td>Age, years; median (range)</td>
<td>60.0 (17-100)</td>
<td></td>
</tr>
<tr>
<td>Male/Female</td>
<td>498/529</td>
<td>48.5/51.5</td>
</tr>
<tr>
<td>B-symptoms at diagnosis</td>
<td>166</td>
<td>16.6</td>
</tr>
<tr>
<td>Elevated LDH at diagnosis</td>
<td>226</td>
<td>29.0</td>
</tr>
<tr>
<td>FLIPI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>335</td>
<td>36.8</td>
</tr>
<tr>
<td>2</td>
<td>257</td>
<td>28.3</td>
</tr>
<tr>
<td>3-5</td>
<td>317</td>
<td>34.9</td>
</tr>
<tr>
<td>Number of treatment lines</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>621</td>
<td>64.2</td>
</tr>
<tr>
<td>2</td>
<td>220</td>
<td>22.7</td>
</tr>
<tr>
<td>3 or more</td>
<td>127</td>
<td>13.1</td>
</tr>
<tr>
<td>Treated patients</td>
<td>968</td>
<td>94.2</td>
</tr>
<tr>
<td>CHOP-like first-line therapy</td>
<td>576</td>
<td>56.0</td>
</tr>
<tr>
<td>Bendamustine-containing first-line therapy</td>
<td>63</td>
<td>6.1</td>
</tr>
<tr>
<td>Fludarabine-containing first-line therapy</td>
<td>27</td>
<td>2.6</td>
</tr>
<tr>
<td>RDT only in first-line</td>
<td>109</td>
<td>10.6</td>
</tr>
<tr>
<td>Rituximab in any of the treatment lines</td>
<td>823</td>
<td>85.0</td>
</tr>
<tr>
<td>Rituximab in first-line treatment</td>
<td>725</td>
<td>70.5</td>
</tr>
<tr>
<td>SCT in any of the treatment lines</td>
<td>66</td>
<td>6.4</td>
</tr>
</tbody>
</table>

Abbreviations: CHOP: cyclophosphamide, doxorubicin, vincristine, prednisone; FLIPI: follicular lymphoma International Prognostic Index; LDH: lactate dehydrogenase; RDT: radiation therapy; SCT: stem cell transplantation.
Table II. Correlation of different clinical factors to risk of SHM according to log-rank test.

<table>
<thead>
<tr>
<th>Clinical factor</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>High FLIPI</td>
<td>0.044</td>
</tr>
<tr>
<td>Age &gt;60 years</td>
<td>0.342</td>
</tr>
<tr>
<td>Haemoglobin &lt;120 g/l</td>
<td>0.283</td>
</tr>
<tr>
<td>Serum LDH, elevated</td>
<td>0.221</td>
</tr>
<tr>
<td>B-symptoms</td>
<td>0.008</td>
</tr>
<tr>
<td>Gender</td>
<td>0.172</td>
</tr>
<tr>
<td>Number of treatment lines received</td>
<td>0.016</td>
</tr>
<tr>
<td>Treatment with or without rituximab</td>
<td>0.302</td>
</tr>
<tr>
<td>Treatment with or without SCT</td>
<td>0.269</td>
</tr>
<tr>
<td>First-line treatment with or without chemotherapy</td>
<td>0.329</td>
</tr>
</tbody>
</table>

P-values <0.05 were considered statistically significant.

Abbreviations: FLIPI: follicular lymphoma International Prognostic Index; LDH: lactate dehydrogenase; SCT: stem cell transplantation; SHM: secondary haematological malignancy.
Figure legends:

Figure 1. Survival curves of all patients according to Kaplan-Meier method, A) progression-free survival (PFS); B) disease-specific survival (DSS); C) overall survival (OS).

Figure 2. Risk of secondary haematological malignancies according to Kaplan-Meier method. A) overall standardized incidence ratio (SIR) 6.2 (95% confidence interval [CI]: 3.4–10.5;) B) according to number of treatment lines SIR for patients with one line of treatment [2.5 (95% CI: 0.8–7.6)], two lines of treatment [6.9 (95% CI: 2.6–18.3)] and three or more lines of treatment [15.9 (95% CI: 7.6–33.4)].
A

Median PFS 46 months
Mean DSS 190 months
Mean OS 165 months

OS of all patients

Months
Risk of secondary haematological malignancies

Median time 62.5 months
Risk of secondary haematological malignancies

- - - One line of treatment
  n=127

- - Two lines of treatment
  n=220

- - Three or more lines of treatment
  n=621

p=0.016

Months

Risk of secondary haematological malignancies

0.10
0.08
0.06
0.04
0.02
0.00

B

…

Three or more lines of treatment
Two lines of treatment
One line of treatment