Therapy-related myelodysplastic syndrome and acute myeloid leukemia after conventional therapy or autologous stem cell transplantation for non-Hodgkin's lymphoma: a systematic review

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Zusammenfassung

Einleitung: Bei Patienten mit non-Hodgkin Lymphomen (NHL) wird eine zunehmende Zahl von Sekundärerkrankungen, wie myelodysplastisches Syndrom und akute myeloische Leukämie, nach erfolgreicher Therapie der Erstneoplasie beobachtet. Viele der publizierten Studien weisen unterschiedliche Angaben zu deren Inzidenz auf. Ziel dieser Arbeit ist es, die Inzidenz von Therapie-assoziierter akuter myeloischer Leukämie (t-AML) und Therapie-assoziiertem myelodysplastischen Syndrom (t-MDS) bei Patienten mit NHL nach konventioneller Therapie bzw. Hochdosistherapie und autologer Stammzelltransplantation (ASCT) zu erfassen.

Methodik: Um dieses Ziel zu erreichen, führten wir einen systematischen Review durch. Geprüft wurden alle publizierten Originalarbeiten in englischer Sprache von 1970 - 2008, die über die Datenbanken MEDLINE, EMBASE und Cochrane Library sowie über Kongreßberichte und Referenzlisten verfügbar waren. Von 4040 potentiell geeigneten Artikeln erfüllten 35 Studien mit einer Gesamtzahl von 93094 NHL Patienten unseren strikten Einschlusskriterien und wurden in die Analyse einbezogen.

Ergebnisse: 32 Arbeiten berichteten über Kohortenstudien, 3 über randomisierte, kontrollierte Studien. In 16/35 Arbeiten war der primäre Endpunkt die Erfassung der Inzidenz von t-MDS/t-AML. Die „crude incidence“ war das am häufigsten verwendete Maß, „actuarial incidence“ und „cumulative incidence“ wurden nur in wenigen Publikationen angeführt. Insgesamt wurden 492 Fälle von t-MDS/t-AML beobachtet. Die „crude incidence“ lag in der Gruppe nach konventioneller Therapie zwischen 0 und 8.2%, in der Gruppe nach Hochdosistherapie und ASCT zwischen 0 und 13.6%. Subgruppenanalysen waren aufgrund unzureichender Daten nicht möglich.

Schlussfolgerung: Die Ergebnisse dieses systematischen Reviews zeigen eine höhere Inzidenz von t-MDS/t-AML nach Hochdosistherapie und ASCT. Dies stellt einen Hinweis für die Rolle von Toxizität in der Pathogenese dieser Sekundärmalignome dar.

Abstract

Background: In patients with non-Hodgkin´s lymphoma (NHL), an increase in long-term sequelae of cytotoxic therapies is being observed. Among those, therapy-related myelodysplastic syndrome (t-MDS) and acute myeloid leukemia (t-AML) are among the most severe ones. However, data on incidence of t-MDS/t-AML following NHL therapies are conflicting. The aim of this review is to systematically evaluate the incidence of t-MDS/t-AML following conventional treatment and high-dose therapy with autologous stem cell transplantation in patients with NHL.

Methods: To pursue this goal, a systematic review was performed. We screened the databases MEDLINE, EMBASE and Cochrane Library as well as congress proceedings and reference lists, to identify original articles, published in English between 1970 and 2008, on t-MDS/t-AML after NHL. Of 4040 potentially appropriate articles, 35 fulfilled our strict inclusion criteria, reporting on a total of 93094 patients with NHL.

Results: Thirty-one original articles reported on cohort studies, 4 on randomized controlled trials. In 16/35 articles the primary endpoint was evaluation of t-MDS/t-AML. “Crude incidence” was the most frequent measure of incidence, “actuarial incidence” and “cumulative incidence” were used occasionally. A total of 492 cases of t-MDS/t-AML were reported. In the group of conventionally treated patients, the crude incidence was between 0 and 8.2%, in the group with high-dose therapy and ASCT, between 0 and 13.6%. Due to missing data, subgroup analyses were not possible.

Conclusion: The results of this systematic review show a higher incidence of t-MDS/t-AML after high-dose therapy and ASCT for patients with NHL as compared to conventional treatment. This indicates a role for toxicity in the pathogenesis of t-MDS/t-AML.

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I. Introduction

I.1 Definition of non-Hodgkin´s lymphoma

Malignant lymphomas are neoplastic disorders of the lymphoid organs and are divided into Hodgkin´s disease (HD) and non-Hodgkin´s lymphomas (NHL). The NHL constitute a heterogeneous group of malignancies (Alexander et al., 2007, Cheson, 1997, Young and Iland, 2007, Rogers, 2006). A dramatic rise in the incidence of NHL during the last decades has been observed (Alexander et al., 2007, Blayney et al., 2003). According to the SEER program (surveillance, epidemiology and end results) of the National Cancer Institute, USA, the incidence of NHL has increased from 12/100.000 in 1974 to 22/100.000 in 2006. Although the etiology of the majority of NHL cases remains unknown, some risk factors could be identified (Young and Iland, 2007). A family history of NHL is associated with an increased lymphoma risk (Alexander et al., 2007). Helicobacter pylori plays an important role in MALT (mucosa-associated lymphoid tissue) lymphomas of the stomach, EBV (Epstein-Barr virus) in Burkitt´s lymphoma and HIV (human immunodeficiency virus) in primary lymphomas of the central nervous system. Finally, some cases of NHL are observed in immunosuppressed patients after solid organ transplantation and those with chronic inflammatory diseases like Sjögren´s syndrome, rheumatoid arthritis, systemic lupus erythematodes or dermatomyositis (Ekstrom-Smedby, 2006, Rogers, 2006, Young and Iland, 2007, Alexander et al., 2007, Ansell and Armitage, 2005).

I.2 Symptoms of NHL

The symptoms of NHL may differ and are dependent on the subtype, i.e. whether it is an aggressive lymphoma or an indolent one, which often exhibit a chronic disease course. Most patients with NHL present with painless, progressive lymphadenopathy. Bone marrow involvement associated with various degrees of peripheral blood cytopenias (anemia, neutropenia, thrombocytopenia) is frequently encountered as well as splenomegaly. Extranodal involvement such as the gastrointestinal tract, the skin, nasal sinuses or the central nervous system may also occur. In general,

lymphomas can develop at almost any site of the body due to the fact that innate or acquired lymphoid tissue is widespread. Often " B- symptoms" are reported by lymphoma patients, which include weight loss of more than 10% within six months, drenching night sweats and fever >38°C unrelated to any infection (Young and Iland, 2007, Ansell and Armitage, 2005).

I.3 Diagnosis of NHL

A careful physical examination and routine laboratory parameters including a peripheral blood and differential count are initial steps in the diagnosis of NHL. In almost all cases, a biopsy of an affected lymph node or extranodal infiltrate is mandatory (Young and Iland, 2007, Armitage et al., 2006). This biopsy should be large enough to allow histologic as well as immunohistochemistry examinations, which are mandatory for an accurate diagnosis of the NHL subtype. As enlargement of thoracic or abdominal/ retroperitoneal lymph nodes may occasionally be the only manifestation of NHL, thoraco- or laparotomy is required in such situations to obtain tissue specimens. In some patients, a "leukemic blood picture" is observed at presentation. Immunophenotyping of neoplastic cells by FACS analysis will establish a proper diagnosis in these individuals without need for invasive procedures.

I.4 Classification of NHL

The first classification system was developed by Henry Rappaport based on the size and shape of lymphoid cells as well as the presence or absence of a follicular growth pattern (Armitage, 2005). After recognition of different lymphocyte subtypes, the Lukes-Collins Classification and the Kiel Classification were developed (Ansell and Armitage, 2005). Whereas the Lukes-Collins Classification was used in the United States, the Kiel Classification was encountered in Europe. This led to some confusion which gave rise to an updated classification system, the Working Formulation. It classified NHL into low-, intermediate- and high-grade lymphomas according to both, malignant cell types and prognosis. The Revised-European-American-Lymphoma (REAL) Classification – a joint project of researchers from both continents –

integrated novel immunologic aspects and formed the basis of the current WHO classification (Armitage et al., 2006, Vardiman et al., 2002). This system aimed at focusing on all aspects of NHL: biology, diagnosis, immunology, genetics and therapy/ prognosis. Currently, there are several subtypes of NHL subdivided into three major categories: indolent, aggressive and very aggressive lymphoma types (Table 1). The two most common types of NHL are “diffuse large B-cell lymphoma” and “follicular lymphoma”, accounting for 30% and 20% of all NHL, respectively.

Table 1: WHO classification of NHL

<u>Indolent NHL:</u>
B-cell:
Follicular lymphoma
Small lymphocytic lymphoma
MALT lymphoma
Nodal marginal zone lymphoma
Lymphoplasmocytic lymphoma (Waldenström´s macroglobulinemia)
T cell and NK cell
<u>Aggressive NHL:</u>
B-cell:
Diffuse large cell lymphoma
Mantle cell lymphoma
Mediastinal large B-cell lymphoma
Intravascular large B-cell lymphoma
Primary effusion lymphoma
Lymphomatoid granulomatosis
T cell and NK cell:
Angioimmunoblastic T-cell lymphoma
Peripheral T-cell lymphoma
Extranodal NK/T-cell lymphoma

Hepatosplenic T-cell lymphoma
Anaplastic large-cell lymphoma

Highly aggressive NHL:

B-cell:

Burkitt´s lymphoma
Lymphoblastic lymphoma

T cell and NK cell:

Lymphoblastic lymphoma

I.5 Staging of NHL

For staging of NHL, the Ann-Arbor system is used (Table 2) (Armitage, 2005). It is an anatomic staging system and was the first widely used staging system for HD and NHL (Armitage et al., 2006). Staging procedures include the case history with evaluation of B-symptoms, a thorough physical examination, computer tomography scanning of the neck, chest, abdomen and pelvis, a bone marrow biopsy, an ENT status and optional PET (positron emmission tomography) scanning (Armitage, 2005).

Table 2: The Ann-Arbor Staging System

Stage I involves only a single lymph node region
Stage II involves two or more lymph node regions on the same side of the diaphragm
Stage III involves lymph node regions on both sides of the diaphragm
Stage IV involves diffuse/ disseminated lymphomatous infiltrates

I.6 The International Prognostic Index

In 1993, the International Prognostic Index (IPI) was developed for patients with diffuse large B-cell lymphoma and emphasizes important patient's characteristics (Armitage, 2005). The IPI is an independent predictor of survival and a useful tool for initial therapeutic decisions (Ansell and Armitage, 2005).

Table 3: International Prognostic Index

Risk factors

Age> 60 years
Number of extranodal sites >2
Stage III or IV
Performance status >2
Serum LDH level
One point is assigned for each risk factor

Risk	Survival at 5 years
Low (0 -1 point)	73%
Low – intermediate (2 points)	50%
High - intermediate (3 points)	43%
High (4 - 5 points)	26%

I.7 Treatment options for NHL

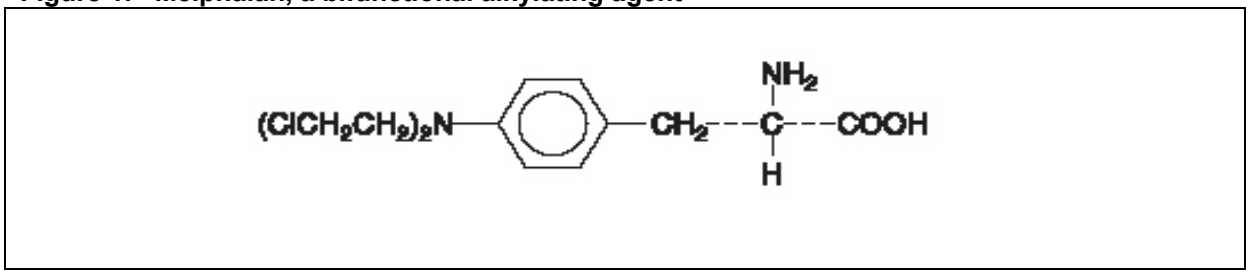
There is a great number of different NHL subtypes (Table 1) and accordingly, many different possibilities to approach patients with NHL exist. One of the fundamental decisions following diagnosis and completion of staging procedures concerns the exact timing of antineoplastic therapy. In cases with curative options, immediate treatment will be recommended. In cases, where this goal is unachievable, a

palliative situation exists. Treatment in these patients will be commenced when one of the following situations occurs: progressive disease, marked cytopenia due to malignant bone marrow infiltration, compression or obstruction of organs due to enlarged lymph nodes and unbearable B-symptoms. The principal parameters that determine, whether a curative or a palliative situation exists, are the NHL subtype, the stage of the disease as well as age and performance status of the patient.

In the following section, a brief overview of the main treatment strategies for patients with NHL will be presented.

Chemotherapy constitutes the basis of lymphoma treatment and alkylating agents - like cyclophosphamide, ifosphamide, melphalan, busulphan, carmustine, cisplatin and mitomycin C – are the most important drugs in this regard. Alkylating agents, which are derived from nitrogen mustard, can be divided into two major classes: monofunctional and bifunctional alkylating agents (Davies, 2001). The former are characterized by one reactive side chain whereas the latter show two reactive chlorethyl chains. Whereas monofunctional agents form DNA adducts, bifunctional alkylators induce in addition DNA intra- and interstrand crosslinks. In any case, DNA damage will induce apoptosis preferentially of neoplastic cells.

Figure 1: Melphalan, a bifunctional alkylating agent



Initially, only alkylator-based therapies were used in the treatment of NHL (Fisher and Shah, 2003, Winter, 2007). Later, polychemotherapy regimens - a combination of several agents belonging to different chemotherapy classes (Pan et al., 2003, Flinn et al., 2000) - were introduced and resulted in improved response rates and significanly longer disease free survival (Zinzani, 2004, Held et al., 2006). The most important is CHOP, consisting of cyclophosphamide, adriamycin, oncovin and prednisolone, which has become the “gold standard” in NHL therapy. Other strategies like PROMACE or MACOP were tested in several studies (Held et al.,

2006, Fisher and Shah, 2003, Gianni et al., 1997). However, their success rates varied and they did not become standard options for NHL therapies. A further significant step forward was the introduction of targeted therapies (see below) which – when combined with CHOP – lead to significantly improved overall survival in several NHL subtypes.

Radiotherapy is also a frequently used approach in the treatment of NHL (Nissen et al., 1983). The principle mechanism of ionising radiation is formation of single and double DNA strand breaks. In contrast to chemotherapy, induction of DNA damage is not restricted to proliferating but also to resting cells ultimately leading to cell death. For reasons poorly understood, tumor cells are more efficiently killed than normal cells. The intention of radiotherapy in NHL can be both, a curative and a palliatve one, and depends mainly on the NHL subtype and stage of the disease. For example, localized stages (I or II acording to Ann-Arbor) of follicular and mantle cell lymphomas can be cured with radiotherapy alone. However, radiotherapy is more often applied in combination with chemotherapy (combined modality treatment) (Nissen et al., 1983). NHL are generally regarded as radiosensitive neoplasias. The exact radiation dose is determined by several factors like NHL subtype, disease extent and whether it is a curative or a palliative approach. Before radiation therapy is commenced, exact planning of the treatment is required. This includes imaging procedures like CT, MR and PET as well as calculation of the 3-dimensional dose distribution. With respect to the radiation field, different modalities exist. Involved field radiation includes only affected lymph node regions whereas with extended field radiation, neighboring non-affected lymph node regions are treated as well. With “mantle field radiation”, all supradiaphragmatic lymph nodes are included, with “inverted Y radiation” all infradiaphragmatic ones. Total body irradiation is another treatment option which has been used since the 1900´s in cancer treatment. Thereby, very low fractionated TBI doses are given several times a week until a cumulative dose has been reached. It was initally used in the primary management of selected patients with advanced NHL. In the 1940´s, systemic chemotherapy was developed and the use of total body irradiation diminished over time until interest in this treatment was revived in the 1980´s when autologous stem cell tranplantation was developed (see below). Patients with diseases primarily limited to nodal sites

have also been treated with total lymph node irradiation. However, this option is not being used nowadays.

Immunotherapy is a novel strategy in the treatment of NHL. It is based on the expression of specific surface/cytoplasmic antigens on lymphoma cells (Thompson et al., 2008). Monoclonal antibodies targeting these antigens were developed in order to improve lymphoma therapy. One of the most successful examples is Rituximab, a monoclonal antibody directed against CD20 (Coiffier, 2006, Abbott, 2006). This antigen is expressed on normal as well as neoplastic B-cells. The addition of Rituximab to the CHOP regimen has signifcantly improved response rate and overall survival of patients with aggressive NHL and has now become a standard approach of many B-cell NHL (Winter, 2007, Andemariam and Leonard, 2006, Sebban et al., 2006, Blayney et al., 2003). Side effects are rare and include anaphylactic reactions like fever, chills and hypotension. Surprisingly, despite a depletion of normal B-cells, Rituximab does not aggravate immunosuppression of lymphoma patients.

Radioimmunotherapy utilizes an antibody labelled with a radionuclide to deliver cytotoxic radiation to a target cell (Hiddemann et al., 2007). It combines the benefits of radiation therapy and immunotherapy, where multiple sites of disseminated disease can be treated simultaneously while minimizing toxicity to normal tissues (Winter, 2007, Bennett et al., 2005). One successful example is ibritumomab tiuxetan, a combination of yttrium 90 and a CD20 directed monoclonal antibody, which has already become an option as front-line therapy for patients with follicular lymphoma (Winter, 2007).

Hematopoietic stem cell transplantation (HSCT) is an important treatment modality for hematologic malignancies and has been used routinely in the treatment of lymphomas since the 1990´s. The basis of this procedure is the delivery of high-dose chemo-radiotherapy for aggressive, resistant or relapsed lymphomas (Thompson et al., 2008). As an unavoidable side effect, bone marrow aplasia occurs necessitating stem cell transplantation for proper hematopoietic reconstitution (Gribben et al., 2005). The stem cell source can be either the patient him-/herself – autologous HSCT – or a related/unrelated donor – allogeneic transplantation. Both procedures have been proven benefical in certain subsets of NHL, however, only

autologous HSCT has become as standard procedure in this patients cohort (Gribben et al., 2005). The major concern with autologous HSCT is the risk of reinfusing occult neoplastic cells which may contribute to disease recurrence while transplant-related mortality is low at 2-4%. The benefit of allogeneic HSCT is a graft devoid of neoplastic cells (Fisher and Shah, 2003). A graft-versus-lymphoma effect, directed at residual lymphoma cells, is a major factor contributing to disease free survival (Ansell and Armitage, 2005, Villanueva and Vose, 2006). However, transplant-related mortality of 20-30% in the allogeneic setting is high and mainly due to infections, toxicity and graft-versus-host disease (Hiddemann et al., 2007). In the latter case, recipient tissues (most frequently the skin, gut and liver) are attacked by donor T-cells. Therefore, the pro and cons of HSCT must be assessed carefully and discussed with the patient in advance.

I.8 Outcome

The outcome of patients with NHL depends on both, patients and lymphoma specific characteristics. The former includes factors like general condition and age, the latter the lymphoma subtype and disease stage. Here, we would like to focus on the two most common NHL subtypes: diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL).

DLBCL is an aggressive lymphoma and accounts for about 30% of all NHL (De Paepe and De Wolf-Peeters, 2007, Kwong, 2007). The standard treatment for patients with DLBCL is CHOP in combination with Rituximab (Ansell and Armitage, 2005, Armitage et al., 2006, Vitolo et al., 2005). Several studies have shown the superiority of a Rituximab containing regimen as compared to CHOP alone (Fisher and Shah, 2003, Coiffier, 2006, Held et al., 2006). Autologous HSCT for DLBCL is performed in high-risk patients as well as those with resistant or relapsed disease. When patients are stratified according to the International Prognostic Index (see above), a 5-year overall survival between 73% (low risk) and 26% (high risk) can be expected.

FL belongs to the indolent group of NHL and accounts for about 20% of all NHL (Kwong, 2007). To date, no standard curative approach for this entity has been developed (Berglund et al., 2000). Therefore, FL patients are offered a wide range of treatment options (Armitage et al., 2006, Hiddemann et al., 2007, McLaughlin et al., 1987, Ladetto et al., 2006, Ersboll et al., 1989). Localized stages are treated with radiotherapy with curative intention (Armitage et al., 2006, Hiddemann et al., 2007, Mac Manus and Hoppe, 1996, Paryani et al., 1984, Ersboll et al., 1989). However, the majority of patients present with advanced stage (stage III or IV according to Ann-Arbor) and therefore, polychemotherapy remains the most important treatment (Armitage et al., 2006, Hiddemann et al., 2007, McLaughlin et al., 1987, Paryani et al., 1984). Different regimens are applied including CHOP and FC (fludarabine, cyclophosphamide). The addition of rituximab to any of the regimen used increases the response rate and prolongs remission duration (Coiffier, 2006, Marcus, 2007). If patients with FL fail initial treatment or relapse, they can be treated with either autologous or allogeneic HSCT. Only the latter option may be associated with long-term disease free survival and possibly cure, although at the price of high transplant-related mortality (Armitage et al., 2006). In general patients suffering from FL show a median survival of 8 - 12 years. In order to assess the prognosis of FL patients more accurately, the follicular lymphoma prognostic index (FLIPI) was developed. Age > 60 years, stage III or IV, hemoglobin concentration of <12mg/ dl ; nodal areas affected > 4 and high serum LDH levels are considered as individual risk factors and scored with one point each. The low risk group shows 0 -1 risk factors, the intermediate 2, and more than 3 risk factors predict high risk. The 10 year overall survival for patients with low risk FL is 71% and drops to 35% in the high risk group.

I.9 Late complications

With the development of cytostatic therapies, improved survival could be achieved for the majority of patients with NHL. Especially with aggressive NHL, long term disease free survival has been demonstrated and a subset of these patients might be regarded cured of their disease. In most patients with indolent lymphomas, this goal is still not achievable, even with high-dose therapy and stem cell transplantation. As

already discussed, the main goal in these patients is mitigation of symptoms, however, a proportion of these patients exhibit also improved survival.

With improved survival – either in complete or partial remission – long term side effects of cancer therapies become apparent. This is due to a non-specific mode of action of almost all agents used to treat patients with malignant diseases (Leone et al., 2007, Tward et al., 2006). Anthracyclins not only cause acute heart injury but may lead to congestive heart failure years after application in a dose dependent manner (Andre et al., 2004, Aviles et al., 2002, Lishner et al., 1991). Cisplatin and methotrexate are associated with chronic renal insufficiency whereas vincaalkaloids may cause central and peripheral neuropathies. Impairment of pulmonary function has been observed after radiotherapy as well as bleomycin (Seymour et al., 2003). Gonadal dysfunction is a more general and often observed long term side effect (Andre et al., 2004). As most cancer drugs target DNA, the induction of persistent mutations is one of the most severe side effects (Leone et al., 2007). If these mutations affect genes involved in growth regulation, clonal evolution may occur and ultimately lead to secondary malignancies (Leone et al., 2007). Indeed, these tumors have been observed with increasing frequency after cytotoxic therapies and comprise i) solid cancers, mainly of the breast and lung, ii) lymphomas – in cases of a primary NHL often of a different subtype - and iii) myelodysplastic syndrome/ acute myeloid leukemia.

Therapy-related myelodysplastic syndrome (t-MDS) and acute myeloid leukemia (t-AML) represent the most severe long term consequences of cytotoxic therapies for a primary, most often malignant disease. They occur at a median of 3-5 years after initiation of the primary treatment (Seymour et al., 2003, Bennett et al., 2005, Kalaycio et al., 2006, Armitage et al., 2003, Mudie et al., 2006) and are associated with a fatal outcome showing 5-year survival rates of less than 10%. The most common primary diseases are lymphomas (NHL and HD), which is most likely due to the fact that lymphomas represent one of those human malignancies with highest survival and cure rates. However, the exact incidence of t-MDS/t-AML following NHL therapy remains a matter of debate and is addressed in a few narrative reviews only. As knowledge of t-MDS/t-AML incidence in association with specific NHL therapeutic

strategies will influence future treatment decisions, we aimed at systematically evaluate this topic.

Table 4: Management algorithm for patients with NHL

Case history and physical examination
Biopsy and classification
Staging
Evaluation of prognostic factors
Timing of NHL therapy: immediate vs. delayed
NHL Therapy
Restaging and re-evaluation
Observation for NHL recurrence and long term side effects

II. Materials and Methods

The majority of NHL patients is treated with conventional therapy that includes chemo-, radio- and immunotherapy as well as combined modality approaches. High dose therapy – i.e. autologous HSCT (ASCT) – is given to high risk patients as part of the initial therapeutic approach and those with resistant or relapsed disease. Different incidence of t-MDS/t-AML is reported with respect to conventional treatment strategies and high-dose therapy with ASCT. Therefore, we aimed at systematically evaluating the incidence of t-MDS/t-AML in these two treatment groups. In order to pursue this goal, a systematic review was performed. A systematic review is a literature review focusing on a specific, clinical question. It tries to identify all relevant research evidence and provides a framework for the integration of the research. A meta-analysis offers a quantitative summary of the results. A systematic review, therefore, can incorporate a meta-analysis or not. This systematic review was

performed according to guidelines described by the Cochrane handbook and the QUOROM (**QU**ality **O**f **R**eporting **O**f **M**eta-analyses) statement (Moher et al., 1999).

II.1 Search strategy

Using the terms listed in Table 5, we identified articles by a systematic literature search between January 1970, when the first study of t-AML following therapy for lymphomas was published, and September 2008. With respect to NHL, all histologies including chronic lymphocytic leukemia and hairy cell leukemia were considered. The following electronic databases were screened: MEDLINE, EMBASE and the Cochrane Library. In addition, reviews and conference proceedings (American Society of Hematology, European Haematology Association, American Society of Clinical Oncology) as well as reference lists were used. The search was restricted to articles published in English, as the full text of all selected articles was studied. We also contacted 26 corresponding authors asking for essential data. Of those, 9 have responded (34%) and provided the information required.

Table 5: Search strategy. Each term of column one was combined with each of column two and three

Non Hodgkin´s lymphoma	therapy related	acute myeloid leukemia
Non Hodgkin´s disease	therapy associated	acute myelogenous leukemia
NHL	therapy induced	AML
Chronic lymphocytic leukemia	secondary	myelodysplastic syndrome
Hairy cell leukemia	treatment related	myelodysplasia
	treatment associated	MDS
	treatment induced	

II.2 Selection Criteria

Eligible studies were those reporting the incidence of t-MDS/t-AML after conventional treatment and autologous HSCT in patients with NHL. Studies reporting on patients who were treated with biological response modifiers only (for example, interferon α) were excluded. The studies were either randomized controlled trials (RCT) comparing conventional treatment with ASCT or cohort studies focusing on either approach. In RCT, subjects are randomly allocated to one of two (or several) different interventions. As long as sufficient numbers of patients are included in either arm, these studies provide a high level of evidence. In our case, a cohort study describes the outcome of a uniformly treated group of patients (cohort) over time. The evidence obtained with a cohort study is inferior as compared to a RCT.

A median follow-up of at least five years from the start of initial therapy was a mandatory inclusion criterion as it reflects the median latency period reported in major clinical studies on t-MDS/t-AML (Kalaycio et al., 2006, Bennett et al., 2005, Mudie et al., 2006). The latency period is defined as the time between the first cytotoxic treatment – not necessarily the time of NHL diagnosis (see above) - and diagnosis of t-MDS/t-AML. Studies reporting data of surviving patients only were excluded. A minimum of 20 patients had to be included into the study in order to be considered for this review. In cases of multiple publications from the same cohort, only the most recent report was included.

II.3 Data Extraction

The following data were sought from the studies selected: 1. *General information*: first author, institution and publication year; type of study: RCT or cohort study; primary study endpoint: lymphoma survival or incidence of t-MDS/t-AML; follow-up of the study population: median, mean, maximum, minimum and standard deviation; type of incidence measure reported: crude, actuarial or cumulative; 2. *Patients´ specific data*: total number of patients, patients´age: median, mean, maximum and minimum and standard deviation; latency period; total number of regimens applied; number of individuals with high-dose therapy and ASCT; 3. *Conventional therapy*:

chemotherapy, radiation and radioimmunotherapy, combined modality. For each category we assessed patients´age, latency period and incidence of t-MDS/t-AML as stated above as well as the number of regimens given; 3. *ASCT:* patients´age, latency period and incidence of t-MDS/t-AML as stated above and pre-ASCT therapy.

III. Results

III.1 Literature search

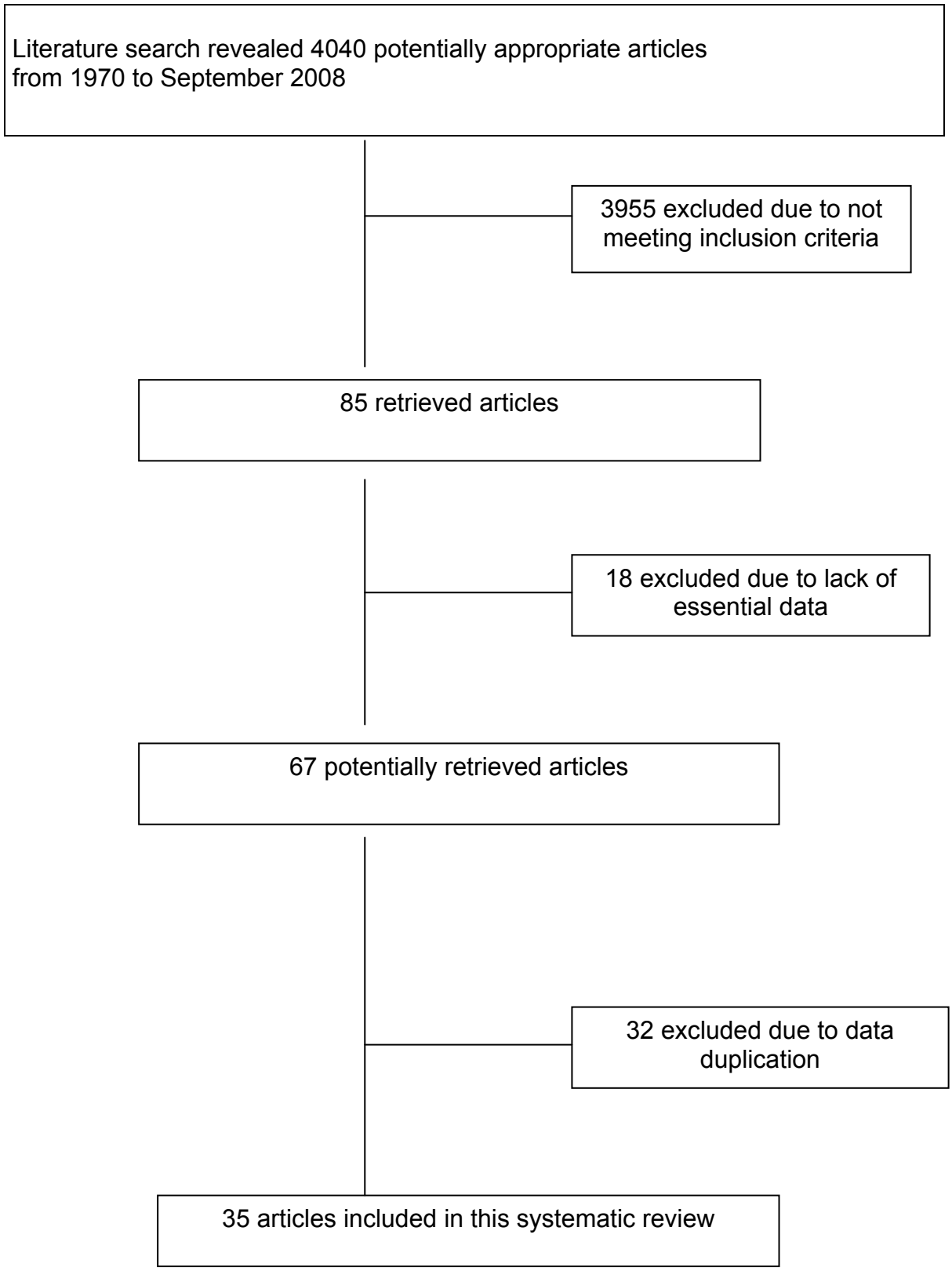
Our literature review using the key words listed in Table 5 revealed 4040 potentially relevant articles. After discussing titles and abstracts, 3955 were excluded due to not fulfilling the inclusion criteria. Of the remaining 85 papers, 18 lacked data mandatory for our analysis. These data constituted either the total number of patients having developed t-MDS/t-AML or the follow-up of the study. Thirty two articles were excluded because of data duplication. As outlined in “Materials and Methods”, only the cohort with the most recent – and therefore longest - follow-up was included. Finally, 35 eligible papers were included in this systematic review (Figure 2).

III.2 Trial characteristics

The first studies reporting on t-MDS/t-AML commenced in 1961. Thirty-two cohort studies were identified among the 35 articles that met the inclusion criteria. In 16 of these, the primary study endpoint was evaluation of the incidence of t-MDS/t-AML. Twenty-two of 31 articles referred to t-MDS/t-AML after conventional therapy and 9 after ASCT. There were three RCT comparing conventional therapy to ASCT. (Sebban et al., 2006, Vitolo et al., 2005, Schouten et al., 2003). However, in the study by Schouten et al., the t-MDS/t-AML incidence was stated in one arm only. Three of 35 studies had a median follow-up of 10 years and more (Seymour et al., 2003, Mora et al., 2003, Aviles et al., 2002). The median patients´ age was stated in

30 articles, two of them reported on pediatric/ adolescent patients with a median age of less than 16 years (Mora et al., 2003, Leung et al., 2001).

Figure 2: Identification of appropriate studies



III.3 Measures of incidence for t-MDS/t-AML

We aimed at assessing three types of measures of incidences: crude, actuarial and cumulative incidence. Crude incidence defines the proportion of patients with an event of interest (i.e. the number of patients diagnosed with t-MDS/t-AML divided by the total number of patients) (Armitage et al., 2003). Actuarial incidence or actuarial risk is based on Kaplan-Meier estimates. It takes into account those patients who died or are lost to follow-up (Armitage et al., 2003, Lavey et al., 1990). Cumulative incidence estimates the percentage of patients who will be diagnosed with t-MDS/t-AML in a certain time interval in the presence of competing risks (Andre et al., 2004).

The results of the 35 studies analyzed are listed in Tables 7 and 8. A total of 93094 NHL patients were included, 91360 undergoing conventional treatment and 1734 ASCT. Four hundred and ninety-two cases with t-MDS/t-AML were identified, 361 following conventional treatment and 131 after ASCT. In the vast majority of studies, only the crude incidence was available. In the group of conventionally treated patients, it showed a range between 0 and 8.2% (Paryani et al., 1984, Travis et al., 1996). In the ASCT group, the crude incidence was between 0 and 13.6% (Taylor et al., 1997, Berglund et al., 2000). However, a higher crude incidence was observed in the ASCT group. Twenty of 24 studies (83%) reporting results of conventional therapy exhibited a crude incidence of t-MDS/t-AML of less than 2%. In contrast, in the ASCT group, this figure is only 4/13 (30.7%). There were two randomized trials with a follow-up of five years or more that reported t-MDS/t-AML in both arms (Sebban et al., 2006, Vitolo et al., 2005). In the conventional treatment group, the crude incidence was 0.0 (Vitolo et al., 2005) and 1.9% (Sebban et al., 2006), respectively, and in the ASCT group, 3.3 (Vitolo et al., 2005) and 1.5 (Sebban et al., 2006), respectively.

We have also analyzed the incidence of t-MDS/t-AML with respect to the endpoint of the studies: “A” in Tables 7 and 8 denotes t-MDS/t-AML as primary study endpoint, “B” lymphoma survival. Whereas “A-studies” showed a crude incidence between 0.21 and 8.17% in the conventional and 0.0 and 11.7% in the ASCT arm, “B-studies” exhibited a range from 0.0 to 11.7% (conventional group) and 0.0 to 13.6% (ASCT

group), respectively. Three studies had a median follow-up of 10 years or more reporting t-MDS/t-AML incidences of 0.85, 1.05 and 1.96%.
The actuarial incidence was stated in two ASCT studies only and was 3.7% at 5 years in one study (Ladetto et al., 2006) and 36.5% at 10 years in the other (Micallef et al., 2000). A cumulative incidence was provided in two studies only being 17% at 15 years after conventional treatment (Travis et al., 1996) and 14.2% at 15 years following ASCT (Brown et al., 2005).

III.4 Subgroup analysis

As it is obvious from the data presented, that ASCT is associated with a higher (crude) incidence of t-MDS/t-AML, we wanted to analyze, whether the transplantation process itself or the extent of previous therapy accounts for this increase. Two parameters may be helpful in this respect. First, the number of conventional treatment cycles before ASCT in comparison to the conventional treatment group and second, the latency period between ASCT and t-MDS/t-AML diagnosis. However, this information was provided in only 3 of the 35 articles (Berglund et al., 2000, Corradini et al., 2004, Seymour et al., 2003) making this approach impossible.

Table 7: t-MDS/ t-AML after Conventional Therapy for NHL

Publication	Study Period	Study Design	Median Follow-up (yr)	Median Age (yr)	Therapy	No. of Cases/ No. of Treated Patients	Incidence (%)		
							Crude	Actuarial	Cumulative
Mac Manus	61 - 94	CS, B	7.7	52	RT	1/177	0.56		
Paryani	61 - 82	CS, B	9.5	46	RT+/-CT	0/66	0.0		
Travis	01/65 - 12/91	CS, A	8.6	50	RT +/- CT	5/61	8.19		17 (15 yr)
Leung	01/70 - 12/97	CS, A	8.4	10.9	CT+/-RT	7/497	1.4		
Ersball	01/70 - 12/79	CS, B	6.4	NR	RT, CT+/-RT	6/127	4.72		
Lavey	01/70 - 09/81	CS, A	5.5	54.6	RT, CT+/-RT	9/686	1.31		
Mora	71 - 90	CS, B	16.5	9.5	CT+/-RT	1/95	1.05		
Robertson	72 - 92	CS, A	7.2	NR	CT	3/1374	0.21		
Mudie	06/73 - 03/00	CS, A	7.7*	46	CT+/-RT	9/2456	0.36		
Nissen	10/73 - 04/78	CS, B	5.0	58	RT+/-CT	0/73	0.0		
Tward	73 - 01	CS, A	7.4	61	NR	243/77876	0.31		
McLaughlin	75 - 82	CS, B	6.5	56	CT+RT	0/74	0.0		
Callea	75 - 00	CS, A	6.5	67	CT	4/389	1.02		
Avilés	01/81 - 03/95	CS, B	13.6	55	CT+/-RT	4/469	0.85		
Seymour	02/84 - 12/92	CS, B	10.0	56	CT+RT	2/102	1.96		
André	02/84 - 01/98	CS, A	6.1	54	CT	11/2837	0.38		
Flinn	NR	CS, A	9.3	NR	CT	1/241	0.41		
Sacchi	88 - 03	CS, A	5.1	60	CT +/- RT	12/563	2.13		
Cheson	10/88 – 03/93	CS, A	5.1, 6.9, 7.4+	60	CT	5/1814	0.27		
Bennett	90 - NR	CS, A	6.0	58	CT, RIT	33/995	3.31		
Pan	07/93 - 08/95	CS, B	6.1	47	CT	0/25	0.0		
Sebban	07/94 - 03/01	RCT, B	7.6	49	CT	4/209	1.91		
Blayney	09/94 - 01/97	CS, B	5.1	52.5	CT	1/88	1.13		
Vitolo	01/96 - 03/01	RCT, B	6.5	43	CT+/-RT	0/66	0.0		

CS, cohort study; RCT, randomized controlled trial; A, primary study endpoint: incidence of t-MDS/t-AML; B: primary study endpoint: lymphoma survival;

*mean; NR, not reported; RT, radiotherapy; CT, chemotherapy; RIT, radioimmunotherapy; + three independent cohorts

Table 8: t-MDS/ t-AML after Autologous Stem Cell Transplantation for NHL

Publication	Study Period	Study Design	Median Follow-up (yr)	Median Age (yr)	Pre-ASCT Therapy	Conditioning	No. of Cases/ No. of Treated Patients	Incidence (%)		
								Crude	Actuarial	Cumulative
Brown	82 - 97	CS, A	9.5	44	CT+/-RT	Cy, TBI	68/605	11.23		14.2 (15 yr)
Taylor	05/84 - 10/95	CS, A	5.1	NR	NR	various +/- TBI	0/62	0.0		
Micallef	01/85 - 11/96	CS, A	6.0	46	CT+/-RT	Cy, TBI	27/230	11.73	36.5 (10 yr)	
Berglund	04/87 - 12/96	CS, B	6.1	45	CT	BEAC +/- TBI	3/22	13.6		
Gribben	89 - 99	CS, B	6.5	49	CT	Cy, TBI	13/137	9.48		
Corradini	90 - 99	CS, B	6.2	47	CT	Mit, Mel	2/60	3.33		
Gopal	01/90- 05/98	CS, , B	7.2	46	CT	HD-RIT	2/27	7.4		
Mahé	92 - 98	CS, B	6.3	46	CT	Cy, TBI	3/49	6.12		
Schouten	08/93- 04/97	RCT, B	5.7	47	CT	Cy,Eto,Mit	0/65	0.0		
Sebban	07/94 - 03/01	RCT, B	7.6	49	CT	Cy, Eto, TBI	2/131	1.52		
Thompson	01/95 - 07/04	CS, B	6.3	48	CT	Cy, Eto, TBI	4/194	2.06		
Vitolo	01/96 - 03/01	RCT, B	6.5	42	CT	Mit, Mel	2/60	3.33		
Ladetto	12/96 - 02/99	CS, A	5.6	NR	CT+/-RT	Mit, Mel	5/92	5.43	3.7 (5 yr)	

BEAC: BCNU, etoposide, cytarabine, cyclophosphamide; TBI, total body irradiation; Bu: busulphan; Eto: etoposide; Cy: cyclophosphamide; Mit: mitoxantrone; Mel: melphalan;

IV. Discussion

IV.1 t-AML/t-MDS

t-MDS/t-AML are clonal hematopoietic stem cell disorders arising after cytotoxic therapies for a primary most often malignant disease. Patients who develop t-MDS/t-AML often complain about fatigue and shortness of breath due to anemia. Less frequently, infections due to neutropenia or hemorrhage due to thrombocytopenia occur. The most common primary diseases are lymphomas (Hodgkin´s disease as well as NHL), followed by breast- and prostate cancer. However, t-MDS/t-AML is also observed after cytotoxic therapies for non-malignant disorders. Autoimmune diseases like rheumatoid arthritis that are refractory to conventional corticosteroid treatment may be treated with repeated courses of cyclophosphamide or methotrexate (Ekstrom-Smedby, 2006, Rogers, 2006, Young and Iland, 2007, Alexander et al., 2007, Ansell and Armitage, 2005). Occasionally, abnormal blood counts on routine follow-up are the first indications of t-MDS/t-AML. Among the primary cytotoxic therapies, alkylating agents, topoisomerase-II- inhibitors and radiotherapy are the most frequent ones (Brown et al., 2005, Robertson et al., 1994, Berglund et al., 2000, Travis et al., 1996, Armitage et al., 2003, Callea et al., 2006).

Diagnosis of t-AML is a straigthforward one with malignant blast cells in the bone marrow or peripheral blood of 20% or more. However, t-MDS is difficult to diagnose as cytopenia and dysplasia can be seen after cytotoxic therapies as a transient phenomenon without evidence of a malignant, secondary disorder. Therefore, additional criteria are mandatory which are clonal cytogenetic aberrations (see below) and/or an increase in blast cells of up to 19% (Leone et al., 2007, Micallef et al., 2000, Kollmannsberger et al., 1998, Davies, 2001, Kantarjian and Keating, 1987, Mahe et al., 2003). According to the WHO classification of “Tumors of the Haematopoietic and Lymphoid Tissues”, t-MDS/t-AML are both subsumed under “Acute Myeloid Leukemia (AML)” and represent about 10% of all cases of AML (Table 9).

Table 9: WHO classification of acute myeloid leukemia (AML)

AML with recurrent cytogenetic abnormalities
AML with multilineage dysplasia
AML and MDS, therapy-related
AML not otherwise categorized
Acute leukemia of ambiguous lineage

Neoplastic cells of patients with t-MDS/t-AML are characterized by numerous genetic abnormalities which develop during the process of malignant transformation (Mahe et al., 2003, Calzone et al., 2006). At the cytogenetic level, balanced as well as unbalanced aberrations occur in more than 90% of cases. Balanced aberrations do not lead to (visible) genetic loss and include translocations leading to pathogeneic fusion genes (Kantarjian and Keating, 1987). The most common ones are t(15;17), t(8;21) and inv(16), which are restriced to cases of t-AML. This type of t-AML develops after a median latency (Armitage et al., 2003) period of three years and exhibits a somewhat better prognosis. However, t-AML characterized by these aberrations is rare and constitutes less than 10% of all cases of t-MDS/t-AML. The vast majority shows unbalanced aberrations, mainly deletions or losses of whole chromosomes. Loss of chromosomes 5 and/or 7 as well as deletions of the long arm of these chromosomes are the most frequent ones (Kantarjian and Keating, 1987, Leone et al., 2007, Micallef et al., 2000, Kollmannsberger et al., 1998, Davies, 2001, Hake et al., 2007). These neoplastic disorders develop after a median latency period of five years (Armitage et al., 2003, Kalaycio et al., 2006, Bennett et al., 2005, Gopal et al., 2003), present with MDS that transform to AML in about 50% of cases. Their prognosis is dismal (Davies, 2001, Hake et al., 2007, Armitage et al., 2003). At the molecular level, t-MDS/t-AML are characterized by various degrees of microsatellite instability (Olipitz et al., 2002). Somatic point mutations are observed in several genes related to growth control or differentiation. The most common ones are the *TP53* and *AML1* genes, respectively (Kwong, 2007).

t-MDS/t-AML are treated by mere supportive care, chemotherapy and stem cell transplantation as well as experimental approaches (Hake et al., 2007). Supportive care includes blood products like red cell and platelet transfusions, antibiotic and

antifungal therapies and growth factors like the granulocyte-colony stimulating factor. However, these approaches do not influence the natural course of the disease. Chemotherapy may delay disease progression but the remission rate is inferior as compared to the de novo MDS or AML. The only potentially curative approach is allogeneic stem cell transplantation (Davies, 2001). Studies available so far have focused on myeloablative transplantation which shows 5-year disease free survival rates of up to 29% and 5-year non-relapse mortality rates of up to 61%. Published data on non-myeloablative transplantation approaches as well as novel antineoplastic therapies are not available for patients with t-MDS/t-AML. In a large, unselected cohort of more than 300 patients, seen at the University of Chicago, the overall 10-year survival rate of patients with t-MDS/t-AML is less than 10%.

IV.2 What is a sytematic review

However, due to increased use of cytotoxic therapies as well as prolonged survival of cancer patients, the number of individuals with t-MDS/t-AML is rising (Mudie et al., 2006, Kollmannsberger et al., 1998, Pedersen-Bjergaard et al., 2000). We therefore aimed at systematically investigate the incidence of t-MDS/t-AML in NHL patients treated with either conventional therapies or ASCT. The outcome of this systematic review may shed light on two important aspects of therapy-related leukemogenesis: toxicity and predisposition. The reasons for focusing on NHL were: 1. NHL patients show long-term survival either in complete or partial remission; 2. NHL is frequently treated by ASCT, therefore, the number of appropriate studies for a systematic review/meta-analysis seems to be sufficient; 3. NHL are among those primary tumors with the highest t-MDS/t-AML rate; 4. an increasing number of alternative therapies to ASCT exhibiting less toxicity are being developed.

In order to pursue this goal, we decided to perform a meta-analysis/systematic review. A meta-analysis/systematic review plays an important role in making decisions about optimal diagnostic strategies or therapeutic options. It focuses on a specific question and can be regarded as an overview of high-quality primary studies (Moher et al., 1999). When performing a meta-analysis/systematic review, it is useful to follow standards and guidelines to improve their quality. The QUOROM group has

published such standards which were also relevant for our study (Moher et al., 1999). In a first step, it is necessary to formulate the problem of interest precisely. Then, eligibility criteria are outlined followed by an extensive literature search. It is indispensable not only to screen major databases but also conference proceedings and reference lists to get all relevant published data. Still a matter of debate is, whether it is sufficient to focus on articles published in English only or to include articles published in other languages, too. We opted for the former approach for the following reasons: 1. for decades, all relevant data are published in English in the medical field and 2. for our question of interest, it was necessary to study the whole article and not only the abstract (usually provided in English). In a next step, from the articles selected, all relevant data are collected and analyzed. In contrast to a systematic review, a quantitative summary of results characterize a meta-analysis. As the majority of studies addressing our question of interest were cohort studies and only a few were randomized, controlled trials, we performed a systematic review without a meta-analysis.

IV.3 Measures of incidences and their problems

When collecting data, we faced another problem: the fact that different measures of incidence were calculated in different articles. Crude incidence refers to the number of cases with t-MDS/t-AML divided by the total number of patients enrolled into the study (Armitage et al., 2003). It obviously increases with time. However, as there is competing risk – death due to lymphoma or other causes – crude incidence underestimates the true incidence. The assessment of actuarial incidence (actuarial risk) is based on Kaplan-Meier estimates but does not take competing risks into account, either. Actuarial risk usually overestimates the true incidence of t-MDS/t-AML (Armitage et al., 2003). Cumulative incidence estimates the percentage of patients who will be diagnosed with t-MDS/t-AML in a certain time interval in the presence of competing risks. It is also described as competing risk analysis or absolute cause-specific risk analysis (Armitage et al., 2003). With respect to our study, crude incidence is by far the most quoted incidence. Actuarial as well as cumulative incidences are only mentioned in two studies each.

Our literature search from 1970, when the first studies on t-MDS/t-AML were published, to September 2008 revealed 4040 potentially appropriate articles. The majority of them were excluded as they did not meet our strict inclusion criteria. Among them, a follow-up period of at least five years was the most important one (Armitage et al., 2003, Mudie et al., 2006, Bennett et al., 2005). This reflects the median latency period of five years between initiation of cytotoxic therapies and diagnosis of t-MDS/t-AML. Studies with a shorter follow-up would underreport t-MDS/t-AML incidence. We have also contacted corresponding authors for essential data and – unexpectedly – faced a high response rate. Nine of 26 authors (34%) responded and provided information that was included in this systematic review. A number of articles were updates of former reports. In these cases, only the most recent one was included into our systematic review in order to avoid data duplication.

IV.4 Discussion of the results

Thirty-five publications were analyzed in detail. Of those, 32 were cohort studies and three randomized controlled trials. In only four studies, actuarial or cumulative incidences were stated in addition to the crude incidence (Travis et al., 1996, Brown et al., 2005, Micallef et al., 2000, Ladetto et al., 2006). Therefore, assessment of t-MDS/t-AML incidence following therapy for NHL is almost exclusively based on crude incidence and should be interpreted with caution as outlined above. The crude incidence of t-MDS/t-AML in patients conventionally treated was between 0 (Paryani et al., 1984) and 8.2% (Travis et al., 1996), in patients undergoing ASCT between 0 (Taylor et al., 1997) and 13.6% (Berglund et al., 2000). There were more studies in the ASCT group with a higher t-MDS/t-AML incidence which is in line with published narrative reviews on this topic. One ASCT study showed an exceedingly high actuarial incidence of 36.5% at ten years (95% confidence interval, 20.6 to 50.1%) (Micallef et al., 2000). This high incidence exemplifies the already discussed problem encountered with actuarial incidence analyses in the presence of competing risk: of 230 NHL patients enrolled in this trial, only 27 remained disease free of whom 21 developed t-MDS/t-AML.

With respect to the higher incidence of t-MDS/t-AML after ASCT, an important question is whether this is due to the pretransplant therapy or a result of the autologous transplantation process itself (Hake et al., 2007). Laboratory evidence pinpoint to pre-ASCT therapy as patients showed abnormal bone marrow metaphases on cytogenetic analysis already at the time before transplant. These individuals developed t-MDS/t-AML posttransplantation at high frequency (Lillington et al., 2001). To approach this question clinically, two parameters are useful in this respect: 1. a comparison between the numbers of conventional chemo-/radiotherapy cycles and t-MDS/t-AML incidences in both arms and 2. a comparison of the latency period calculated from the first cytotoxic therapy and ASCT, respectively. A comparably similar interval would speak for a major role of the ASCT process. However, we were faced with the fact that these informations were not available to a sufficient extent in the articles selected. Screening the published literature on this question revealed evidence for a possible contribution of conventional therapies as well as ASCT. Patients who are selected for ASCT are often those with resistant and relapsed disease. They therefore have already undergone several regimens of cytotoxic therapy which certainly induce genetic damage in haematopoietic stem cells (Kalaycio et al., 2006, Kantarjian and Keating, 1987). On the other hand, these cells may be damaged during priming chemotherapy and the concomitant application of the granulocyte-colony stimulating factor, which is a prerequisite for ASCT. Finally, despite “myeloablative” conditioning regimens, some hematopoietic stem cells may survive giving rise to a malignant clone (Schouten et al., 2003). Despite several attempts, it is still not possible to unambiguously clarify this issue. One reason is related to the fact, that many different therapies are applied during the course of the disease and ASCT is never compared upfront in a randomized way. However, during this systematic review, we have also encountered weaknesses of studies which not only made a comparison between conventional therapies and ASCT impossible but also lead to exclusion of articles otherwise appropriate for a systematic review. We have therefore summarized these essential data in Table 10 and would encourage future authors to include them into their reports.

Table 10: Data necessary for proper assessment of t-MDS/AML risk

Median follow-up from time of the primary disease
Median latency period from first cytotoxic therapy
Median latency period form ASCT
Median age at first treatment
Median age at diagnosis of t-MDS/t-AML
Number of cytotoxic therapies pre-ASCT
Cumulative doses of chemo- and radiotherapy
Stem cell mobilisation regimen +/- G-CSF
Conditioning for ASCT
Crude incidence of t-MDS/t-AML
Cumulative incidence of t-MDS/t-AML

The group of patients treated with ASCT shows a higher incidence of t-MDS/t-AML than the conventionally treated one. This suggests that toxicity plays a major role in the pathogenesis of these secondary leukemias. It nevertheless raises the question on host related factors influencing t-MDS/t-AML risk. There is increasing evidence that t-MDS/t-AML develops on the basis of genetic predisposition. First observations came from patients with neurofibromatosis 1 who are at risk for a number of primary malignancies. If these tumors are treated by cytotoxic therapies, an elevated rate of t-MDS/t-AML is observed (Maris et al., 1997). Neurofibromatosis 1 is a hereditary disorder caused by mutations in the *Nf1* gene. In addition, when heterozygous *Nf1* mice are treated with alkylating agents or radiotherapy, an elevated rate of neural crest tumors and myeloid malignancies is observed (Chao et al., 2005). Several groups have investigated the role of genetic polymorphisms in the pathogenesis of t-MDS/t-AML. The most likely candidate genes are those which encode proteins involved in drug metabolism and DNA repair. However, although some polymorphisms were described that alter t-MDS/t-AML risk, the results are conflicting and are not being used as clinical parameters in patients undergoing cytotoxic therapies (Seedhouse and Russell, 2007). Our own group has recently discovered two germline mutations in the *C-RAF* proto-oncogene which are transforming and anti-apoptotic. One of them was inherited over at least four generations, the other a de novo mutation. Although rare, these mutations were the first germline mutations

described in patients with t-MDS/t-AML who do not belong to any of the known tumor predisposition syndromes (Zebisch et al., 2006).

IV.5 Outlook

In summary, we have performed the first systematic review on incidences of t-MDS/t-AML following conventional therapy and ASCT for NHL. The data obtained pinpoint to a higher incidence following high-dose therapy and imply a role for toxicity in the pathogenesis of these secondary disorders. However, we have faced a number of essential data missing which made a proper analysis of risk factors impossible and recommend inclusion of these data into further studies. The fact that toxicity plays a role in t-MDS/t-AML risk may also have clinical implications (Tward et al., 2006, Sacchi et al., 2008). Novel therapeutic strategies in NHL patients aim at proper risk assessment for patients with certain lymphoma entities (Brown et al., 2005). For example, the international prognostic index, initially developed for patients with high-grade lymphomas, is now also available for patients with follicular and mantle cell lymphoma. These parameters have clinical implications with respect to high-dose regimens or novel approaches like radioimmunotherapies, which are applied early in the course of the disease in high-risk patients. By reducing toxicity it might be possible to reduced t-MDS/t-AML risk. However, ultimately it is desirable to directly assess genetic risk factors for t-MDS/t-AML which may influence the therapeutic algorithm for patients with NHL (Calzone et al., 2006).

V. Appendix: summary of included articles

Author	Andre 2004
Studydesign	1
Study group	2837(2837 CTX)
Median follow-up(mo)	74
Latency period(y)	*nd
Cases of t-MDS/t-AML	11

Author	Aviles 2002
Studydesign	1
Study group	469(218CTX, 251 CRTX)
Median follow-up(mo)	163,2
Latency period(y)	nd
Cases of t-MDS/t-AML	4

Author	Blayney 2003
Studydesign	1
Study group	88(CTX)
Median follow-up(mo)	61,2
Latency period(y)	nd
Cases of t-MDS/t-AML	1

Author	Berglund 2000
Studydesign	1
Study group	22(ASCT)
Median follow-up(mo)	74
Latency period(y)	5
Cases of t-MDS/t-AML	3

Author	Brown 2005
Studydesign	1
Study group	605(ASCT)
Median follow-up(mo)	114
Latency period(y)	nd
Cases of t-MDS/t-AML	68

Author	Corradini 2004
Studydesign	1
Study group	60(ASCT)
Median follow-up(mo)	74,4
Latency period(y)	4mo 1y
Cases of t-MDS/t-AML	2

Author	Ladetto 2006
Studydesign	1
Study group	92(ASCT)
Median follow-up(mo)	68
Latency period(y)	nd
Cases of t-MDS/t-AML	5

Author	Mc Laughlin 1987
Studydesign	1
Study group	74(CRTX)
Median follow-up(mo)	78
Latency period(y)	nd
Cases of t-MDS/t-AML	0

Author	Mc Manus 1996
Studydesign	1
Study group	177(RTX)
Median follow-up(mo)	92,4
Latency period(y)	nd
Cases of t-MDS/t-AML	1

Author	Leung 2001
Studydesign	1
Study group	497(259CTX, 238CRTX)
Median follow-up(mo)	100,8
Latency period(y)	nd
Cases of t-MDS/t-AML	7

Author	Mahé 2003
Studydesign	1
Study group	49(ASCT)
Median follow-up(mo)	75,6
Latency period(y)	nd
Cases of t-MDS/t-AML	3

Author	Micallef 2000
Studydesign	1
Study group	230(ASCT)
Median follow-up(mo)	72
Latency period(y)	nd
Cases of t-MDS/t-AML	27

Author	Mora 2003
Studydesign	1
Study group	95 (33CTX,62CRTX)
Median follow-up(mo)	198
Latency period(y)	nd
Cases of t-MDS/t-AML	1

Author	Nissen 1983
Studydesign	1
Study group	73 (33RTX,40CRTX)
Median follow-up(mo)	60
Latency period(y)	nd
Cases of t-MDS/t-AML	0

Author	Seymour 2003
Studydesign	1
Study group	102(CRTX)
Median follow-up(mo)	120
Latency period(y)	5,45
Cases of t-MDS/t-AML	2

Author	Taylor 1997
Studydesign	1
Study group	62 (ASCT)
Median follow-up(mo)	61,2
Latency period(y)	nd
Cases of t-MDS/t-AML	0

Author	Paryani 1984
Studydesign	1
Study group	66(53RTX ,13CRTX)
Median follow-up(mo)	114
Latency period(y)	nd
Cases of t-MDS/t-AML	0

Author	Vitolo 2005
Studydesign	2
Study group	126 (60ASCT, 66 Conv Tx)
Median follow-up(mo)	78
Latency period(y)	nd
Cases of t-MDS/t-AML	2/ 0

Author	Travis 1996
Studydesign	1
Study group	61 (20 RTX, 41 CRTX)
Median follow-up(mo)	103,2
Latency period(y)	nd
Cases of t-MDS/t-AML	5

Author	Pan 2003
Studydesign	1
Study group	25 (25 CTX)
Median follow-up(mo)	74,3
Latency period(y)	nd
Cases of t-MDS/t-AML	0

Author	Ersbol 1989
Studydesign	1
Study group	127 (73CTX; 23RTX; 25CRTX)
Median follow-up(mo)	76,8
Latency period(y)	nd
Cases of t-MDS/t-AML	6

Author	Lavey 1990
Studydesign	1
Study group	686 (322 CTX; 72RTX; 292 CRTX)
Median follow-up(mo)	66
Latency period(y)	nd
Cases of t-MDS/t-AML	9

Author	Robertson 1994
Studydesign	1
Study group	1374(CTX)
Median follow-up(mo)	86,4
Latency period(y)	nd
Cases of t-MDS/t-AML	3

Author	Tward 2006
Studydesign	1
Study group	77876 (Conv.)
Median follow-up(mo)	88,8
Latency period(y)	nd
Cases of t-MDS/t-AML	243

Author	Callea 2006
Studydesign	1
Study group	389 (CTX)
Median follow-up(mo)	78
Latency period(y)	nd
Cases of t-MDS/t-AML	4

Author	Flinn 2000
Studydesign	1
Study group	241 (CTX)
Median follow-up(mo)	111,6
Latency period(y)	nd
Cases of t-MDS/t-AML	1

Author	Sacchi 2008
Studydesign	1
Study group	563 (CTX, CRTX)
Median follow-up(mo)	61,2
Latency period(y)	nd
Cases of t-MDS/t-AML	12

Author	Cheson 1999
Studydesign	1
Study group	1814 (CTX)
Median follow-up(mo)	61,2; 82,8; 88,8
Latency period(y)	nd
Cases of t-MDS/t-AML	5

Author	Bennett 2005
Studydesign	1
Study group	995 (CTX, RIT)
Median follow-up(mo)	72
Latency period(y)	nd
Cases of t-MDS/t-AML	33

Author	Sebban 2006
Studydesign	2
Study group	340 (209 CTX; 131 ASCT)
Median follow-up(mo)	91,2
Latency period(y)	nd
Cases of t-MDS/t-AML	2 (CTX) /4 (ASCT)

Author	Gribben 2005
Study design	1
Study group	137 (ASCT)
Median follow-up(mo)	78
Latency period(y)	nd
Cases of t-MDS/t-AML	13

Author	Thompson 2008
Study design	1
Study group	194 (ASCT)
Median follow-up(mo)	75,6
Latency period(y)	nd
Cases of t-MDS/t-AML	4

Author 2003	Schouten
Study design	2
Study group	89(24Conv.; 65 ASCT)
Median follow-up(mo)	68,4
Latency period(y)	nd
Cases of t-MDS/t-AML	0

Author	Mudie 2006
Study design	1
Study group	2456
Median follow-up(mo)	92,4
Latency period(y)	nd
Cases of t-MDS/t-AML	9

Author	Gopal 2003
Study design	2
Study group	27(HD-RIT)
Median follow-up(mo)	86,4
Latency period(y)	nd
Cases of t-MDS/t-AML	2

*nd… not determined

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