Eye involvement in **haematologic malignancies**

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Eye involvement in haematologic malignancies

Betrokkenheid van het oog bij hematologische maligniteiten
(met een samenvatting in het Nederlands)

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Paranimfen

Viera Kalinina Ayuso

Ymkje Hettinga

Ithaka

Als je doel Ithaka is en je vertrekt daarheen,
dan hoop ik dat je tocht lang zal zijn,
en vol nieuwe kennis, vol avontuur.

Vrees geen Laistrigonen en Kyclopen, of een woedende Poseidon;
je zult ze niet tegenkomen op je weg, als
je gedachten verheven zijn, en emotie
je lichaam en geest niet verlaat.
Laistrigonen en Kyclopen, en de razende Poseidon
zul je niet tegenkomen op je weg,
als je ze al niet meedroeg in je ziel, en
je ziel ze niet voor je voeten werpt.

Ik hoop dat je tocht lang mag zijn,
de zomerochtenden talrijk zijn, en
dat het zien van de eerste havens
je een ongekende vreugde geeft.
Ga naar de warenhuizen van Fenicië,
neem er het beste uit mee.
Ga naar de steden van Egypte, en
leer van een volk dat ons zoveel te leren heeft.

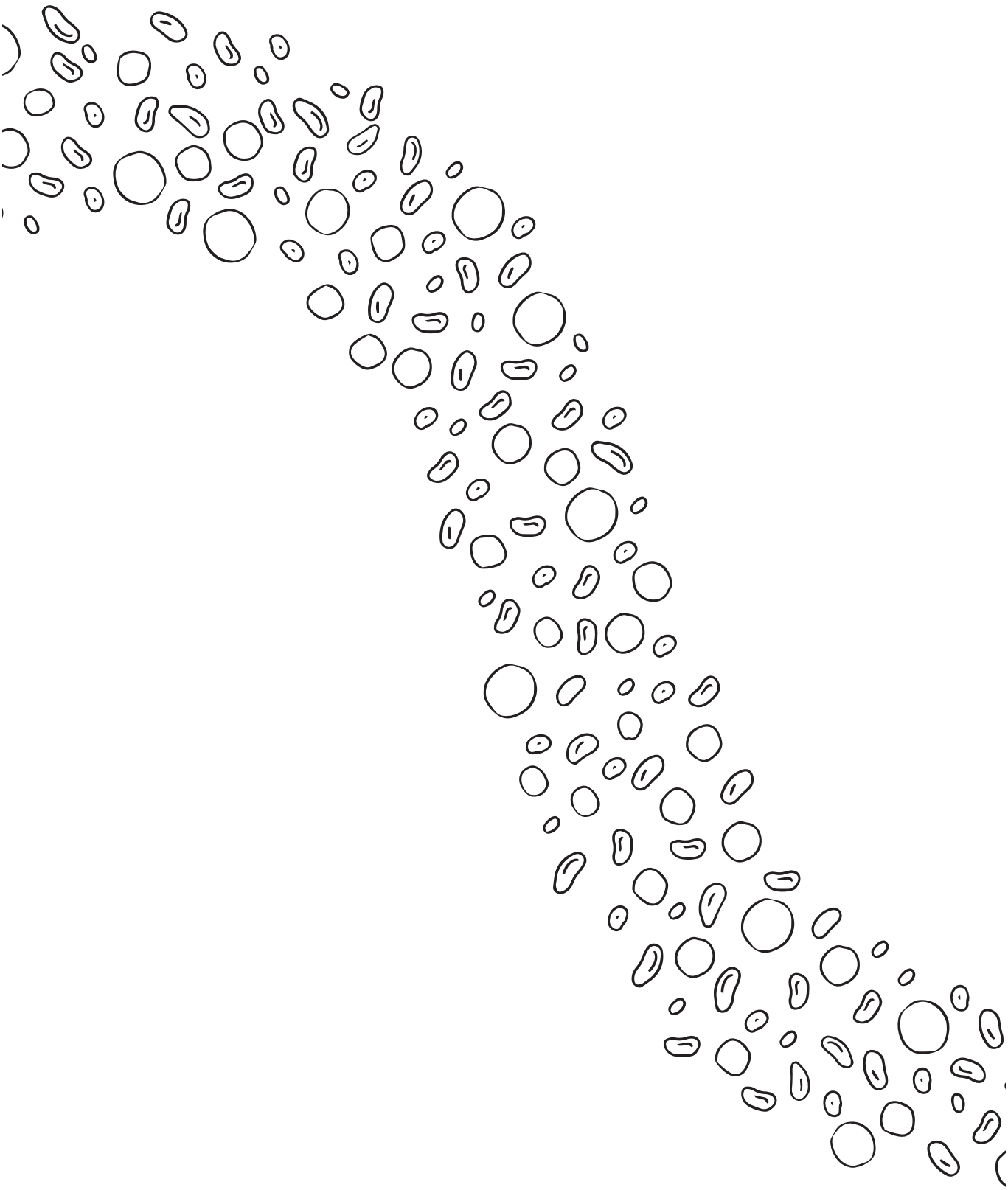
Verlies Ithaka niet uit het oog;
daar aankomen was je doel.
Maar haast je stappen niet;
het is beter dat je tocht duurt en duurt
en je schip pas ankert bij Ithaka,
wanneer je rijk geworden bent
van wat je op je weg hebt geleerd.

Verwacht niet dat Ithaka je meer rijkdom geeft.
Ithaka gaf je een prachtige reis;
zonder Ithaka zou je nooit vertrokken zijn.
Het gaf je alles al, meer geven kan het niet.

En mocht je vinden dat Ithaka arm is,
denk dan niet dat het je bedroog.
Want je bent een wijze geworden, hebt intens geleefd,
en dat is de betekenis van Ithaka.

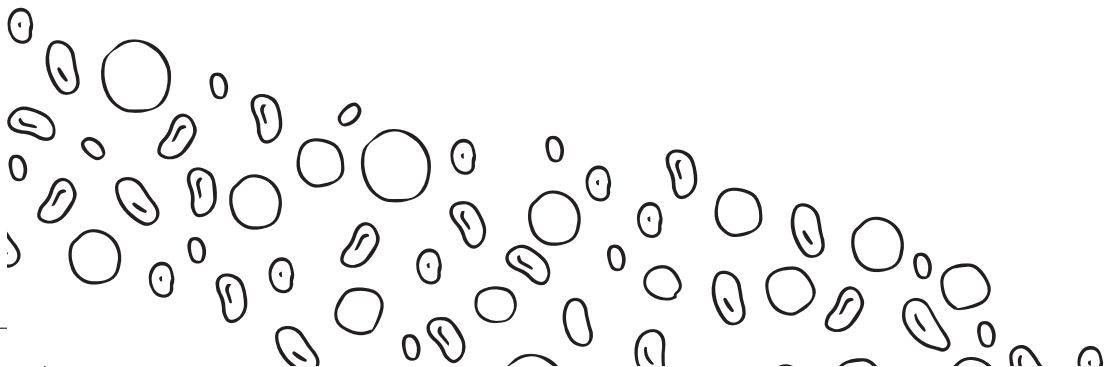
Konstantinos Kavafis (1863-1933)

Contents



Chapter 1

General introduction and aims



General introduction

Ocular involvement in patients with haematological malignancies remains frequently unrecognised and is in consequence not adequately treated. These patients are commonly severely ill and ocular problems might be overshadowed by more severe and acute systemic problems. However, ocular involvement in these malignancies may cause permanent damage of visual acuity and its timely recognition and treatment can prevent unnecessary visual loss.

In this thesis we focus on two major ophthalmologic topics in haematological malignancies: intraocular lymphoma and ocular graft versus host disease.

Primary vitreoretinal lymphoma

Background

Haematological malignancies that can metastasize to the eye include acute and chronic leukaemia, (non) Hodgkin lymphomas and multiple myeloma[1]. Table 1 shows an overview of the most common subtypes of these haematological malignancies. Leukaemia is the haematological malignancy that most commonly occurs in the eye. Autopsy studies reveal localisation of leukaemia in the eye in 28% to 50% of patients with this haematological malignancy, which mainly consists of choroidal involvement. However a much lower number of patients present with clinical symptoms[2-4]. In multiple myeloma, cysts of the ciliary body as well as retinal involvement have been reported in up to 50% of patients[1]. Although the eye can be involved in Hodgkin's lymphoma, the involvement in different subtypes of Non-Hodgkin's lymphoma is more common and includes predominantly diffuse large B-cell lymphoma (DLBCL), mucosa-associated lymphoid tissue (MALT) lymphoma and T-cell lymphoma. The eye is most frequently involved in DLBCL and its involvement might represent either a primary localisation or metastasis.

Primary localisation of lymphoma in the eye might be referred to as primary intraocular lymphoma (PIOL). Primary vitreoretinal lymphoma (PVRL) is often described as a subtype of this disease. The terminology in the literature is not consistent and there are no international guidelines on which term should be used in specific situations. In this thesis the term PVRL will be used for primary intraocular manifestation of non-Hodgkin lymphoma. In most cases PVRL consists of a diffuse large B-cell lymphoma (DLBCL), though in scarce cases T-cell lymphoma might be diagnosed. Vitreoretinal lymphoma has been described to be a subtype of primary nervous system lymphoma (PCNSL); however PVRL presents *de novo* in the ocular tissue. The eye, likewise the CNS, is an immune privileged site, which makes the pathogenesis of PVRL intriguing because the immune system behaves in a different way than in sites that are not immunoprivileged.

Clinical presentation

PVRL mimics the symptoms and signs of uveitis and is therefore known to be a "masquerade" syndrome, which often causes a delay in the diagnosis (figure 1 and 2). PVRL most often occurs in elderly patients (median age 60-70 years) however it is also seen in younger individuals, particularly in those who are immunocompromised (e.g. with HIV infection or following organ transplantation). PVRL should be suspected in elderly patients with uveitis refractory to standard treatment. The most common symptoms include blurred or decreased vision and floaters[5-7]. Patients occasionally report ocular pain, a foreign body sensation or photopsia.

Lymphoma	
Hodgkin lymphoma	Classic Hodgkin lymphoma
	Nodular sclerosis Hodgkin lymphoma
	Mixed cellularity Hodgkin lymphoma
	Lymphocyte-depleted Hodgkin lymphoma
	Lymphocyte-rich classic Hodgkin lymphoma
	Nodular lymphocyte-predominant Hodgkin lymphoma
Non-Hodgkin lymphoma	B-Cell Lymphoma Subtypes
	Diffuse large B-cell lymphoma
	Follicular lymphoma
	Mucosa-associated lymphatic tissue (MALT) lymphoma
	Small cell lymphocytic lymphoma/chronic lymphocytic leukemia
	Mantle cell lymphoma
	Mediastinal (thymic) large B-cell lymphoma
	Lymphoplasmacytic lymphoma and Waldenstrom macroglobulinemia
	Nodal marginal zone B-cell lymphoma
	Splenic marginal zone lymphoma
	Extranodal marginal zone B-cell lymphoma
	Intravascular large B-cell lymphoma
	Primary effusion lymphoma
	Burkitt lymphoma-Burkitt leukemia
	Lymphomatoid granulomatosis
	T-Cell and Natural Killer Cell Lymphoma Subtypes
	Peripheral T-cell lymphoma, not otherwise specified
	Cutaneous T-cell lymphoma (Sezary syndrome and mycosis fungoides)
	Anaplastic large cell lymphoma
	Angioimmunoblastic T-cell lymphoma
	Lymphoblastic lymphoma (can sometimes be a B-cell subtype)
	NK-cell lymphoma
Leukemia	
Myelogenous	Acute myeloid leukaemia (AML)
	Chronic myeloid leukaemia (CML)
	Chronic myelomonocytic leukaemia (CMML)
	Juvenile myelomonocytic leukaemia (JMML)
Lymphocytic	Acute lymphoblastic leukaemia (ALL)
	Chronic lymphocytic leukaemia (CLL)
	Hairy cell leukaemia
Myeloma	
	Multiple myeloma
	Plasmacytoma
	Localized myeloma
	Extramedullary myeloma

Table 1.
Overview of major myeloid and lymphoid haematological malignancies

Typical signs include clumps or sheets of cells in the vitreous humour[6]. These cells consist of a combination of lymphoma cells and reactive inflammatory cells. Less frequently multifocal, cream-colored, subretinal lesions are observed, as well as pigmentary changes giving a tigroid aspect to the choroid (figure 3 and 4)[5, 6]. Other less common signs include retinal detachment, optic neuropathy and a variety of chorioretinal abnormalities. Anterior segment findings are rare and generally include corneal precipitates, mild anterior flare and occasionally pseudohypopyon. In addition elevated intraocular pressure in an eye with retinal detachment is occasionally encountered. The most common fluorescent angiographic findings are granularity, late staining, and small foci of blockage at the level of the retinal pigment epithelium (RPE)[8]. In some cases thickening of the choroid can be disclosed on ultrasonography[9].

PVRL may be unilateral or bilateral on initial presentation, but ultimately 80% to 90% of patients will have bilateral involvement[10].

Diagnosis

Clinical diagnosis is often difficult and requires experience in the interpretation of ophthalmologic imaging because symptoms and signs of PVRL can mimic many other intraocular conditions ("masquerade" syndrome). Confirmation of the clinical suspicion of PVRL requires ocular fluid or tissue sampling followed by morphological and immunocytological analysis by an experienced pathologist.

Vitreous tissue can be obtained by vitreous biopsy or pars plana vitrectomy (PPV). The diagnostic yield is usually better in PPV and since new PPV techniques are associated with less complications and offer a large volume of the sample, PPV is usually preferred. Unfortunately, cytology of vitreous specimens is frequently not informative, does not contain any neoplastic cells and only inflammatory reaction can be encountered (for example in cases with minimal vitreal involvement)[11]. Multiple vitreous biopsies are often needed to confirm the diagnosis and sometimes even chorioretinal biopsy is needed to confirm the diagnosis of PVRL. This diagnostic quest can cause both a delay in diagnosis and consequently in start of treatment. An 8-21 months delay in diagnosis is commonly described[8, 12-18].

It is of great importance that the vitreous sample obtained for diagnosing intraocular lymphoma is properly handled, there should be as little delay as possible between aspiration and examination of the sample; adding a culture medium (e.g. foetal calf serum) to the vitreous sample can improve the survival and viability of the malignant cells[18-20]. Treatment with corticosteroids prior to sampling might be associated with decreased diagnostic outcome since steroids might decrease the number of lymphoma cells and less viable lymphoma cells might remain because of the cytolytic effect of these agents on lymphoma cells[20].

Although cytology is being considered as a mainstay of the diagnosis of PVRL, flow cytometry was recently reported as a useful diagnostic tool (sensitivity of 82.4% and specificity of 100%) [21]. Flow cytometry requires a smaller biopsy volume and should therefore be considered as a diagnostic tool for PVRL when the volume of the vitreous sample is limited. If sufficient volume of the vitreous sample remains, other

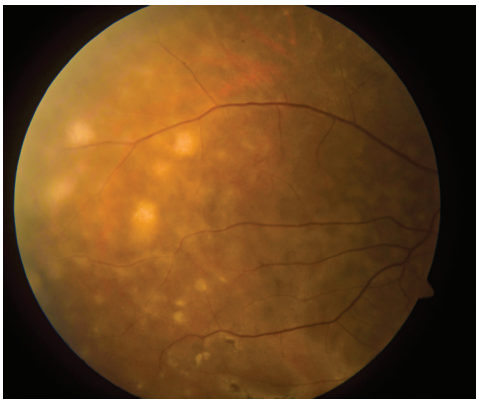


Figure 1.
Primary vitreoretinal lymphoma presenting as masquerade syndrome with multifocal white dots resembling multifocal chorioretinitis

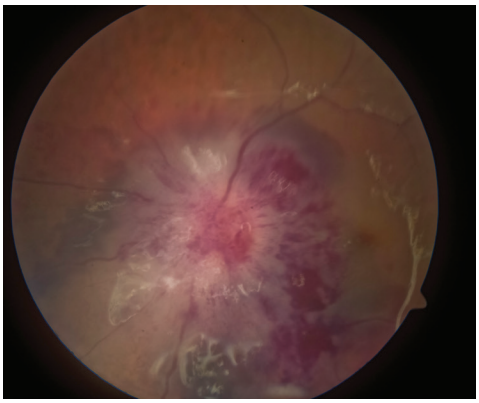


Figure 2.
Swelling and haemorrhages of optic nerve head caused by lymphoma infiltration of the optic nerve

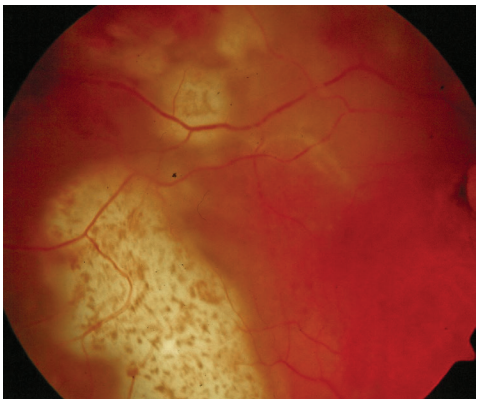


Figure 3.
Cream-colored subretinal lesions with retinal pigment alterations in primary vitreoretinal lymphoma

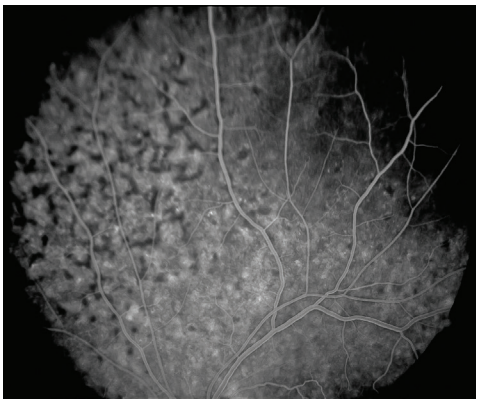


Figure 4.
Indocyanine green angiography: retinal pigment changes with a typical tygroid aspect in a patient with primary vitreoretinal lymphoma

tests, such as immunohistochemistry, cytokine measurements, and micro dissection combined with the PCR may confirm the diagnosis. Another supporting measure is a high IL-10 to IL-6 ratio (greater than 1) because lymphoma B-cells produce IL-10, whereas in uveitis IL-6 is usually elevated. However IL-10 to IL-6 ratio does not confirm the diagnosis PVRL, but was proposed to be valuable for monitoring the effect of treatment[22-30]. In patients with confined retinal or subretinal lesions and negative vitreous samples for PVRL, (sub)retinal biopsy should be considered to confirm the diagnosis of PVRL.

If the diagnosis PVRL is confirmed, patients always need to undergo staging, with a special emphasis on lymphoma localisation in the brain and/or the cerebrospinal fluid.

Differential diagnosis

Differential diagnosis is very broad and includes all types of uveitis involving the posterior eye segment. PVRL should especially be suspected in patients with signs of uveitis of unknown aetiology after screening for uveitis and those who do not respond to standard treatment regimens.

Relationship of ocular and CNS involvement

Approximately 80% of the patients with PVRL subsequently develop lymphoma of the cerebral parenchyma, spinal cord or meninges[18, 31-33]. Conversely, it has been reported that patients with PCNSL develop ocular manifestation in 15-25%[8, 34-37]. The close association of vitreoretinal lymphoma (VRL) and CNSL is not surprising, considering the embryological origin of these two organs. Whether oculocerebral lymphoma is a consequence of direct infiltration along the optic nerve, metastatic spread or multifocal tumour development remains to be clarified. Involvement of the CNS can be focal and/or diffuse and frequently occurs in the frontal lobe. Interestingly, VRL and CNSL usually remain limited to the CNS and the eyes. The reason for this confinement is as yet unclear; however, to some extent it may be explained by the chemokine receptor expression pattern of the neoplastic cells or immunoprivileged character of both sites[38-40].

Impact and prognosis

PVRL has a high mortality rate and a major impact on the quality of life because a large proportion of patients develop subsequent CNSL. Patients might suffer from CNS damage caused directly by the lymphoma or from the diverse treatment regimens, for example because of the development of radiation encephalopathy after irradiation of the brain.

Currently the median survival of patients treated for PCNSL ranges from approximately 9 to 27 months[41-43]. Prognostic factors for PCNSL include age, performance status[44-45], lactate dehydrogenase (LDH) and CSF protein level and deep brain structure involvement[46]. The majority of patients that develop PCNSL are older than 60 years which is associated with a worse survival than age younger than 60 years[47-49].

The median survival for PVRL has been reported to be 58 months[50-51]. Another important factor that specifically influences the prognosis is the delay in diagnosis and treatment of PVRL with anti-inflammatory medications because it is often wrongly diagnosed as uveitis. Patients are often treated as having uveitis until CNSL manifestations develop which deteriorates the prognosis dramatically. Since PVRL is a rare disease no prognostic model could yet be developed; factors that assumingly influence the prognosis are localisation (worse prognosis if PVRL is breaking through retinal pigment epithelium or infiltrates in vitreous and/or optic nerve) and treatment sensitivity[52]. It is not known whether prognostic factors for CNSL can be applied to PVRL and whether uni- or bilateral disease does influence the prognosis.

Treatment of combined PCNS and VRL

Treatment of PCNSL greatly changed within the last decades: its treatment modalities are rapidly evolving but reported treatment series are usually small[31]. The issues that must be considered include the efficacy of treatment against the overall poor prognosis of the disease, the extent of the disease, and the side effects of treatment (e.g. dementia). In the past various systemic therapies have been employed, combined with and without the use of radiotherapy, and have achieved remissions, although relapses were common. Conventional therapies, such as CHOP (cyclophosphamide, adriamycin, vincristine, and prednisone) and high-dose methotrexate have also been used[31]. Hematopoietic stem-cell rescue has been employed after intense chemotherapy for refractory disease[53-54]. In the Netherlands eligible patients may be included in the Hovon 105 study protocol[55]. Patients with a newly diagnosed PCNSL are randomized at entry between Arm A: 2 courses of MBVP chemotherapy (methotrexate, BCNU (carmustine), Vumon (teniposide) and prednisone) or Arm B: 2 courses of MBVP chemotherapy combined with intravenous rituximab. Patients that respond to MBVP chemotherapy are consolidated with high dose-Ara-C chemotherapy. In addition patients younger than 60 years will be further consolidated with whole brain radiotherapy. Patients with PVRL and lymphoma cells in cerebrospinal fluid (CSF) are also included in this study protocol.

Treatment of PVRL

In contrast to PCNSL, guidelines for PVRL treatment are lacking and many diverse approaches are being used. It is not known whether PVRL should be treated as a separate entity limited to the eye(s) with local treatment or whether systemic treatment should be used accordingly to the PCNSL protocol.

Local treatment possibilities for the eye include radiotherapy of the eye(s), intravitreal methotrexate and / or rituximab[56]. Due to possible cataract formation and retinal complications of radiation and the fact that this treatment cannot be repeated if the patient relapses, intravitreal treatment has become more popular for both isolated and recurrent ocular disease. Intravitreal methotrexate has been used successfully in some patients to treat local recurrences, but disease relapse is common when therapy is discontinued and resistant cases have occurred[57]. Most PVRLs are B-cell lymphomas that express CD20 on their cell surface. CD20 is an optimal target because it is neither shed nor internalized and is not found unbound in circulation. Rituximab, a humanized monoclonal antibody directed against CD20 antigen, has recently been used both systemically and locally for PVRL with promising results. In scarce case reports of patients with PVRL, intraocular rituximab was used and the results were encouraging since several patients responded well to this treatment modality[58-60].

Local versus Systemic treatment of PVRL

Grimm *et al*. reported 83 patients with intraocular lymphoma that were classified in a local treatment group (intra-ocular methotrexate, ocular radiotherapy) or an extensive treatment group (systemic chemotherapy, whole brain radiotherapy)[50]. The authors concluded that local therapy alone did not increase the risk of brain relapse in patients with PVRL. In a population of 83 patients median progression-free and overall

survival were 29.6 and 58 months, respectively, these were unaffected by treatment type (local or extensive). However in these 83 patients classified as isolated PVRL, a substantial group already had positive CSF for malignant cells. Therefore the data are not reliable.

In 2008, the international PCNSL collaborative group analysed 221 patients with PCNSL with intraocular involvement, from 16 centers. They reported that patients who were treated with ocular treatment in 102/221 patients (79 with ocular radiotherapy, 22 with intravitreal methotrexate, and 1 with both) in addition to systemic treatment of their CNSL (systemic chemotherapy and/or whole brain radiotherapy) had a better progression free survival compared to systemic treatment only. However overall survival was not influenced by treatment type (local versus systemic)[51].

Challenges

As PVRL is associated with a poor survival, with most patients dying of CNS disease, early diagnosis and appropriate treatment strategy are of crucial value. Treatment of PVRL is aimed at eradicating ocular lymphoma cells and preventing lymphoma localisation in the CNS. The therapeutic strategy for patients with intraocular lymphoma, without proven intracerebral localisation with MRI imaging and/or laboratory studies of cerebrospinal fluid, is not clear. There are two distinct approaches to these patients. The first approach favours the application of early aggressive systemic treatment (systemic chemotherapy and/or whole brain radiotherapy) and is based on the presumption that the patients already have developed microscopic locations of lymphoma in the brain at the time of the diagnosis of PVRL. However, systemic treatment might be too aggressive in patients without any proof of CNS involvement and its efficacy for PVRL is not evaluated. The second approach is local therapy and is aimed at eradication of lymphoma cells in the eye; nonetheless local therapy has no effect on possible subclinical manifestations elsewhere and does not prevent lymphoma development in the CNS or the contralateral eye. The crucial issue that remains to be elucidated for finding the best treatment strategy is whether PVRL and CNSL are of multifocal origin and originate independently in both sites or whether this lymphoma has one site of origin and spreads subsequently from one site to the other.

Ocular Graft-versus-Host Disease

The survival of patients with haematological malignancies has greatly improved in the last decades due to the novel treatment options, including autologous and allogeneic hematopoietic stem cell transplants (figure 5). Allogeneic hematopoietic stem cell is abbreviated as allo-SCT or HSCT; in this thesis both abbreviations are used depending on the preference of co-authors and/or journal. The curative effect of allo-SCT is achieved by the so-called graft-versus-tumour (GvT) effect. A major drawback of allo-SCT is represented by the graft versus patient activity of the new implanted immune system, the so-called graft-versus-host disease (GvHD), which is the major reason for non-relapse mortality and morbidity. The recognition of host antigens as foreign by donor T lymphocytes is the major underlying mechanism of GvT and GvHD, and maintaining GvT while dampening GvHD is the crucial challenge of current investigations. Acute GvHD develops in up to 40% after HLA-matched allo-SCT and is fatal in 10-20% of the patients[61]. Chronic GvHD may develop in up to 70% of the patients, especially when full stem cell grafts are used without T cell depletion[62]. In those cases cumulative incidence of mortality may increase to 20% during follow-up[62]. Also quality of life may be strongly impaired because tissues such as mucosal tissue, skin, liver, lungs, intestines and connective tissue are being affected.

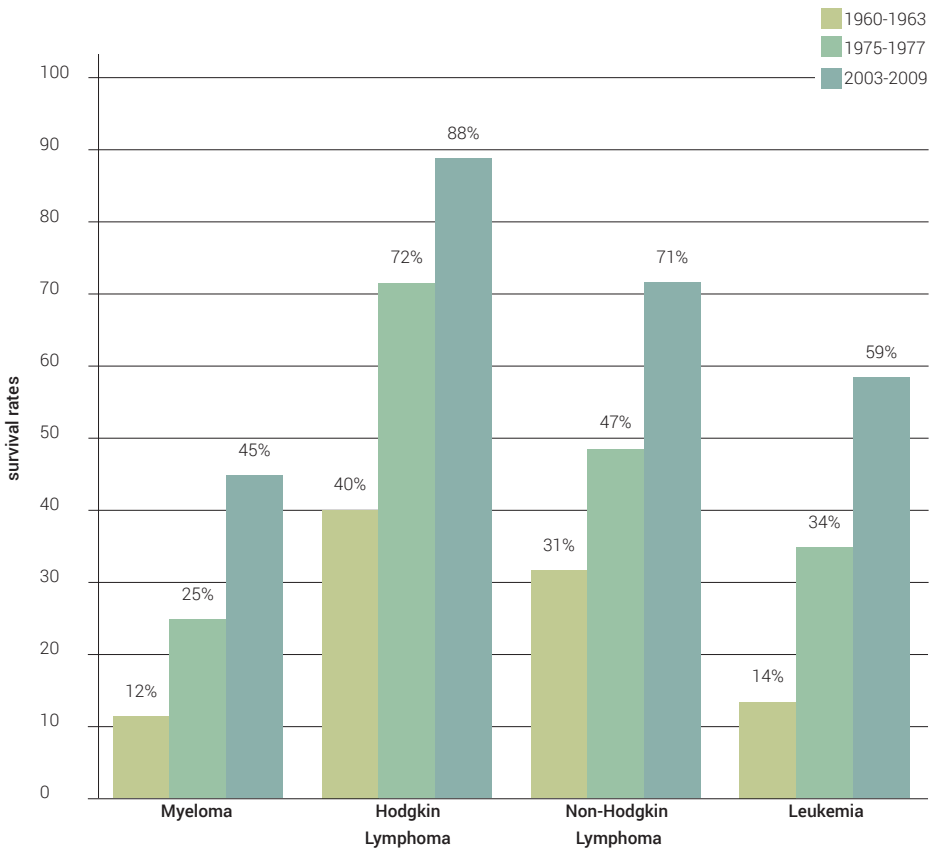
Preventive measures for systemic GvHD consist of immunosuppressive agents such as Cyclosporin A, mycofenolate mofetil and prednisolone, administered generally until 6 months after allo-SCT. Other options to prevent GvHD are in vivo or in vitro T cell depletion of the stem cell graft[63-67]. Because of the development of new allo-SCT techniques and growing knowledge on this treatment modality, the survival of patients after allo-SCT has greatly improved. However, patients may still develop acute and/or chronic GvHD and the downside of improved survival is that patients might suffer from long-term chronic complications of GvHD.

In the eye, the lacrimal gland and/or conjunctival tissue can be involved in GvHD and damage of these structures can cause severe dry eye syndrome (DES)[68-71]. So far, curative treatment options are not available and the main aim of treatment is suppression and relief of symptoms. Since the life expectancy after allo-SCT is much better than before, many patients may suffer from chronic DES for many years. All diverse topical treatment options for ocular GvHD suppress mainly the symptoms and are not curative or preventive[72-81]. Once the lacrimal gland has been scarified, its function cannot be recovered. Systemic treatment during the post-transplant period may not penetrate well enough into the tissue of the lacrimal gland and therefore local application of immunosuppressive agents might be more effective.

Challenges

So far, adequate diagnostic guidelines and the classification of ocular GVHD are lacking, which possibly leads to underdiagnosis and delay of treatment. Systematic studies on prevalence of ocular GVHD, clinical

manifestations, severity and the impact on the quality of life in SCT survivors are lacking. The exact pathogenesis and the sequence of immunological processes occurring in diverse types of ocular GvHD are not identified. Since the survival after allo-SCT has greatly increased there is a need to clarify the long term severity and impact on quality of life in allo-SCT survivors. Although the patients undergoing allo-SCT are being treated systemically with immunosuppressive agents to prevent GvHD, these agents may not sufficiently penetrate into the ocular tissues. Preventive options for ocular GvHD were not systematically studied and the treatment of ocular GvHD is today limited to symptom alleviation. The identification of effective preventive treatment, preferably by local administration, might protect from painful ocular symptoms and unnecessary visual loss in patients who survived allo-SCT.



Sources: SEER (Surveillance, Epidemiology and End Results) Cancer Statistics Review, 1975-2010. National Cancer Institute; 2013 and Fact sheet Leukemia and Lymphoma Society; aug 2013.

Figure 5.
Five year survival rates by year of diagnosis 1960-1963 vs. 1975-1977 vs. 2003-2009

Aims of this thesis

To investigate the most favourable treatment options for PVRL in a multicenter study (chapter 2)

To report on patients exhibiting the association of DLBCL in diverse immunnoprivileged sites such as the eye, testes and central nervous system (chapter 3)

To give an overview of ocular GVHD and point out the state of art of recent treatment options for ocular GvHD (chapter 4)

To define new diagnostic criteria as well as the severity parameters for chronic ocular GvHD in an international setting (chapter 5)

To evaluate the long term visual quality of life in patients after allo-SCT and to identify the visual impact of ocular GVHD (chapter 6)

To investigate the profile of cytokines in tear fluid of patients and hereby gain more insight into pathogenesis of ocular GVHD after allo- SCT and determine the relationship between the tear fluid levels of cytokines and the presence and manifestations of ocular GvHD (chapter 7)

To study the risk factors for ocular GvHD in both adults and children after allogeneic HSCT (chapter 8)

To investigate the safety and potential efficacy of topical Cyclosporin A 0.05% (Restasis) application in preventing ocular GVHD and compare it to artificial tears, one of the standard treatment options (chapter 9)

To investigate the prevalence of intraocular infections after allo-SCT (chapter 10)

References

1. Lin P, Mruthyunjayahoebe P. Retinal Manifestations of Oncologic and Hematologic Conditions. International ophthalmology clinics. 2012; 52 (1): 67–91.

2. Nelson CC, Hertzberg BS, Klintworth GK. A histopathologic study of 716 unselected eyes in patients with cancer at the time of death. Am J Ophthalmol. 1983 Jun; 95 (6): 788-93.

3. Allen RA, Straatsma BR. Ocular involvement in leukemia and allied disorders. Arch Ophthalmol. 1961 Oct; 66: 490-508.

4. Leonardy NJ, Rupani M, Dent G, Klintworth GK. Analysis of 135 autopsy eyes for ocular involvement in leukemia. Am J Ophthalmol. 1990 Apr 15; 109 (4): 436-44.

5. Choi JY, Kafkala C, Foster CS. Primary intraocular lymphoma: A review. Seminars in ophthalmology. 2006; 21 (3): 125–33.

6. Buggage RR, Chan CC, Nussenblatt RB. Ocular manifestations of central nervous system lymphoma. Current opinion in oncology. 2001; 13 (3): 137–42.

7. Coupland SE, Heimann H, Bechrakis NE. Primary intraocular lymphoma: a review of the clinical, histopathological and molecular biological features. Graefes archr clin exp ophthalmol. 2004; 242 (11): 901–13.

8. Cassoux N, Merle-Beral H, Leblond V, *et al*. Ocular and central nervous system lymphoma: clinical features and diagnosis. Ocular immunology and inflammation. 2000; 8 (4): 243–50.

9. Chan C-C, Gonzales JA. Chapter 6 imaging. In: Chan C-C, Gonzales JA. Primary Introcular Lymphoma. E-book; 2007: 79–93.

10. Hormigo A, Abrey L, Heinemann M-H, DeAngelis LM. Ocular presentation of primary central nervous system lymphoma: diagnosis and treatment. British journal of haematology. 2004; 126 (2): 202–8.

11. Chan C-C, Gonzales JA. Chapter 7 Pathology. In: Chan C-C, Gonzales JA. Primary Introcular Lymphoma. E-book; 2007: 94–112.

12. Appen RE. Posterior uveitis and primary cerebral reticulum cell sarcoma. Arch Ophthalmol. 1975; 93: 123–124.

13. Barr CC, Green WR, Payne JW *et al*. Intraocular reticulum-cell sarcoma: clinico-pathologic study of four cases and review of the literature. Surv Ophthalmol, 1975; 19: 224–239.

14. Brown SM, Jampol LM, Cantrill HL. Intraocular lymphoma present- ing as retinal vasculitis. Surv Ophthalmol. 1994; 39: 133–140.

15. Char DH, Ljung BM, Miller T, Phillips T. Primary intraocular lymphoma (ocular reticulum cell sarcoma) diagnosis and management. Ophthalmology. 1988; 95: 625–630.

16. Freeman LN, Schachat AP, Knox DL *et al.* Clinical features, laboratory investigations, and survival in ocular reticulum cell sarcoma. Ophthalmology. 1987; 94: 1631–1639.

17. Peterson K, Gordon KB, Heinemann MH, DeAngelis LM.The clinical spectrum of ocular lymphoma. Cancer. 1993; 72: 843–849.

18. Whitcup SM, de Smet MD, Rubin BI *et al*.Intraocular lymphoma. Clinical and histopathologic diagnosis. Ophthalmology. 1993; 100: 1399–1406.

19. Lobo A, Lightman S. Vitreous aspiration needle tap in the diagnosis of intraocular inflammation. Ophthalmology. 2003; 110 (3): 595–9.

20. Blumenkranz M, Ward T. Applications and limitations of vitreoretinal biopsy techniques in intraocular large cell lymphoma. Retina. 1992; 12 (3): S64–S70.

21. Missotten T, Tielemans D, Bromberg JE, *et al*. Multicolor flowcytometric immunophenotyping is a valuable tool for detection of intraocular lymphoma. Ophthalmology. 2013; 120 (5): 991–6.

22. Wolf L, Reed G, Buggage R. Vitreous cytokine levels. Ophthalmology. 2003: 1671–1672.

23. Matsuoka M, Yoshida H, Kinoshita Y, Nishimura T. Two cases of intraocular lymphoma diagnosed by analyses of vitreous and infusion fluid. Clinical ophthalmology (Auckland, N.Z.). 2013; 7: 691–4.

24. Fisson S, Ouakrim H, Touitou V, *et al*. Cytokine profile in human eyes: contribution of a new cytokine combination for differential diagnosis between intraocular lymphoma or uveitis. PloS one. 2013; 8 (2): e52385.

25. Wang Y, Shen D, Wang VM, Sen HN, Chan C-C. Molecular biomarkers for the diagnosis of primary vitreoretinal lymphoma. International journal of molecular sciences. 2011; 12 (9): 5684–97.

26. Zaldivar RA, Martin DF, Holden JT, Grossniklaus HE. Primary intraocular lymphoma: clinical, cytologic, and flow cytometric analysis. Ophthalmology. 2004; 111: 1762–1767.

27. Cassoux N, Giron A, Bodaghi B, *et al*. IL-10 measurement in aqueous humor for screening patients with suspicion of primary intraocular lymphoma. Invest Ophthalmol Vis Sci. 2007; 48: 3253–3259.

28. Chan CC, Wallace DJ. Intraocular lymphoma: update on diagnosis and management. Cancer Control. 2004; 11: 285–295.

29. Intzedy L, Teoh SC, Hogan A, *et al*. Cytopathological analysis of vitreous in intraocular lymphoma. Eye. 2008; 22: 289–293.

30. Merle-Beral H, Davi F, Cassoux N, *et al*. Biological diagnosis of primary intraocular lymphoma. Br J Haematol. 2004; 124: 469–473.

31. Jahnke K, Thiel E, Abrey LE, Neuwelt E a, Korfel A. Diagnosis and management of primary intraocular lymphoma: an update. Clinical ophthalmology (Auckland, N.Z.). 2007; 1 (3): 247–58.

32. Freeman LN, Schachat AP, Knox DL, *et al*. Clinical features, laboratory investigations, and survival in ocular reticulum cell sarcoma. Ophthalmology. 1987; 94: 1631–9.

33. Akpek EK, Ahmed I, Hochberg FH, *et al*. Intraocular-central nervous system lymphoma. Clinical features, diagnosis and outcome. Ophthamology. 1999; 106: 1805–10.

34. DeAngelis LM .1994. Primary central nervous system lymphoma. Recent Results Cancer Res. 135: 155–169.

35. Hochberg FH, Miller DC. Primary central nervous system lymphoma. J Neurosurg. 1988; 68: 835–853.

36. Peterson K, Gordon KB, Heinemann MH, DeAngelis LM. The clin-ical spectrum of ocular lymphoma. Cancer. 1993; 72: 843–849.

37. Verbraeken HE, Hanssens M, Priem H *et al*. Ocular non-Hodgkin's lymphoma: a clinical study of nine cases. Br J Ophthalmol. 1997; 81: 31–36.

38. Ferguson T a, Griffith TS. A vision of cell death: insights into immune privilege. Immunological reviews. 1997; 156 (1): 167–84.

39. Pe'er J Rowe JM, Frenkel S, Dann, EJ. Testicular lymphoma, intraocular (vitreoretinal) lymphoma, and brain lymphoma: Involvement of three immunoprivileged sites in one patient. American Journal of Hematology. 2010 : 611–633.

40. Riemersma SA, Schuuring E, Kluin PM, *et al*. Extensive genetic alterations of the HLA region, including homozygous deletions of HLA class II genes in B-cell lymphomas arising in Extensive genetic alterations of the HLA region, including homozygous deletions of HLA class II genes in B-cell lymphoma. 2012: 3569–3577.

41. Panageas KS, Elkin EB, DeAngelis LM, *et al*. Trends in survival from primary central nervous system lymphoma, 1975–1999: a population-based analysis. Cancer. 2005; 104 (11): 2466–2472.

42. DeAngelis LM, Seiferheld W, Schold SC, *et al*. Combination chemotherapy and radiotherapy for primary central nervous system lymphoma: Radiation Therapy Oncology Group Study 93–10. J Clin Oncol. 2002; 20 (24): 4643–4648.

43. Xie H, Ahluwalia MS, Peereboom DM. The Cleveland Clinic Experience With Primary Central Nervous System Lymphoma. Am J Clin Oncol. 2013 Apr 19. [Epub ahead of print]

44. Bessell EM, Graus F, Punt JA, *et al*. Primary non-Hodgkin's lymphoma of the CNS treated with BVAM or CHOD/BVAM chemotherapy before radiotherapy. J Clin Oncol. 1996; 14 (3): 945–954.

45. Blay JY, Conroy T, Chevreau C, *et al*. High-dose methotrexate for the treatment of primary cerebral lymphomas: analysis of survival and late neurologic toxicity in a retrospective series. J Clin Oncol.1998; 16 (3): 864–871.

46. Ferreri AJ, Blay JY, Reni M, *et al*. Prognostic scoring system for primary CNS lymphomas: the International Extranodal Lymphoma Study Group experience. J Clin Oncol.2003; 21 (2): 266–272.

47. Reni M, Ferreri AJ, Garancini MP, Villa E. Therapeutic management of primary central nervous system lymphoma in immunocompetent patients: results of a critical review of the literature. Ann Oncol.1997; 8 (3): 227–234.

48. Shibamoto Y, Tsuchida E, Seki K, *et al*. Primary central nervous system lymphoma in Japan 1995–1999: changes from the preceding 10 years. J Cancer Res Clin Oncol.2004; 130 (6): 351–356.

49. Jahnke K, Korfel A, Komm J, *et al*. Intraocular lymphoma 2000–2005: results of a retrospective multicentre trial. Graefes Arch Clin Exp Ophthalmol.2005; 244 (6): 663-9.

50. Grimm SA, Pulido JS, Jahnke*et al*. Primary intraocular lymphoma: An International Primary Central Nervous System Lymphoma Collaborative Group report. Ann Oncol. 2007; 18: 1851–1855.

51. Grimm SA, McCannel CA, Omuro AM *et al*. Primary CNS lymphoma with intraocular involve- ment: International PCNSL Collaborative Group report. Neurology. 2008; 71: 1355–1360.

52. Chan C-C, Gonzales JA. Chapter 11 Prognosis in PIOL. In: Chan C-C, Gonzales JA. Primary Introcular Lymphoma. E-book: 2007; 94–112.

53. Soussain, C, Hoang-Xuan, K, Taillandier, L, Fourme, E, Choquet, S. . Intensive chemotherapy followed by hematopoietic stem-cell rescue for refractory and recurrent primary CNS and intraocular lymphoma: Société Française de Greffe de Moëlle Osseuse-Thérapie Cellulaire. Jclin oncol. 2008; 26 (15), 2512-8.

54. Soussain, B. C., Suzan, F., Hoang-xuan, K., Cassoux, N., Levy, V., Azar, N., Belanger, C., *et al*. . Results of Intensive Chemotherapy Followed by Hematopoietic Stem-Cell Rescue in 22 Patients With Refractory or Recurrent Primary CNS Lymphoma or Intraocular Lymphoma. J clin oncol.2001; 19 (3), 742-749.

55. Rituximab in Primary Central Nervous system Lymphoma. A randomized HOVON / ALLG intergroup study PROTOCOL Principal Investigator: J.K. Doorduijn Co-investigators : J.E.C. Bromberg, S. Issa (ALLG). Version 17 dec 2012.

56. Vosganian, G. S., Boisot, S., Hartmann, K. I., Freeman, W. R., Sharpe, R. W., Tripuraneni, P., & Saven, A.. Primary intraocular lymphoma: a review. Journal of neuro-oncology. 2011; 105 (2), 127-34.

57. Fishburne BC, Wilson DJ, Rosenbaum JT, Neuwelt EA. Intravitreal methotrexate as an adjunctive treatment of intraocular lymphoma. Arch Ophthalmol. 1997 Sep; 115 (9): 1152-6.

58. Kitzmann, a S., Pulido, J. S., Mohney, B. G., Baratz, K. H., Grube, T., Marler, R. J., Donaldson, M. J., *et al*.. Intraocular use of rituximab. Eye (London, England). 2007; 21 (12), 1524-7.

59. Itty S, Olson JH, O'Connell DJ, Pulido JS. Treatment of primary intraocular lymphoma (PIOL) has involved systemic, intravitreal or intrathecal chemotherapy and/or radiotherapy. Retina. 2009 Mar; 29 (3): 415-6.

60. Ohguro N, Hashida N, Tano Y. Effect of intravitreous rituximab injections in patients with recurrent ocular lesions associated with central nervous system lymphoma. Arch Ophthalmol. 2008 Jul; 126 (7): 1002-3.

61. Ferrara JL, Levine JE, Reddy P, Holler E. Graft-versus-host disease. Lancet. 2009 May 2; 373 (9674): 1550-61.

62. Pérez-Simón JA, Encinas C, Silva F, Arcos MJ *et al*. Prognostic factors of chronic graft-versus-host disease following allogeneic peripheral blood stem cell transplantation: the national institutes health scale plus the type of onset can predict survival rates and the duration of immunosuppressive therapy. Biol Blood Marrow Transplant. 2008 Oct; 14 (10): 1163-71.

63. Alpdogan O, van den Brink MR. Immune tolerance and transplantation. Semin Oncol. 2012 Dec; 39 (6): 629-42.

64. Pidala J, Perez L, Anasetti C. Have we improved in preventing and treating acute GVHD? Current opinion in hematology. 2011; 18 (6): 408–413.

65. Jakubowski A a, Small TN, Kernan N a, *et al*. T cell-depleted unrelated donor stem cell transplantation provides favorable disease-free survival for adults with hematologic malignancies. Biology of blood and marrow transplantation. 2011; 17 (9): 1335–42.

66. Ho VT. Ex vivo T cell depletion of allogeneic PBSC as acute and chronic GVHD prophylaxis after myeloablative HCT: time to reconsider? Biology of blood and marrow transplantation. 2011; 17 (8): 1112–3.

67. Devine SM, Carter S, Soiffer RJ, *et al*. Low risk of chronic graft-versus-host disease and relapse associated with T cell-depleted peripheral blood stem cell transplantation for acute myelogenous leukemia in first remission: results of the blood and marrow transplant clinical trials network protocol 0303. Biology of blood and marrow transplantation. 2011; 17 (9): 1343–51.

68. Westeneng AC, Hettinga Y, Lokhorst H, Verdonck L, van Dorp S, Rothova A. Ocular graft-versus-host disease after allogeneic stem cell transplantation. Cornea. 2010; 29 (7): 758–63.

69. Tabbara KF, Al-Ghamdi A, Al-Mohareb F, *et al*. Ocular findings after allogeneic hematopoietic stem cell transplantation. Ophthalmology. 2009; 116 (9): 1624–9.

70. Ogawa Y, Kuwana M. Dry eye as a major complication associated with chronic graft-versus-host disease after hematopoietic stem cell transplantation. Cornea. 2003; 22 (7 Suppl): S19–27.

71. Balaram M, Rashid S, Dana R. Chronic ocular surface disease after allogeneic bone marrow transplantation. Ocul Surf 2005; 3: 203–211.

72. Russo PA, Bouchard CS, Galasso JM. Extended-wear silicone hydrogel soft contact lenses in the management of moderate to severe dry eye signs and symptoms secondary to graft-versus-host disease. Eye Contact Lens 2007; 33: 144–147.

73. Takahide K, Parker PM, Wu M, *et al*. Use of fluid-ventilated, gas-permeable scleral lens for management of severe keratoconjunctivitis sicca secondary to chronic graft-versus-host disease. Biol Blood Marrow Transplant 2007; 13: 1016–1021.

74. Robinson MR, Lee SS, Rubin BI, *et al*. Topical corticosteroid therapy for cicatricial conjunctivitis associated with chronic graft-versus-host disease. Bone Marrow Transplant 2004; 33: 1031–1035.

75. Chiang CC, Lin JM, Chen WL, Tsai YY. Allogeneic serum eye drops for the treatment of severe dry eye in patients with chronic graft-versus-host disease. Cornea 2007; 26: 861–863.

76. Ogawa Y, Okamoto S, Mori T, *et al*. Autologous serum eye drops for the treatment of severe dry eye in patients with chronic graft-versus-host disease. Bone Marrow Transplant 2003; 31: 579–583.

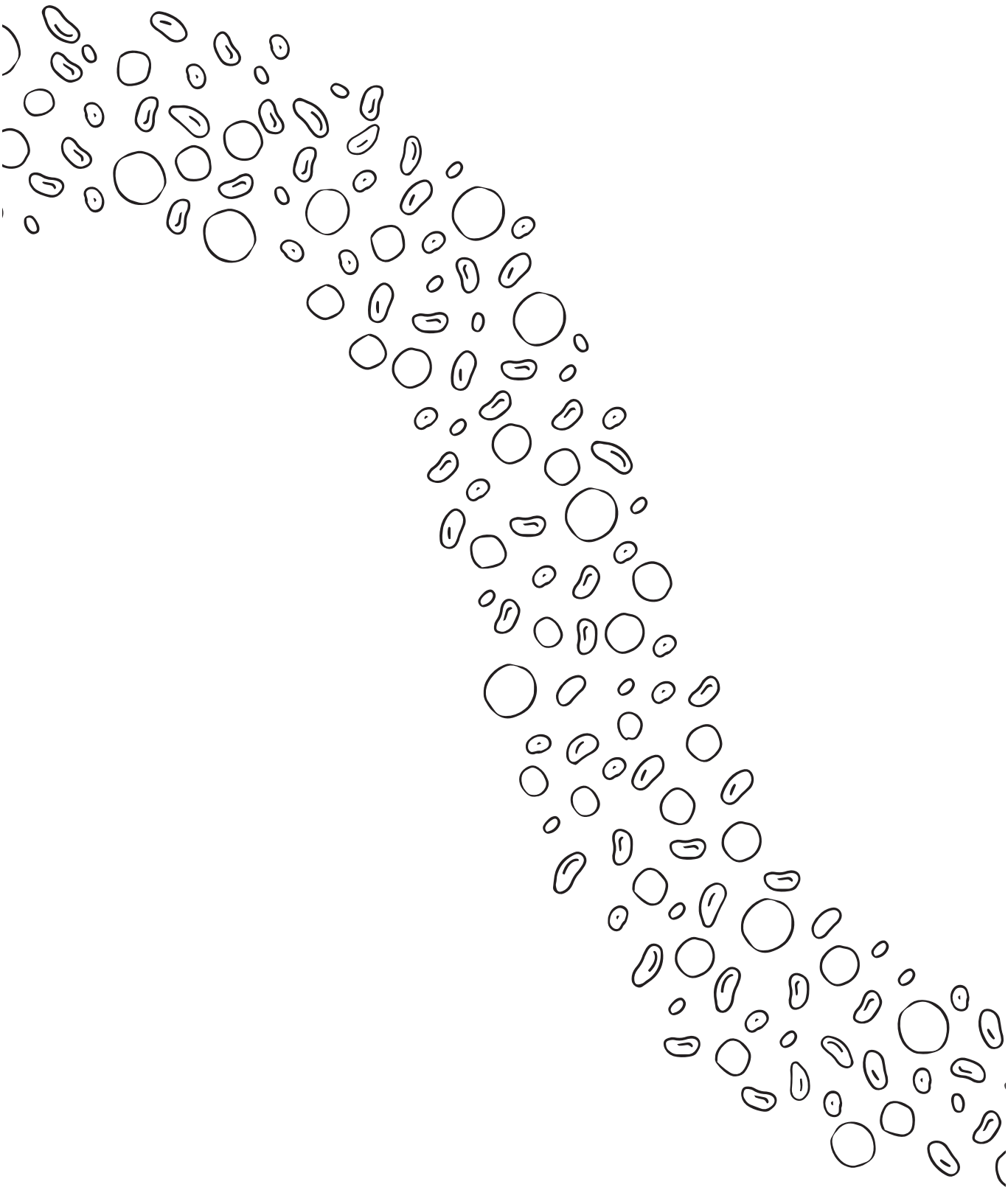
77. Kiang E, Tesavibul N, Yee R, *et al*. The use of topical Cyclosporin A in ocular graft-versus-host-disease. Bone Marrow Transplant 1998; 22: 147–151.

78. Lelli GJ Jr, Musch DC, Gupta A, *et al*. Ophthalmic cyclosporine use in ocular GVHD. Cornea 2006; 25: 635–638.

79. Rao SN, Rao RD. Efficacy of topical cyclosporine 0.05%in the treatment of dry eye associated with graft versus host disease. Cornea 2006; 25: 674–678.

80. Tam PM, Young AL, Cheng LL, Lam PT. Topical 0.03% tacrolimus ointment in the management of ocular surface inflammation in chronic GVHD. Bone Marrow Transplant 2010; 45: 957–958.

81. Ogawa Y, Dogru M, Uchino M, *et al*. Topical tranilast for treatment of the early stage of mild dry eye associated with chronic GVHD. Bone MarrowTransplant. 2010; 45: 565–569.



Chapter 2

Treatment strategies in primary vitreoretinal lymphoma: toward prevention of subsequent central nervous system lymphoma. A European Study

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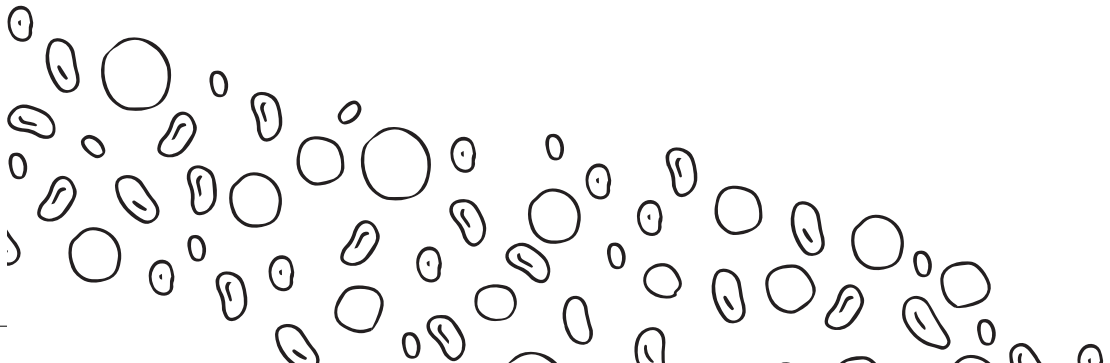


Abstract

An optimal treatment option for primary vitreoretinal lymphoma (PVRL) without signs of CNS involvement on MRI or in cerebrospinal fluid (CFS) remains unknown and we therefore studied 95 patients in a retrospective study engaging 17 participating centres in Europe. The incidence of central nervous system lymphoma (CNSL) was the primary outcome measure and was evaluated in different treatment groups defined and ascribed as local ocular treatment (radiotherapy and/or intravitreal chemotherapy (n=31; 33%)), extensive treatment (intravenous and/or intrathecal chemotherapy and/or cerebral irradiation (n=21; 22%)) or a combination of both treatments (n=23; 24%). Additionally, 20 patients were initially not treated for their PVRL. The median follow-up was 57 months.

CNSL developed in 45/95 (47%) of all patients; 17/20 (85%) of the untreated group and 32-43% of patients in all treated groups. Incidence of CNSL development was similar among the three treatment groups (p=0.76). Kaplan-Meier ten-year survival curves with CNS manifestations as outcome showed no significant differences when the three treated groups were compared (p=0.91). The five-year cumulative survival rate was similar in all treatment groups (P=0.10). The median progression-free survival (end point CNSL manifestations) was 47 months (range 7-152) in all treated patients. There was no significant difference in overall and progression-free survival among the three treatment groups (p=0.74 and p=0.15, respectively). Treatment complications developed in 9/40 (23%) patients receiving systemic chemotherapy with acute renal failure being the most common (5/40; 13%). Ocular treatment was employed in 53 patients and complications within the eye(s) occurred in 15 patients (28%).

In the present series, the additional benefit of an extensive treatment approach for PVRL in patients without evidence of CNSL on MRI or CSF examinations could not be proven and was associated with more severe adverse effects.

Introduction

Opinions are divided on how to treat primary intraocular lymphoma in the absence of central nervous system lymphoma (CNSL). Primary intraocular lymphoma is an uncommon malignancy, manifesting first in the retina and/or vitreous in one or both eyes and is currently classified as primary vitreoretinal lymphoma (PVRL)[1,2]. PVRL presents predominantly as diffuse large B-cell lymphoma (DLBCL) and has a high association with CNS and occasionally with testicular manifestations[3-6]. CNSL has been reported to develop in approximately 80% of patients with PVRL and determines the survival of the PVRL-affected patients. The median overall survival of patients with PVRL is currently reported to be 58 months[7].

The best therapeutic approach for patients with isolated PVRL without documented CNS manifestations has not achieved consensus, particularly because of a paucity of outcome data. Basically, two distinct approaches to patients with isolated PVRL are employed. The first approach consists of aggressive treatment regimens such as are used for CNSL including high dose methotrexate (HD-MTX)-based chemotherapy with or without intrathecal treatment and/or whole brain radiation[8-12]. This treatment is aimed at both local control of PVRL and at prevention for subsequent CNSL manifestations. The second approach consists of only local ocular treatments. Systemic treatment as used for CNSL, with intravenous (and sometimes intrathecal) chemotherapy may be associated with severe systemic adverse effects, while local ocular treatment such as ocular radiotherapy and intravitreal chemotherapy with methotrexate and/or rituximab lacks systemic adverse effects. The first approach is based on the presumption that the patients already have developed subclinical lymphoma in the CNS, which could not as yet be substantiated by MRI or cerebrospinal fluid (CSF) examinations. The second approach, local ocular therapy, is aimed at eradication of lymphoma cells in the eye but has no effect on possible subclinical manifestations elsewhere. Only limited evidence is available on the efficacy of both approaches. Herein we retrospectively evaluate the outcomes of diverse treatment regimens used for PVRL in 95 patients from various European centers and focus on incidence of CNS manifestations during the follow up.

Patients and methods

Patients

This retrospective study includes immunocompetent, HIV- negative patients diagnosed with isolated PVRL between January 1991 and January 2013 from all the university medical centres in the Netherlands as well as the Rotterdam Eye hospital, Jules Gonin Eye hospital in Switzerland and Moorfields Eye hospital and Bristol Eye hospital in the United Kingdom, departments of ophthalmology from Tuebingen university hospital and Heidelberg university hospital in Germany, Istanbul university, faculty of medicine in Turkey, Pitié-Salpêtrière hospital in France, university hospital of León in Spain and Centre Hospitalier Universitaire

St-Pierre and Brugman in Belgium. All data were reviewed for completeness and consistency. The study is in accordance with the Declaration of Helsinki and was approved by the institutional ethical committee of the University Medical Center Utrecht, which concluded that the Dutch Medical Research Involving Human Subjects Act did not apply and written informed consent was not needed.

For inclusion into the study, the minimal duration of follow-up needed to be at least one year after the first symptoms of PVRL. Patients with previous systemic lymphoma were excluded. Isolated PVRL was diagnosed by ocular tissue biopsy, whereas cerebrospinal fluid (CSF) examination and imaging of the brain were used to exclude CNSL at the moment of first symptoms of PVRL.

In 17 patients the diagnosis of PVRL was initially suspected but could not be confirmed by ocular tissue biopsy during the early stage of the disease and was histologically confirmed at the stage when CNSL had already developed. In 13/17 of these patients the diagnosis was confirmed by ocular fluid and/ or tissue analysis at the moment when CNSL had already developed and in four/17 the diagnosis was made by a brain biopsy. These 17 patients were excluded from the analyses on CNSL incidence and survival.

The following data were collected: age, gender, date of onset of ocular complaints, date of diagnosis of PVRL, type and date of treatment, all relevant ocular and systemic treatment complications, date of PVRL relapse, date of manifestation of CNSL, and interval to CNSL manifestations from first ocular symptoms, survival in years from first ocular symptoms and cause of death.

Statistical analysis

The primary outcome of this retrospective study is the development of CNSL and its association with type of PVRL treatment used. Secondary end points are relapse of PVRL, survival and cause of death. The data were analysed using IBM SPSS Statistics 20.

Differences in the distribution of individual features among treatment groups were analysed by means of the chi-square test or Fisher's exact test for categorical variables and the Mann-Whitney U test or Kruskall-Wallis test for continuous nonparametric variables. Survival was defined as the time from first symptoms of PVRL to death or the last follow-up assessment.

Survival rates were calculated using the Kaplan-Meier method and differences were compared with the use of the log-rank test. The Cox proportional-hazards model was used for multivariate survival analyses.

Results

Characteristics of the study patients

The patient characteristics are shown in Table 1. The median age at diagnosis of PVRL was 62 years (range 33-86) and 41/95 (43%) were male. PVRL at onset was bilateral in 59 (62%) patients and unilateral in 36 (38%) patients. The median duration from symptoms to diagnosis PVRL was 12 months (range 0-63). Of 106 patients with isolated PVRL, data on treatment were available in 97 patients. One of these patients

	All cases	No extensive or ocular treatment	Ocular treatment only[x]	Extensive treatment only	Combination of ocular and extensive treatment	P-value All patients[#]	P-value Treatment Groups[+]
	N=95	N=20	N=31	N=21	N=23		
Age, years							
Median	62	54	65	59	62		
Range	33-86	33-83	41-86	43-73	39-83	P=0.04*	P=0.07*
Male gender, N (%)	41 (43%)	9 (45%)	12 (39%)	8 (38%)	12 (52%)	P=0.75**	P=0.54**
Interval between onset of symptoms to diagnosis, months							
Median	12	25	10	10	7		
Range	0-63	9-49	1-42	2-41	0-63	P=0.002*	P=0.65*
Bilateral PVRL N (%)	59 (62%)	17 (85%)	14 (45%)	12 (57%)	16 (70%)	P=0.03**	P=0.22***
CNSL development N (%)	45 (47%)	17 (85%)	10 (32%)	9 (43%)	9 (39%)	P=0.002**	P=0.76**
Interval between first manifestations PVRL and CNSL, months							
Median	35	64	28	29	46		
Range	7-133	21-114	7-66	20-133	11-126	P=0.14*	P=0.77*
Recurrence of vitreoretinal lymphoma during follow-up, N (%)							
	18 (19%)	1 (5%)	4 (13%)	6 (29%)	7 (30%)	p=0.32***	P=0.53***
Follow up from first manifestations PVRL, months							
Median	57	97	48	44	78		
Range	12-260	17-179	15-260	17-133	17-133	P=0.11*	P=0.69*

X includes two patients who received also CHOP (Cyclophosphamide, Hydroxydaunorubicin, Oncovin (vincristine), Prednisone) and were considered as not systemically treated. #all 95 patients included in this study. +all patients with exclusion of patients that were not treated for PVRL. ; *P-value calculated with Kruskal-Wallis Test, **P-value calculated with Pearson Chi-square, *** P-value calculated with Fisher's Exact Test; PVRL: primary vitreoretinal lymphoma; CNSL: central nervous system lymphoma

Table 1.
Patient demographics and clinical characteristics

had a malignancy of the testicle (unknown type) 7 years prior to PVRL and another had a cutaneous lymphoma one year prior to PVRL. These two patients were excluded because primary location of VRL was uncertain and 95 patients were included for detailed analysis. The diagnosis of isolated PVRL was confirmed by ocular tissue biopsy in 91/95 patients. In 4/95 patients the diagnosis of PVRL was confirmed by brain biopsy after CNSL already developed. The follow up from first symptoms of PVRL in all included patients ranged from 12-260 months (median 57 months).

Among the 95 patients diagnosed with PVRL, 45 (47%) patients subsequently developed CNSL and 34 out of all 95 (36%) patients died during follow-up. Death was strongly associated with the development of CNSL (p=≤0.001). The median time interval from the onset of the first symptoms of PVRL to the onset of CNSL was 35 months (range 7-133).

Patients with and without CNSL manifestations were similar in male-to-female ratio and bilaterality of PVRL (P= 0.41 and P=0.41 respectively). Patients who developed CNSL were younger than patients who did not develop CNSL (median age 56; range 33-86 versus median age 65; range 41-86, p=0.01). The follow-up of patients with CNSL was longer than of those without CNSL (median 50 months; range 12-246 versus median 39 months; range 7-134; p=0.03).

Therapeutic approaches

Treatment regimens were principally classified into four categories: no treatment, only ocular treatment, extensive treatment and a combination of ocular and extensive treatment (Table 2).

Ocular treatment included local radiotherapy, intravitreal application of methotrexate and/or rituximab. Extensive treatment regimens are listed in Table 3 and consisted of different regimens of systemic and intrathecal chemotherapy, whole-brain radiotherapy (WBRT) and peripheral blood stem cell transplantation (PBSCT). CHOP (Cyclophosphamide, Hydroxydaunorubicin, Oncovin (vincristine), Prednisone) chemotherapy was not considered as receiving systemic treatment since it is not effective for CNS lymphoma (Table 2)[13-15].

The patient characteristics per treatment group are summarized in Table 1. Of all included patients 31/95 (33%) patients received only ocular treatment. These patients were treated either with radiotherapy (16/31, 52%), intravitreal MTX and/or rituximab (12/31, 39%) or a combination of radiotherapy and intravitreal chemotherapy (2/31, 6%) and one of the 31 (3%) affected eyes was enucleated. Extensive treatment including intravenous and/or intrathecal chemotherapy and/or cerebral irradiation was given in 21/95 (22%) patients and a combination of ocular and extensive treatment was given to 23 /95 (24%) patients. Additionally, 20 patients were not treated initially for their PVRL, because the diagnosis was proven in retrospect at the time of CNS manifestations (n=17) and because of preference of the patient (n=2), or physician (n=1).

Type of treatment	No. of patients
No treatment, N (%)	20 (21%)
Extensive treatment, N (%)	21 (22%)
Local (ocular) treatment **, N (%)	31 (33%)
Irradiation eyes	18
Intravitreal MTX and/or Rituximab	10
Irradiation eyes and intravitreal chemotherapy	2
Enucleation	1
Combination of ocular and extensive treatment, N (%)	23 (24%)
Intravitreal and intravenous chemotherapy	4
Intravitreal and intrathecal chemotherapy	1
Intravitreal and intravenous chemotherapy and cerebral radiotherapy	1
Intravitreal and intravenous chemotherapy and PBSCT	1
Ocular radiotherapy and intravenous chemotherapy	6
Ocular radiotherapy and intrathecal chemotherapy	3
Ocular and cerebral radiotherapy	2
Ocular radiotherapy and intravenous and intrathecal chemotherapy	2
Ocular and cerebral radiotherapy and intravenous chemotherapy	2
Ocular and cerebral radiotherapy and intravenous and intrathecal chemotherapy	1

** includes two patients who received also CHOP (Cyclophosphamide, Hydroxydaunorubicin, Oncovin (vincristine), Prednisone) and were considered as not systemically treated.
CNS: central nervous system; PBSCT: peripheral blood stem cell transplantation.

Table 2.
Types of treatment given for Primary Vitreoretinal Lymphoma

Patient characteristics in specific treatment groups

The general characteristics among specific treatment groups did not differ, however the untreated patients were older than those treated (p=0.04) and the duration from the onset of symptoms to diagnosis of PVRL was longer (p=0.002). The three treated groups were similar for diverse variables including gender (p=0.54), age (p=0.07) duration from the onset of symptoms to diagnosis of PVRL (p= 0.65), interval from the onset symptoms of PVRL to the onset of CNSL (p=0.77) and duration of follow-up (p=0.69), (Table 1). Furthermore, the duration of follow-up was similar for patients receiving ocular radiotherapy and chemotherapy (P=0.18).

Type of treatment	N (%)
Type of intravenous treatment	40 (42%)
CHOP	2 (5%)
HD-MTX based	16 (40%)
Cytarabine based	4 (10%)
MTX and Cytarabine	5 (13%)
Rituximab	1 (3%)
Rituximab and Cytarabine	2 (5%)
Rituximab, MTX and Cytarabine	3 (8%)
Rituximab, MTX, Cytarabine and PBSCT	4 (10%)
COPADEM-protcol *	3 (8%)
Type of intrathecal treatment	10 (11%)
Methotrexate	7 (70%)
Methotrexate and Cytarabine	3 (30%)
Type of cerebral treatment	6 (6%)
Radiotherapy	6 (100%)

CHOP: Cyclophosphamide, Hydroxydaunorubicin Oncovin (Vincristine) and Prednisone; HD-MTX: High Dose-Methotrexate; MTX: Methotrexate; PBSCT: peripheral blood stem cell transplantation
* Blay JY, Bouhour D, Carrie C, Bouffet E, Brunat-Mentigny M, Philip T, Biron P. The C5R protocol: a regimen of high-dose chemo-therapy and radiotherapy in primary cerebral non-Hodgkin's lymphoma of patients with no known cause of immunosuppression. Blood.1995 Oct 15; 86 (8): 2922-9.

Table 3.
Systemic, meningeal and cerebral treatment regimens used for treatment of Primary Vitreoretinal Lymphoma (PVRL)

Effect of treatment PVRL on CNSL manifestations

CNS lymphoma developed in 45/95 (47%) of all patients; specifically in 17/20 (85%) of the untreated group (which included patients in whom the diagnosis of PVRL was proven in retrospect at the time of CNSL manifestations) and among 32-43% in all treated groups (p=0.76; Table 1). Furthermore, no significant differences in CNSL manifestations were observed when ocular therapy was compared with solely systemic chemotherapy and ocular treatment with combination of systemic chemotherapy and ocular therapy (p=0.53 and p=0.45, respectively). In addition, development of CNSL manifestations was similar in patients who received ocular chemotherapy and ocular radiotherapy (p=0.23). Two patients in the ocular therapy group were treated with additional CHOP chemotherapy which has negligible activity in the CNS. Both patients did not develop CNSL after a follow-up of 33 and 96 months respectively.

Type of treatment and adverse effects	No. of patients (%)
Extensive treatment	
Systemic chemotherapy	40
Total with adverse effects	9 (23%)
Acute renal failure	5 (56%)
Anemia and Thrombocytopenia	1 (11%)
Nausea	1 (11%)
Fever	1 (11%)
Sepsis	1 (11%)
Intrathecal chemotherapy with methotrexate	10
Total with adverse effects	1 (10%)
Meningitis/arachnoiditis	1 (100%)
Local (ocular) treatment	
Irradiation eyes	34
Total with adverse effects	10 (29%)
Radiation retinopathy	1 (10%)
Retinal detachment	2 (20%)
Cystoid macular edema	1 (10%)
Cataract	3 (30%)
Keratoconjunctivitis sicca	2 (20%)
Conjunctivitis	1 (10%)
Intravitreal MTX and/or Rituximab	19
Total with adverse effects	5 (26%)
Superficial keratitis	3 (60%)
Raised intraocular pressure	2 (40%)

Table 4.
Side effects of treatment regimens given for primary vitreoretinal lymphoma

Kaplan-Meier ten-year survival curves (calculated from the onset of PVRL symptoms to the development of CNS manifestations) showed no significant differences when the three treated groups were compared (p=0.91, Figure 1). Furthermore, no differences were observed between the specific treatment groups (ocular treatment only versus extensive treatment only, p=0.96; ocular treatment only versus a combination of extensive and ocular treatment, p=0.56; and between extensive treatment only and combination of systemic chemotherapy and ocular treatment, p=0.80).

Ocular relapse of PVRL

Ocular relapse of PVRL occurred in 18/95 patients (19%) within 12-131 months after the first symptoms of PVRL (median 28 months). Risk of ocular relapse was similar among the specific treatment groups (p=0.53). The incidence of local relapse in patients who received ocular therapy versus patients who received systemic chemotherapy solely did not differ (p=0.31). No difference in ocular relapses was observed between the patients who received ocular chemotherapy and ocular radiotherapy (p=0.60).

Treatment complications

Systemic chemotherapy was used in 40 patients (23 extensive treatment only and 17 receiving a combination of ocular and extensive treatment). One or more complications developed in 9/40 (23%) patients receiving systemic chemotherapy (Table 4) with acute renal failure being the most common (5/40; 13%). Ocular radiotherapy was employed in 34 patients. One or more complications occurred in 10 patients (29%). Intravitreal chemotherapy was given to 19 patients and complications developed in 5

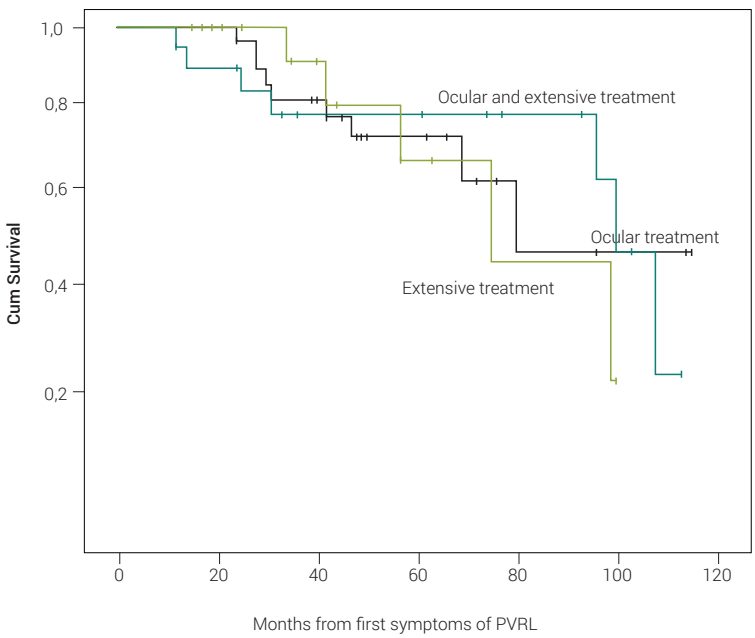


Figure 1.

Kaplan-Meier 10-year survival estimates (with central nervous system lymphoma as outcome) by specific treatment group in 95 patients with primary vitreoretinal lymphoma.

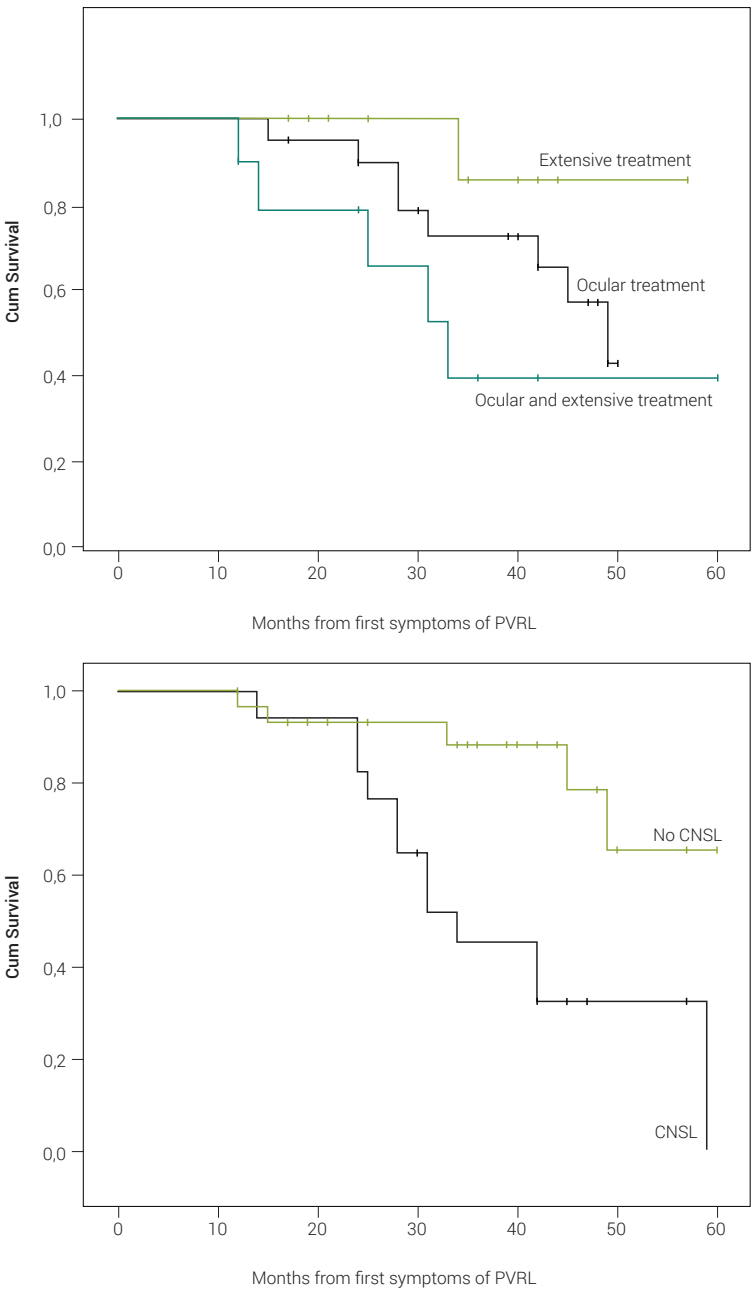


Figure 2.

Kaplan-Meier 5-year overall survival estimates following treatment for primary vitreoretinal lymphoma by treatment group (top) and overall survivall for primary vitreoretinal lymphoma in patients that developed central nervous system lymphoma and patients that did not develop central nervous system lymphoma (bottom).

Cause of death	N (%)
Total number of patients who died	**34/95 (36%)**
Direct complications of CNSL	12/34 (36%)
Indirect complications of CNSL	6/34 (18%)
Other causes of death	4/34 (12%)
Heart failure	1/34 (3%)
Prostate carcinoma	1/34 (3%)
Small cell lung carcinoma	1/34 (3%)
Aneurysm abdominal aorta	1/34 (3%)
Unknown cause of death	12/34 (36%)

CNSL: Central nervous system lymphoma

Table 5.
Mortality in patients with Primary Vitreoretinal Lymphoma

patients (26%, Table 4). Radiation encephalopathy did not develop in our patients treated for PVRL with whole brain radiotherapy (n=6), however this complication did develop in five patients that were treated for subsequent CNSL.

Cause of death

Out of all 95 patients, 34 (35%) died (Table 5); 12 patients died from CNSL, six patients from indirect complications of CNSL including complications of treatment, four patients died of lymphoma-unrelated causes and in 12 patients' data was not available. Patients untreated for PVRL were more likely to die than treated patients (p=0.04); but when the three treatment groups were compared their death rates did not significantly differ (p=0.07). CNSL was highly associated with death (25/ 45 patients who developed CNSL; 56% versus 9/50 patients who did not develop CNSL; 18%; p<0.001).

Overall survival and progression-free survival

The median overall survival from onset of symptoms of PVRL till death was 44 months (range 11-113) in patients that did not develop CNSL and 34 months (range 12-131) in patients that developed CNSL (p=0.7). The five-year cumulative survival rate was lower in patients with CNSL compared to patients without CNSL (35% (95% CI 50% to 86%) versus 68% (95% CI 19% to 51%) p=0.003, Figure 2a) and was similar among all treatment groups (P=0.10, Figure 2b), The median CNSL-free survival was 47 months (range 7-152) in all treated patients. There was no significant difference in overall and progression-free survival among the three treatment groups (p=0.74 and p=0.15, respectively).

Discussion and conclusion

Our study illustrates the lack of a consistent treatment approach for patients with PVRL and the data provides current outcomes of a similar prevalence (32-43%) of CNSL manifestations among patients with PVRL treated with various ocular and extensive treatment regimens. In our study, the benefit of extensive treatment strategies in patients with PVRL could not be substantiated at least at the time when CNS involvement could not be documented despite adequate analysis and a median follow-up duration of 57 months.

This largest contemporary series of patient outcomes is particularly pertinent as it presents a homogenous group of patients with PVRL without signs of CNS involvement. Grimm *et al*. conducted a retrospective study in 83 patients with PVRL from 16 centers in 7 countries[7]. In contrast to our study, the classification of PVRL in their series included 11 patients with CSF examinations positive for lymphoma cells. Furthermore the median follow-up in Grimm's study was 32 months and no minimal duration of follow-up was provided. In contrast, we included PVRL patients without any signs of CNS involvement at onset (i.e. negative MRI and negative CSF examinations) with a minimal follow-up of 1 year and median follow-up of 57 months. In line with our findings, initial treatment regimens in Grimm's series varied widely and consisted of local therapy in 28% of patients (which included ocular radio- and chemotherapy), extensive therapy in 64% of patients (systemic chemotherapy, whole brain radiotherapy) and no treatment in 7% of patients. CNSL developed in 47% of all included patients and local therapy alone (without systemic treatment or brain radiotherapy) did not increase the risk of brain relapse compared to an intensive treatment regimen (p=0.4). These results are entirely in accordance with our findings and moreover indicate also that ocular treatment of PVRL was not associated with excess of CNS manifestations compared to more aggressive (systemic) treatment regimens. Furthermore, our patients were selected from ophthalmology departments rather than neuro-oncology departments as in the series by Grimm *et al*. eliminating possible selection bias in favour of developing CNSL.

In the past, the standard treatment for PVRL included ocular radiotherapy. Most published studies on local PVRL treatment focus on the effect on PVRL itself and do not have sufficient follow-up to report on incidence of subsequent CNS manifestations. Berenbom *et al*. (2007) described limited side effects of ocular radiotherapy in 12 PVRL patients and stated that this treatment modality does not prevent CNSL relapse[16]. Hormigo *et al*. (2004) compared in a study on 17 patients with PVRL chemotherapy (n=4), chemotherapy combined with ocular radiotherapy (n=10) and ocular radiotherapy only (n=3) and concluded that ocular radiotherapy diminished the risk of a relapse of PVRL but did not prevent the occurrence of CNSL[8]. Teckie *et al*. (2013) reported on 12 patients treated with ocular radiotherapy alone, seven of which remained relapse-free during 30 months of follow up[17]. Lymphoma recurred in five patients in eye and/or brain and could be effectively treated with chemotherapy and/or radiotherapy at that time. However, all these studies have limited numbers of patients and inconsistent follow-up, whereas comparison of local

treatment to other treatment modalities was not systematically performed.

In our series follow-up duration of patients treated solely with ocular radiotherapy was 47 months and 29% of patients developed various side effects. However, one should realize that our series included patients from 1991 onwards. In the early nineties radiotherapy treatment regimens differed in intensity compared to recent strategies[18]. In the past, radiation regimens frequently induced cataract formation, radiation retinopathy and keratoconjunctivitis sicca[10,18]. At present, radiotherapy is of lower intensity (36 Gray), therapy is fractionated and although dry eye complaints during the treatment and post-radiotherapy cataract develop regularly, retinopathy is uncommon[18,19]. Theckie *et al* indicate that in their series no serious side effects of radiation developed and counter the claim that permanent visual loss might occur[17]. More recently, intraocular chemotherapy has gained popularity and includes diverse regimens of methotrexate and rituximab. Unfortunately, studies comparing ocular radiotherapy with intraocular chemotherapy are lacking. Side effects of administration of these agents only occur within the eye and include hyperemia, keratopathy, cataract, glaucoma, iridocyclitis, vitreous hemorrhages, retinal detachment, maculopathy and endophtalmitis[20-22]. In addition, proof of superiority of intraocular chemotherapy above radiotherapy is lacking and so far only limited data on follow-up is available. Obviously, advantages of this local chemotherapy to intravenous treatment are higher levels of chemotherapeutic agents in the eye and the lack of systemic, possibly life-threatening, adverse effects.

Hashida *et al* (2012) report a prospective study on 13 patients with CD-20 positive PVRL (one with CNSL) who were treated with intravitreal rituximab[20]. This treatment was efficacious on short term (reducing signs of PVRL) however ocular disease recurred in nearly half of the patients within one year and 69% of patient developed subsequent CNSL manifestations during follow up[20]. Frenkel *et al* (2008) describe the results of intravitreal methotrexate in 44 eyes with vitreoretinal lymphoma[23]. In all eyes clinical remission was established after a mean of 6.4 (range 2-16) intravitreal injections. However 16 patients already had CNSL at the start of treatment . Ferreri *et al* found that in patients with PCNSL with VRL (n=22) addition of ocular radiotherapy to systemic intravenous chemotherapy resulted in better control of ocular disease[24].

To date, the pathogenesis of VRL-CNS lymphoma is not known and the mechanism of its origin and subsequent spread has not been elucidated. It is not known whether CNS and ocular manifestations occur independently or whether this type of lymphoma originates at one site, subsequently spreading other sites. Our results, as well as the frequent bilateral involvement in VRL and the long time intervals (up to 10 years) between manifestations of large B-cell lymphoma at different immune-privileged sites (eyes, testes and CNS) favour the possibility of a multifocal origin[25]. In addition, spreading from one site to others might concurrently occur. The multifocal origin of lymphoma at various immune privileged sites has been ascribed to an ineffective immune response to lymphoma cells at these protected sites[26]. Booman *et al*. studied the genomic alteration of both DLBCL of the testes and CNSL and showed that DLBCL in these two locations exhibits both shared and site specific genetic alterations[27]. They conclude that these findings underline the concept of immune privileged site lymphoma but that CNS and testes lymphoma do not form

a single entity. The future analysis of lymphoma tissue from eyes and (subsequent) CNS involvement for histological, immunological and genetic comparisons as well as the clonal identity might clarify this issue. PVRL is known to be a "masquerade" disease presenting as inflammation i.e. uveitis, which may cause a substantial delay in correct diagnosis. multiple ocular biopsies (vitreous and/or retina) are often needed before the diagnosis-once suspected-can be confirmed. Samples often do not contain any neoplastic cells and show only an aspecific inflammatory reaction. This phenomenon has been noted earlier and is also illustrated in our study by a median duration of 12 months from first ocular symptoms to definitive diagnosis of PVRL8.

Our study obviously has the shortcomings of a retrospective study design. The reasoning for the choice of treatment in individual patients could not be retraced. However the different hospitals tended to use the same treatment modalities for all their patients. As the patient characteristics did not differ between treatment groups and the choice of treatment was mainly based on which treatment option was conventional per treatment center, the bias seems to be limited. The interval from first symptoms of PVRL to CNSL development was longer, though not significant, in the patients that were not treated (Table 1). This unexpected trend –if any- might have been caused by the limited number of patients included. For survival analysis, we used the onset of symptoms as starting point. This point was chosen because if the moment of diagnosis would have been used the results might have been heavily biased by the accuracy of diagnosis by the ophthalmologists. When the data were adapted to the time of diagnosis of PVRL, the results did not differ. In the ten-year survival estimates with CSNL as outcome, a tendency towards a better prognosis is seen in the group treated with both extensive and ocular treatment, this trend could be further explored in a prospective study with more patients included. We show that patients who developed CNSL were younger at onset of PVRL and had a longer follow-up duration. It might be possible that the development of CNS manifestation in the (elderly) patients would have been higher after a longer follow-up. In our study, the rate of local recurrences was similar for local and systemic treatment regimens. However, our study was designed to register CNS manifestations. The details of ocular involvement, including data on activity, chronicity and reactions to treatments were not specifically registered. For the evaluation and comparison of various treatment strategies on ocular disease activity and recurrences another study design would be required. Extensive treatment modalities such as systemic chemotherapy and WBRT are associated with severe adverse effects and their use, especially in elderly people, should be supported by its benefits. A prospective study design would be needed to evaluate the value of extensive treatment approaches in patients with PVRL. This would require an international cooperation and a long time-span since PVRL is a rare disorder and secondary manifestations might develop late.

In conclusion, development of CNS manifestations after PVRL in our study with a median follow-up of 57 months was similar in PVRL patients treated with local ocular treatments and in patients with extensive treatment regimens. In the present series, the additional benefit of an extensive treatment approach could not be proven and was associated with more and severe adverse effects.

References

1. Chan, C.-C. *et al*. Primary vitreoretinal lymphoma: a report from an International Primary Central Nervous System Lymphoma Collaborative Group symposium. The oncologist 16, 1589–99 (2011).

2. Coupland, S. E. Molecular pathology of lymphoma. Eye (London, England) 44, 1–10 (2012).

3. Davis, J. W., Moriarty, R. P., Schlossberg, S. M. & Schellhammer, P. F. Bilateral testicular lymphoma treated with chemotherapy and radiation without orchiectomy: complete response relapsed at 52 months in the vitreous humor. Urology 57, 555 (2001).

4. Pe'er, Jacob; Rowe, Jacob; Frenkel, Shara, Dann, E. Testicular lymphoma, intraocular (vitreoretinal) lymphoma, and brain lymphoma: Involvement of three immunoprivileged sites in one patient. American Journal of Hematology 85, 631–633 (2010).

5. Aliferis, K., Chan, C. C., Donati, G. & Baglivo, E. Intraocular lymphoma following a primary testicular lymphoma in remission for 10 years. International ophthalmology 28, 439–40 (2008).

6. Wallace, D. J. *et al*. Primary testicular and intraocular lymphomas: two case reports and a review of the literature. Survey of ophthalmology 51, 41–50 (2006).

7. Grimm, S. a *et al*. Primary intraocular lymphoma: an international primary central nervous system lymphoma collaborative group report. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO 18, 1851 (2007).

8. Hormigo, A., Abrey, L., Heinemann, M.-H. & DeAngelis, L. M. Ocular presentation of primary central nervous system lymphoma: diagnosis and treatment. British journal of haematology 126, 202–8 (2004).

9. Batchelor, T. T., Kolak, G., Ciordia, R., Foster, C. S. & Henson, J. W. High-dose methotrexate for intraocular lymphoma. Clinical cancer research : an official journal of the American Association for Cancer Research 9, 711–5 (2003).

10. Jahnke, K., Thiel, E., Abrey, L. E., Neuwelt, E. a & Korfel, A. Diagnosis and management of primary intraocular lymphoma: an update. Clinical ophthalmology (Auckland, N.Z.) 1, 247–58 (2007).

11. Jahnke, K. *et al*. Intraocular lymphoma 2000-2005: results of a retrospective multicentre trial. Graefe's archive for clinical and experimental ophthalmology = Albrecht von Graefes Archiv für klinische und experimentelle Ophthalmologie 244, 663–9 (2006).

12. Vosganian, G. S. *et al*. Primary intraocular lymphoma: a review. Journal of neuro-oncology 105, 127–34 (2011).

13. Mead, G. M. *et al*. A medical research council randomized trial in patients with primary cerebral non-Hodgkin lymphoma: cerebral radiotherapy with and without cyclophosphamide, doxorubicin, vincristine, and prednisone chemotherapy. Cancer 89, 1359–70 (2000).

14. Deckert, M. *et al*. Modern concepts in the biology, diagnosis, differential diagnosis and treatment of primary central nervous system lymphoma. Leukemia 25, 1797–807 (2011).

15. Ferreri, A. J. M. How I treat primary CNS lymphoma. Blood 118, 510–22 (2011).

16. Berenbom, a, Davila, R. M., Lin, H.-S. & Harbour, J. W. Treatment outcomes for primary intraocular lymphoma: implications for external beam radiotherapy. Eye (London, England) 21, 1198–201 (2007).

17. Teckie, S. & Yahalom, J. Primary intraocular lymphoma: treatment outcomes with ocular radiation therapy alone. Leukemia & lymphoma 1–7 (2013).

18. Durkin, S. R., Roos, D., Higgs, B., Casson, R. J. & Selva, D. Ophthalmic and adnexal complications of radiotherapy. Acta ophthalmologica Scandinavica 85, 240–50 (2007).

19. Monroe, A. T., Bhandare, N., Morris, C. G. & Mendenhall, W. M. Preventing radiation retinopathy with hyperfractionation. International journal of radiation oncology, biology, physics 61, 856–64 (2005).

20. Hashida, N., Ohguro, N. & Nishida, K. Efficacy and Complications of Intravitreal Rituximab Injection for Treating Primary Vitreoretinal Lymphoma. Translational Vision Science & Technology 1, 1 (2012).

21. Levy-Clarke GA, Chan CC, Nussenblatt RB . Diagnosis and management of primary intraocular lymphoma . Hematology/ Oncology Clinics of North America 19, 739 – 749 (2005).

22. Smith, J. R., Rosenbaum, J. T. & Wilson, D. J. Role of Intravitreal Methotrexate in the Management of Primary Central Nervous. Ophthalmology 109, 1709–1716 (2002).

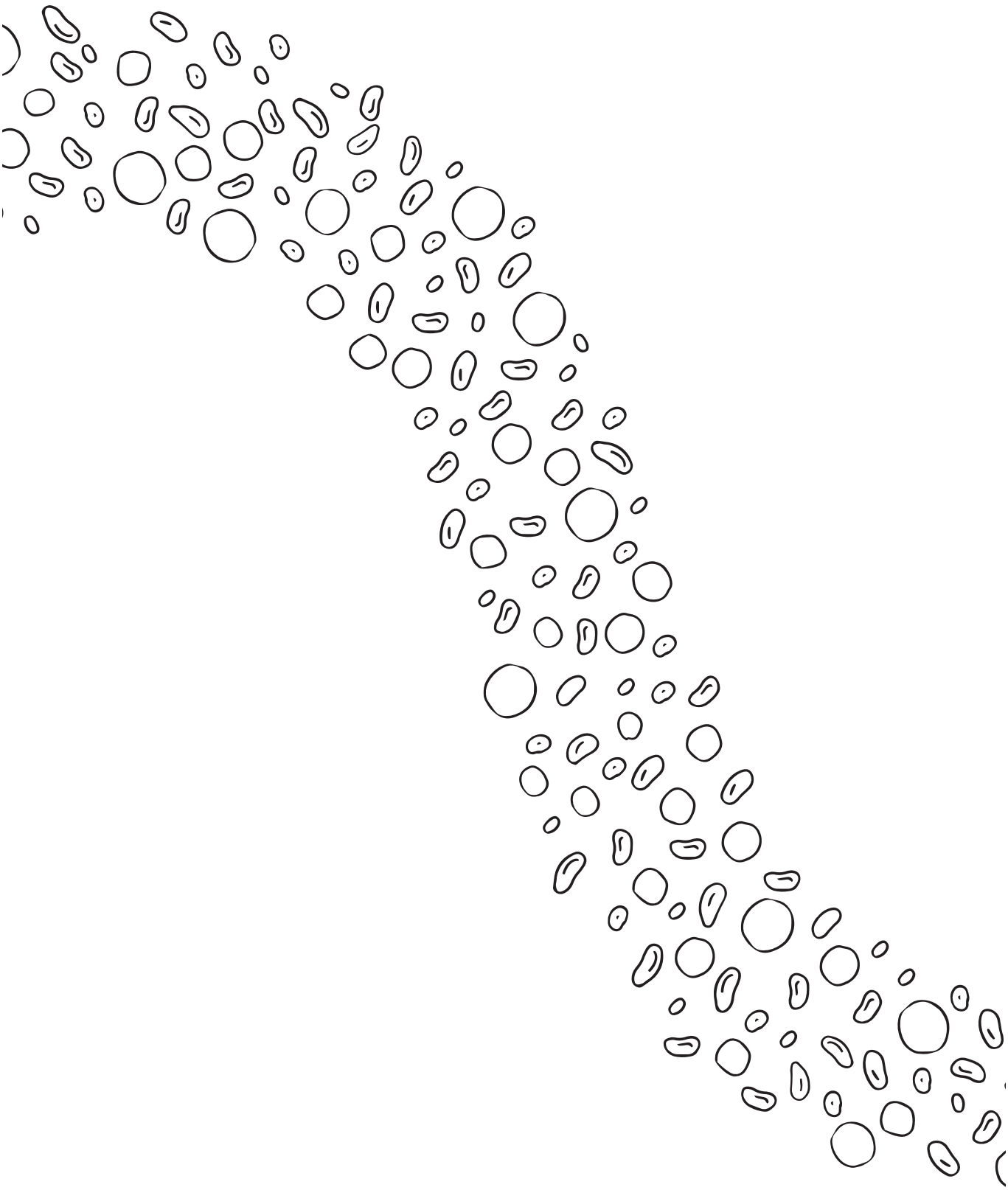
23. Frenkel, S., Hendler, K., Siegal, T., Shalom, E. & Pe'er, J. Intravitreal methotrexate for treating vitreoretinal lymphoma: 10 years of experience. The British journal of ophthalmology 92, 383–8 (2008).

24. Ferreri, a. J. M. Relevance of intraocular involvement in the management of primary central nervous system lymphomas. Annals of Oncology 13, 531–538 (2002).

25. Riemens, A. *et al*. Diffuse large B-cell lymphoma in immunoprivileged sites: association of vitreoretinal, testicular and central nervous system lymphoma. Acta ophthalmologica 1–2 (2013).

26. Riemersma, S. A. *et al*. Extensive genetic alterations of the HLA region, including homozygous deletions of HLA class II genes in B-cell lymphomas arising in Extensive genetic alterations of the HLA region, including homozygous deletions of HLA class II genes in B-cell lymphoma. Blood 96, 3569–3577 (2012).

27. Booman, M. *et al*. Genomic alterations and gene expression in primary diffuse large B-cell lymphomas of immune-privileged sites : the importance of apoptosis and immunomodulatory pathways. Journal of Pathology, The 209–217 (2008).



Chapter 3

Diffuse large B-cell lymphoma in immunoprivileged sites: association of vitreoretinal, testicular, and central nervous system lymphoma.

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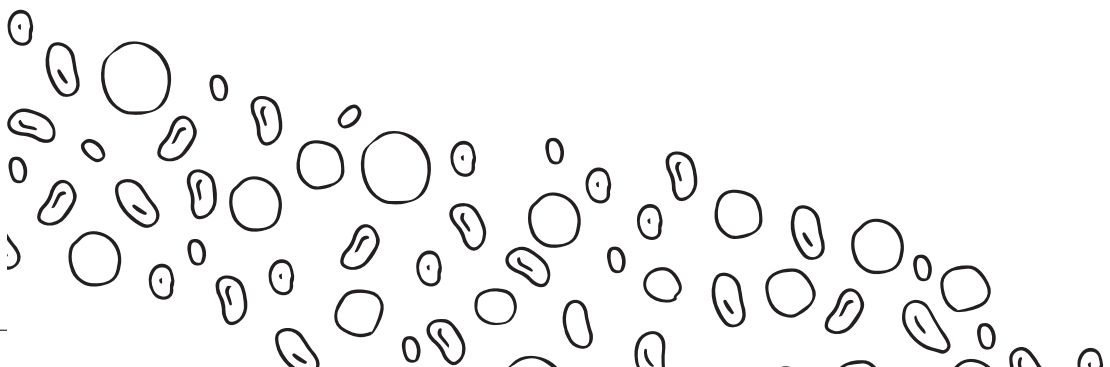


Abstract

Purpose

To describe the association between vitreoretinal, testicular and central nervous system (CNS) lymphoma in 9 patients and report on their clinical manifestations and prognosis.

Methods

Retrospective observational case series of 9 patients with a combination of vitreoretinal and testicular lymphoma of whom 7 with CNS involvement. We assessed diverse clinical data as age at onset, course of the disease, uni/bilateral testicular and ocular lymphoma location, ocular signs, CNS involvement and other sites of spread, interval to other lymphoma localisation, types of treatment and mortality.

Results

At onset, 7 out of 9 patients manifested with primary testicular lymphoma, 1 with primary central nervous system lymphoma and 1 with primary vitreoretinal lymphoma (PVRL). All were classified as diffuse large B-cell (DLBC) lymphoma. The mean age at diagnosis of first lymphoma manifestation was 66 years (sd 8.3). Eight patients without ocular involvement at onset developed vitreoretinal lymphoma (VRL) after a mean interval of 55 months (sd. 39; range 12-120). CNS involvement occurred in 7 out of 9 patients (one at onset and 6 after a mean interval of 61 months; Sd 51 range 3-119). Six patients died and the mean interval from onset of first manifestation of DLBC lymphoma to death was 86 months (range 25-205 months). Ocular involvement in all patients was first diagnosed as uveitis.

Conclusions

We report on 9 patients with intraocular DLBC lymphoma that also developed lymphomas in other immune privileged sites. These findings favour the existence of immune privileged-site lymphoma. The awareness of the existence of this type of lymphoma might improve it's recognition in the future.

Introduction

Primary vitreoretinal lymphoma (PVRL) is a rare type of lymphoma, which is highly associated with central nervous system lymphoma (CNSL)[1]. Primary CNSL represents a subcategory of non-Hodgkin lymphoma (NHL) which is confined to the brain, spinal cord, (lepto)meninges and eyes. The vast majority of PNCSL can be subtyped as diffuse large B-cell lymphomas (DLBCL) and is an aggressive histological type of NHL which has a poor prognosis[2]. Primary vitreoretinal lymphoma (PVRL) and testicular lymphoma are also typically classified as DLBCL[3-6]. Both primary testicular and PVRL are highly associated with CNSL[7]. So far it is not certain whether PVRL and PCNSL represent multiple manifestations of one entity or whether they are different disorders, one originating in the eye(s) and the other in the brain. Primary testicular lymphoma represents about 1-2% of all NHLs and is associated with CNS and contralateral testis[8].

The central nervous system (CNS), eyes and testes are immune privileged sites (so called immune sanctuaries) and have been considered to represent sites where lymphoma cells might escape from T lymphocyte-mediated immunosurveillance and where chemotherapy might have reduced efficacy[9-11]. In this study we present 9 patients diagnosed with a combination of testicular and vitreoretinal DLBCL of whom 7 with concurrent CNS manifestations.

Methods

The study is in accordance with the Declaration of Helsinki and was approved by the institutional ethical committee of the University Medical Center Utrecht, which concluded that the Dutch Medical Research Involving Human Subjects Act did not apply and written informed consent was not needed.

All patients with a combination of primary DLBCL of the testicle(s) and eye(s) that were diagnosed between 1990 and 2012 were selected from 5 tertiary ophthalmic centres from the Netherlands and Slovenia; University Medical Centre Utrecht (n=2), Academic Hospital Maastricht (n=1), Erasmus Medical Centre Rotterdam (n=2), Eye Hospital Rotterdam (n=2) and University Hospital Ljubljana, Slovenia (n=2).

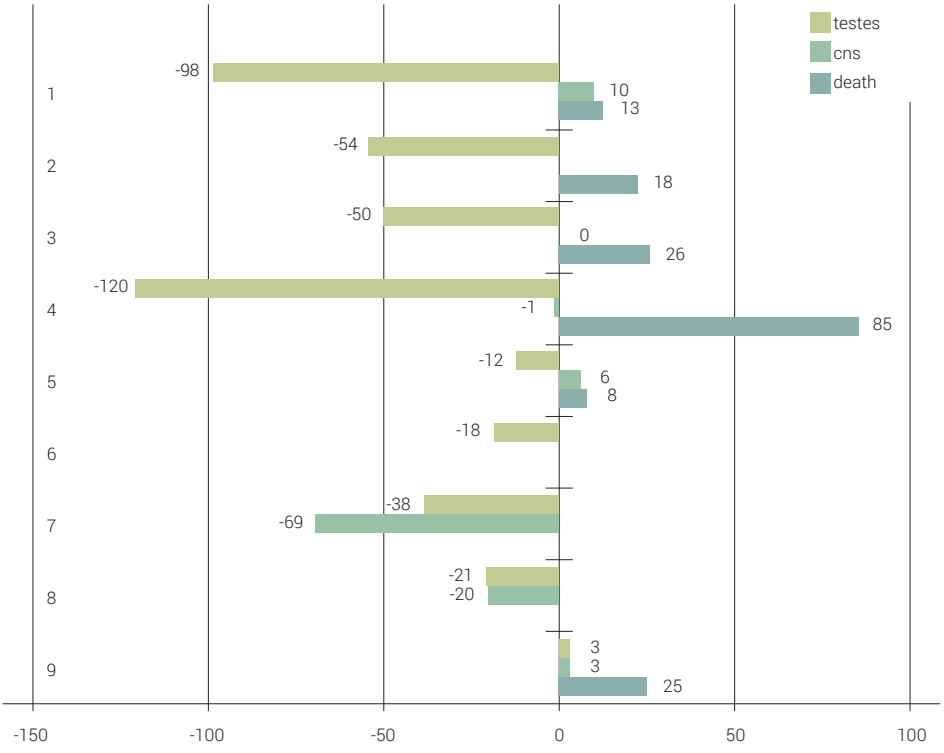
Diagnosis was based on tumour biopsy from the testes and on vitreous fluid obtained by vitrectomy from the eye. CNS localisation was based on combination of neuroimaging (MRI), biopsy and/or cytological analyses of cerebrospinal fluid.

The collected data included various clinical characteristics such as age, uni/bilateral testicular and ocular lymphoma location, date of diagnosis, types of treatment, CNS involvement, other sites of lymphoma, ocular signs, date of diagnosis ocular lymphoma, treatment of ocular lymphoma, possible morbidity, mortality. The data were entered and analysed using SPSS software version 20.0 (IBM Inc, Chicago, Illinois, USA).

Results

The general and clinical data of all 9 male patients are given in Table 1. The mean follow-up time was 86 months (sd. 67).

The histological type of NHL was DLBCL in all patients. Seven patients were first diagnosed with primary testicular NHL of which one patient had concurrent bilateral axillary lymph node and abdominal lymph node localisation, one had lymphoma cells in spinal fluid and one patient was diagnosed with CNS involvement one month after the diagnosis of testicular lymphoma. One additional patient was primarily diagnosed with PVRL and developed testicular lymphoma 13 months later. Another patient presented with PCNSL (n=1) in which testicular lymphoma developed 31 months later.



0 = development vitreoretinal lymphoma.
Horizontal axis: time in months.
Vertical axis: case number of patient.

Figure 1.
Interval from vitreoretinal lymphoma to testicular and central nervous system lymphoma and death

The mean age at the moment of first diagnosis lymphoma was 66 years (sd 8.3). Although only one patient had PVRL at onset, 8 patients developed intraocular lymphoma after a mean interval of 55 months (sd. 39; range 12-120 months). Testicular lymphoma was bilateral in 2 patients and unilateral in 7 patients. Ocular lymphoma was bilateral in 5 patients and unilateral in 4 patients. The mean interval between onset of symptoms and diagnosis of VRL was 12 months (sd 24, range 0-76).

CNS involvement developed in 7 out of 9 patients, in one patient at onset, in one patient with concurrent testes lymphoma and 5 additional patients developed CNS manifestation after a mean interval of 61 months (Sd 51 range 3-119) (Table 1). One patient developed metastases in the lung 2 years after CNS manifestation. Two patients without CNS manifestations had however limited follow-up time (23 months and 72 months).

Diverse treatment regimens are given in Table 1. Out of 9 patients, at the first diagnosis of lymphoma, 4/9 (44%) primarily received a combination of local, intrathecal and systemic treatment, 2/9 (22%) a combination of local and systemic treatment, 1/9 (11%) intrathecal and systemic treatment, 1/9 (11%) local treatment only and 1/9 (11%) was not treated. Treatments for testicular lymphoma included hemicastration, systemic and/or intrathecal chemotherapy and radiotherapy (Table 1). Of all patients with primary testicular lymphoma none relapsed in the (contralateral) testicle. Primary CNS lymphoma (n=1) was treated with radiotherapy of the brain and CNS recurrences (n=5) with diverse combinations of brain irradiation, intrathecal methotrexate (MTX), and chemotherapy (Table 1). CNS and ocular lymphoma recurrences occurred without any additional signs of systemic localization elsewhere. The treatment for intraocular lymphoma is described in Table 1. Primary VRL (n=1) was not treated and the remaining 8 patients with secondary ocular involvement were treated with varied combinations of treatments including systemic, intrathecal and intraocular chemotherapy and radiotherapy of the eyes and/or brain (Table 1).

Ocular involvement in all patients was first diagnosed as uveitis. Ocular manifestations were at onset unilateral in 4 patients and bilateral in 5 patients. All patients reacted well to treatment (radiation, chemotherapy) and no recurrence of ocular lymphoma was reported.

During the follow-up, 6/9 (67%) patients died. The mean interval from the first manifestation of lymphoma to death was 86 months (range 25-205 months), from the onset of testicular and death was 86 months (range 22-205 months) from onset of VRL and death was 42 months (range 13-85 months), the mean interval from onset CNSL and death was 28 months (range 1-86 months). Figure 1 depicts the interval from VRL to testicular and CNS lymphoma and death in all 9 cases. Three patients are still alive with mean follow up of 27 months (range 24-30 months), of these patients one was diagnosed with CNS lymphoma one month after testes lymphoma and one patient had primary CNS lymphoma.

The intervals between the manifestations in diverse immunoprivileged sites were in 3 cases longer than 5 years.

No.	Age at onset of first lymphoma manifestation (years)	Primary lymphoma manifestation	Treatment primary lymphoma location	Onset to VRL[1] (months)	Treatment secondary VRL	CNSL[2]	Onset primary tumor to -CNSL (months)	Death	Survival testicular lymphoma (months)	Survival VRL (months)	Survival CNSL (months)	Interval eye-testes	Interval eye-CNS
1*	63	Testes	Hemi-castration, radiotherapy 5x contralateral testis, CHOP[3] 6x	98	MTX[5] intavitreal 6 times, bilateral	Yes	108	Yes	111	13	3	-98	10
2	70	Testes	Hemi-castration, radiotherapy lymph nodes (26 Gy[4] total, 13 doses), CHOP[3] 8x	54	Radiotherapy skull, eyes and meningen, MTX[5] intrathecal 6 times	No	na	Yes	72	18	na	-54	
3	68	Testes, liquor	CHOP[3], radiotherapy testes (40 Gy[4]), intrathecal MTX[5]	50	MTX[5] intrathecal (freq unknown) en radiotherapy	Yes	50	Yes	76	26	26	-50	0
4	75	Testes**,	8 x CHOP[3] en intrathecal MTX[5]	120	Chemotherapy	Yes	119	Yes	205	85	86	-120	1
5	73	Testes	Hemicastration, R CHOP[6] 4x, intrathecal chemotherapy	12	Radiotherapy planned	Yes	18	Yes	20	8	2	-12	6
6	67	Testes	Hemicastration, radiotherapy contralateral testis,R CHOP[3] 4x, intrathecal chemotherapy	18	Systemic R CHOP[3] and MTX[5] high dose, intrathecal chemotherapy	No	na	No	na	na	na	-18	
7	51	Brain	Radiation brain (40 Gy[4])	69	2 MBVP[7] courses*	Yes	na	No	na	na	na	-38	-69
8	71	Testes and brain***	Hemicastration, CHOP[3] 8x, intrathecal MTX[5] (12x), radiotherapy brain (30 Gy[4])	21	Radiotherapy eyes 30 Gy[4]	Yes	1	No	na	na	na	-21	-20
9	56	Eye	No treatment	0	Not applicable	Yes	3	Yes	22	25	22	3	3

*This patient also developed DLBCL in the neck (43 months after primary testes lymphoma). Treatment: DHAP-VIM[8]-DHAP, mabthera and leucoferese) ** At the moment of diagnosis testicular lymphoma the axillary lymph nodes were also involved. *** Only one month time difference between testes and brain localisation. [1]VRL: vitreoretinal lymphoma; [2]CNSL: central nervous system lymphoma; [3] CHOP: cyclophosphamide-hydroxydaunorubicin-oncovin-prednisolone; [4]Gy: Gray; [5]MTX: methotrexate; [6]R CHOP: rituximab cyclophosphamide-hydroxydaunorubicin-oncovin-prednisolone; [7] MBVP: Methotrexate, BCNU (Carmustine), Vumon (Teniposide) and Prednisone [8] DHAP-VIM: cisplatin-cytarabine-dexamethasone-etoposide-ifosfamide-methotrexate.

Table 1. **Patient characteristics and treatment primary lymphoma**

Discussion

In this study we present 9 patients with DLCB lymphoma manifesting in testes and eyes of whom 7 also had CNS manifestations. This combination of locations is intriguing since testes, eyes and CNS are immune privileged sites.

Only occasional cases of concurrent vitreoretinal and testicular lymphoma have been reported previously[12-16]. Interestingly the first case of intraocular lymphoma was described in1951 by Cooper *et al*. and subsequently developed a testicular lymphoma[16].

Primary testicular lymphoma accounts for 1-9% of the testicular malignancies but is the most common testicular malignancy in males older than 50 years of age[3,17,18]. Primary testicular lymphoma is associated with extranodal site lymphoma's of which the CNS is the most common site (6%-16.5%); other locations include the skin, Waldeyer's ring, lung and pleura. It is a very aggressive type of malignancy with a relapse rate >90% and a 5-year survival of 20% to 25%[19].

PVRL arises within the eye and has a poor prognosis, which is partly due to the late diagnosis, frequent (subsequent) involvement of the CNS, limited sensitivity of the lymphoma to therapy and aggressive treatment modalities with significant complications. PVRL commonly masquerades as posterior uveitis and is difficult to diagnose which often results in a substantial delay of diagnosis[20,21]. The mean interval between onset of symptoms and diagnosis of VRL was in our patients 12 months (sd 24, range 0-76). PVRL often starts as unilateral disease, but becomes bilateral in approximately 80% of cases[22], in our study (P)VRL was bilateral at onset in 56% of patients. Grimm *et al*. report that the median survival of PVRL with brain involvement was 31 months and isolated PVRL had a median survival of 57 months[23,24]. In our study the interval from onset of VRL to death was 34 months (range 13-85 months). However Thiel *et al*. describe that with the use of modern aggressive therapies the median survival of PVRL with CNSL has increased from 1-1.5 years to over 3 years [25].

Coupland *et al*. demonstrate that one single B-cell clone is responsible for the manifestation of bilateral ocular lymphoma and CNSL and hereby show that the lymphomatous manifestations in oculocerebral lymphoma consist of the identical neoplastic B cell population and that they derive from the same tumour precursor cell[26].

Associations of testes and CNS NHL and of ocular and CNS NHL have been reported previously. Because all 3 locations represent immune privileged sites the hypothetical existence of so called "immune privileged site diffuse large B-cell lymphoma (IP-DLBCL) has been put forward. The blood-brain barrier, blood-retina barrier and testicular cells prevent the entrance of lymphocytes and antibodies from the peripheral blood. It was hypothesized, that in immune privileged sites, the malignant cells can escape the immunosurveillance and thereby the destruction initiated by the immune system[9,10]. Features of IP-DLBC lymphoma include involvement of other immune privileged sites. and relatively poor prognosis, possibly due to reduced efficacy of chemotherapy[27,28]. In our study we describe 9 patients in which lymphoma developed in 2 or 3 of the immune privileged sites in one and the same patient. However one patient had abdominal and axillar lymph node involvement which suggests lymphoma spread is not restricted to immune privileged sites.

The crucial issue that remains to be elucidated is whether the IP-DLBCL is of multifocal origin and originates independently in various immune privileged sites or whether this lymphoma has one site of origin and spreads subsequently from one site to the other. Our study suggests that IP-DLBCL is a multifocal condition since the time interval between primary lymphoma and secondary lymphoma was quite extensive, specifically 3 cases had a time interval between the diverse sites longer than 5 years. Booman *et al*. describe that both shared and site-specific genomic aberrations are present in testicular and CNS lymphoma, which suggests that these are not a homogeneous entity[29]. Wallace *et al*. demonstrated that in one patient ocular and testicular lymphoma cells had a different IgH gene rearrangement, however they could not confirm this finding by sequencing because of a small quantity of cells[15].On the contrary, CNS prophylaxis seems to be effective in primary testicular DLBCL, which could suggest that the lymphoma is already present in the CNS at the moment of diagnosis of testicular lymphoma. The comparison of the genetic profile and clonal identity of lymphoma cells in diverse sites is crucial for the elucidation of this question, but unfortunately could not be performed in our retrospective series. In conclusion, the pathogenesis of IP-DLBCL is at present unknown and requires future elucidation.

In this case series, we report on 9 patients with intraocular DLBC lymphoma that also developed other immunprivileged sites (testes and CNS). Our findings advocate the existence of IP-DLBC lymphoma and the awareness of this type of lymphoma might improve it's recognition in the future.

References

1. Coupland SE & Damato B: Understanding intraocular lymphomas. Clinical & experimental ophthalmology.2008; 36: 564–78.

2. Baraniskin A, Deckert M, Schulte-Altedorneburg G, Schlegel U, Schroers R. Current strategies in the diagnosis of diffuse large B-cell lymphoma of the central nervous system. British journal of haematology. 2012; 156 (4): 421–32.

3. Horne MJ, Adeniran AJ. Primary diffuse large B-cell lymphoma of the testis. Archives of pathology & laboratory medicine. 2011; 135 (10): 1363–7.

4. Miedler JD, MacLennan GT. Primary testicular lymphoma. The Journal of urology. 2007; 178 (6): 2645.

5. Lagrange J, Ramaioli A, Theodore C, *et al*. Original article Non-Hodgkin's lymphoma of the testis: A retrospective study of 84 patients treated in the French anticancer centres. 2001: 1313–1315.

6. Al-Abbadi M, Hattab EM, Tarawneh M, Orazi A, Ulbright TM. Primary testicular and paratesticular lymphoma: a retrospective clinicopathologic study of 34 cases with emphasis on differential diagnosis. Archives of pathology & laboratory medicine. 2007; 131 (7): 1040–6.

7. Choi JY, Kafkala C, Foster CS. Primary intraocular lymphoma: A review. Seminars in ophthalmology. 2006; 21 (3): 125–33.

8. Freeman C, Berg JW, Cutler SJ. Occurrence and prognosis of extranodal lymphomas. Cancer. 1972 Jan; 29 (1): 252-60.

9. Riemersma SA, Jordanova ES, Schop RF, *et al*. Extensive genetic alterations of the HLA region, including homozygous deletions of HLA class II genes in B-cell lymphomas arising in immune-privileged sites. Blood.2000; 96: 3569–77.

10. Garrido F, Ruiz-Cabello F, Cabrera T, *et al*. Implications for immunosurveillance of altered HLA class I phenotypes in human tumours. Immunology today. 1997; 18 (2): 89–95.

11. Ruiz-Cabello F, Garrido F. HLA and cancer: from research to clinical impact. Immunology today. 1998; 19 (12): 539–42.

12. Davis, J. W., Moriarty, R. P., Schlossberg, S. M., and Schellhammer, P. F. Bilateral testicular lymphoma treated with orchiectomy: complete response relapsed at 52 months in the vitreous humor. Urology. 2001; 57 (3): 555.

13. Pe'er,J, Rowe, J, Frenkel,S, and Dann, E. Testicular lymphoma, intraocular (vitreoretinal) lymphoma, and brain lymphoma: Involvement of three immunoprivileged sites in one patient. American Journal of Hematology.2010; 85 (8): 631-3.

14. Aliferis K, Chan CC, Donati G, Baglivo E. Intraocular lymphoma following a primary testicular lymphoma in remission for 10 years. International ophthalmology. 2008; 28 (6): 439–40.

15. Wallace DJ, Altemare CR, Shen DF, *et al*. Primary testicular and intraocular lymphomas: two case reports and a review of the literature. Survey of ophthalmology. 2006; 51 (1): 41–50.

16. Cooper, E.L, Riker, J.L. Malignant lymphoma of the uveal tract. American journal of ophthalmology. 1951; 34 (8): 1153-8.

17. Shahab N, Doll DC. Testicular lymphoma. Semin Oncol. 1999; 26 (3): 259-69.

18. Zucca E, Roggero E, Bertoni F, Cavalli F. Primary extranodal non-Hodgkin's lymphomas. Part IT Gastrointestinal, cutaneous and genitourinarylymphomas. Annals of Oncology. 1997; 8 (8): 727–737.

19. Vitolo U, Ferreri AJM, Zucca E. Primary testicular lymphoma. Critical reviews in oncology/hematology. 2008; 65 (2): 183–9.

20. Ishida T, Ohno-matsui K, Kaneko YUH, *et al*. Patterns in eyes with primary intraocular lymphoma. Retina. 2010; 30 (1): 23-32.

21. Karma A, von Willebrand EO, Tommila P V, Paetau AE, Oskala PS, Immonen IJ. Primary intraocular lymphoma: improving the diagnostic procedure. Ophthalmology. 2007; 114 (7): 1372–7.

22. Buggage RR, Chan CC, Nussenblatt RB. Ocular manifestations of central nervous system lymphoma. Current opinion in oncology. 2001; 13 (3): 137–42.

23. Grimm S a, Pulido JS, Jahnke K, *et al*. Primary intraocular lymphoma: an International Primary Central Nervous System Lymphoma Collaborative Group Report. Annals of oncology: official journal of the European Society for Medical Oncology / ESMO. 2007; 18 (11): 1851–5.

24. Grimm S a, McCannel C a, Omuro a MP, *et al*. Primary CNS lymphoma with intraocular involvement: International PCNSL Collaborative Group Report. Neurology. 2008; 71 (17): 1355–60.

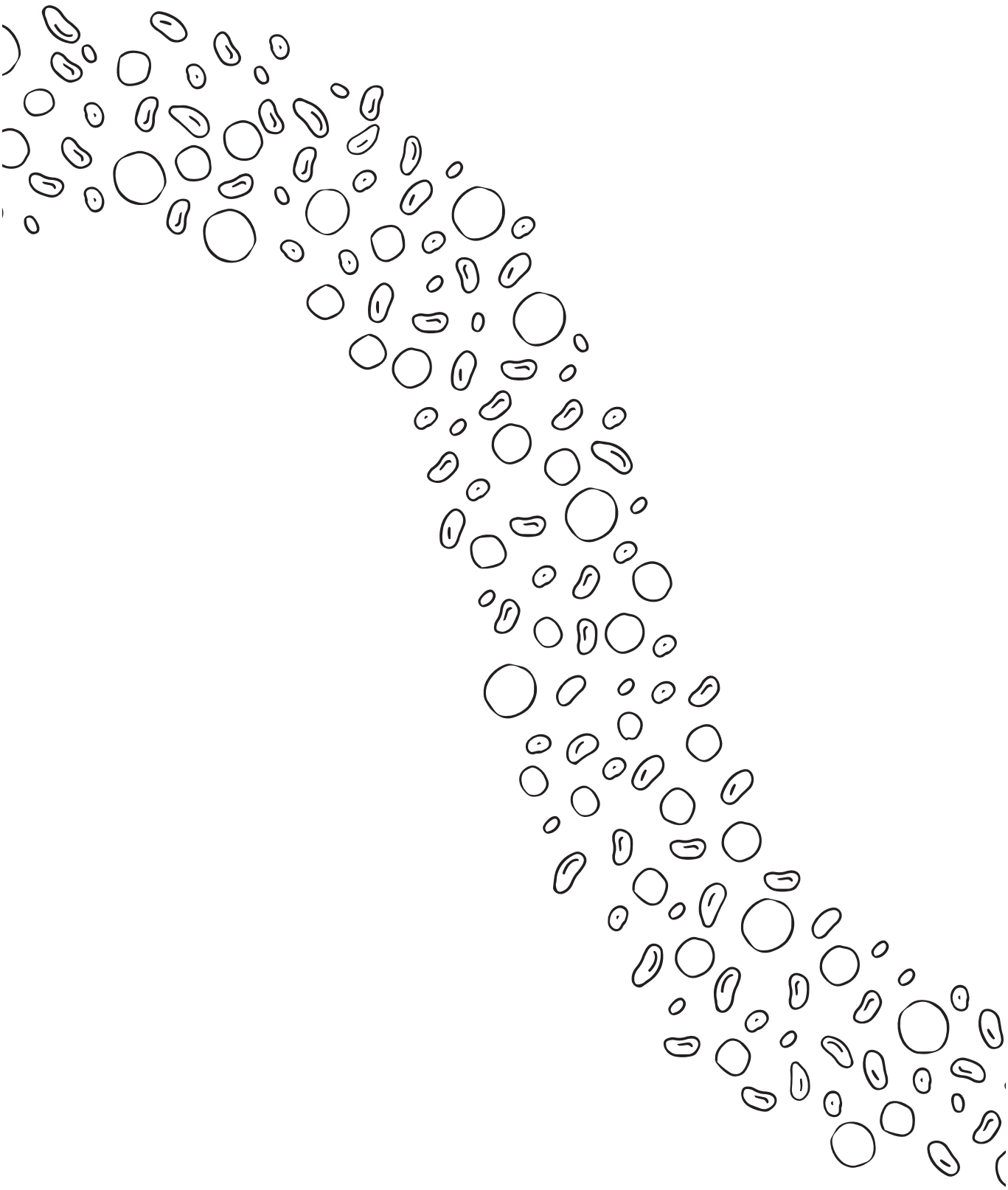
25. Thiel E, Korfel A, Martus P, *et al*. High-dose methotrexate with or without whole brain radiotherapy for primary CNS lymphoma (G-PCNSL-SG-1): a phase 3, randomised, non-inferiority trial. The lancet oncology. 2010; 11 (11): 1036–47.

26. Coupland SE, Hummel M, Stein H, Willerding G, Jahnke K & Stoltenburg-Didinger G: Demonstration of identical clonal derivation in a case of "oculocerebral" lymphoma. The British journal of ophthalmology 2005; 89 (2): 238–9.

27. Fonseca R, Habermann TM, Colgan JP, *et al*. Testicular lymphoma is associated with a high incidence of extranodal recurrence. Cancer. 2000; 88 (1): 154–61.

28. Zucca E. Patterns of Outcome and Prognostic Factors in Primary Large-Cell Lymphoma of the Testis in a Survey by the International Extranodal Lymphoma Study Group. Journal of Clinical Oncology. 2003; 21 (1): 20–27.

29. Booman M, Szuhai K, Rosenwald A, *et al*. Genomic alterations and gene expression in primary diffuse large B-cell lymphomas of immune-privileged sites : the importance of apoptosis and immunomodulatory pathways. Journal of Pathology, The. 2008; 216 (2): 209-17.



Chapter 4

Current insights into ocular graft-versus-host disease

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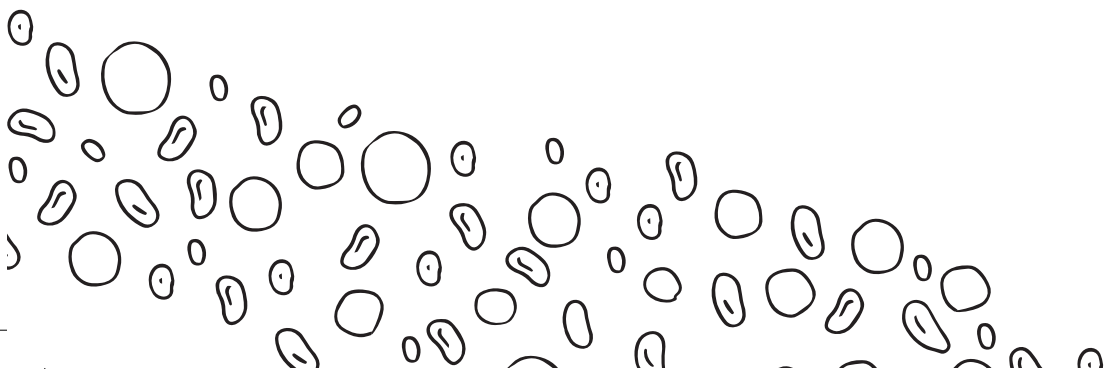


Abstract

Purpose of review

To update our knowledge on hematopoietic stem cell transplantation (SCT) and Graft-versus-Host Disease (GVHD) and summarize the current treatment options for ocular GVHD.

Recent findings

Allogeneic (allo)-SCT represents a treatment option for a number of hematological malignancies and bone marrow disorders; the indications for this procedure are still increasing. Ocular GVHD develops in 40-60% of patients after allo-SCT, can cause severe ocular surface disease (OSD) and has a negative impact on quality of life. There are no widely accepted guidelines for the treatment of ocular GVHD. In addition to the usual treatment with artificial tears, topical steroids, punctal occlusion and contact lenses, recent treatment options include anti-inflammatory medications including topical Cyclosporin A and tacrolimus. Unfortunately, none of the treatment regimens are completely satisfactory and systematic data on the efficacy of these agents are lacking. The preventive treatment possibilities for ocular GVHD have not been defined.

Summary

This review summarizes current data on ocular GVHD and focuses on novel treatment options for this severe ocular disorder. More data on the impact of ocular GVHD and the development of therapeutic and preventive measures are needed.

Introduction

Allogeneic hematological stem cell transplantation (allo-SCT) from a HLA-matched related or unrelated donor is used as a curative therapy for a large number of malignant and nonmalignant hematological diseases. The first successful allo-SCTs in humans were performed in 1968 and ever since many novel strategies have been developed in this field. The major curative effect of allo-SCT is achieved by the so called Graft-versus-Leukemia (GvL) effect. However, despite new transplantation strategies, the down side of the graft versus patient activity of the new implanted immune system, the so called Graft-versus-Host-Disease (GVHD), is still the major reason for non-relapse mortality and morbidity. The recognition of host antigens as foreign by donor T lymphocytes is the major underlying mechanism of GvL and GVHD, and maintaining GvL while dampening GVHD is the crucial challenge of current investigations. Approximately 40% of HLA-matched patients develop acute systemic GVHD and 30-70% of patients develop chronic systemic GVHD[1,2].

Systemic GVHD: Classification

The classification of acute or chronic GVHD is nowadays based on clinical symptoms rather than time interval. Previously, patients developing symptoms in less than 100 days after transplantation were normally classified as having acute GVHD (aGVHD) and patients with symptoms manifesting after 100 days were classified as having chronic GVHD (cGVHD)[3,4]. More recently 2 new categories were added: persistent, recurrent, or late-onset aGVHD (>3 months) and overlap syndrome (no time limit) in which features of chronic and acute GVHD appear together[5] (Table 1).

Category	Time interval between the SCT and onset of GVHD	Presence of acute GVHD features	Presence of chronic GVHD features
Acute GVHD			
Classic	≤ 100 days	Yes	No
Late-onset	> 100 days	Yes	No
Chronic GVHD			
Classic	No time limit	No	Yes
Overlap syndrome	No time limit	Yes	Yes

GVHD, graft-versus-host disease; SCT, stem cell transplantation. Adapted from [5].

Table 1.
Classification of Acute and Chronic GVHD (adapted from Filipovich et al, 2005[5])

Systemic GVHD: Clinical manifestations and diagnosis

Classical aGVHD involves usually three organ systems: skin, gastrointestinal tract and liver (Table 2). cGVHD develops within 3-6 months after SCT, and frequently during the tapering off of systemic immunosuppression or after its discontinuation, but it can also manifest itself up to 3 years after transplantation. cGVHD is often preceded by a history of aGVHD and can be restricted to one single organ or widespread in several organs. The most common sites involved at the initial diagnosis are skin (75%), mouth (51-63%) and liver (29-51%) and involvement of the eye occurs in 40-60% of the patients[2] (Table 2). Although cGVHD manifestations are heterogeneous at onset, the frequency of specific organ manifestations appears to be similar for all treatments i.e. after peripheral blood and bone marrow grafts, with the use of related or unrelated donors, with myeloablative or reduced-intensity conditioning, and in adults or children[6]. To diagnose GVHD at least one clinical sign or manifestation must be present and it is recommended that the diagnosis should be confirmed by tissue biopsy in order to exclude other possible diagnoses such as infections or drug toxicity[7,8].

Systemic GVHD: Prevalence and high risk factors

The incidence of aGVHD is directly related to the degree of HLA mismatch, but even in high-resolution HLA matching, aGVHD can still develop[9]. Systemic treatment for aGVHD is necessary in 40% of patients with HLA-matched grafts and in 60-80% of patients receiving one-antigen HLA-mismatched graft[2].Conditioning regimens during allo-SCT with high doses of total body irradiation can induce aGVHD most likely due to the severe tissue damage which results in the presentation of self-antigens to the donor immune system. Therefore, a delay in the application of immune cells has been recommended after allo-SCT[10]. Chronic GVHD can develop after aGVHD but it can also develop de novo. Risk factors for developing cGVHD include a history of aGVHD, the donor and/or recipient being older, the use of female donors for male recipients and the source of SCT (bone marrow, umbilical cord or peripheral blood).

Systemic GVHD: Pathogenesis

Both acute and cGVHD are caused by donor T cells derived from the graft. The pathogenesis of aGVHD is described as a three-phase process. The first step involves the activation of antigen-presenting cells (APCs) by damage usually caused by the allo-SCT conditioning regimen. Damaged host tissues respond by producing “damage associated danger” signals, including proinflammatory cytokines such as TNF-alpha, IL-1, IL-6, chemokines and increased expression of adhesion molecules MHC antigens and co-stimulatory molecules on host APCs[11]. The core of the GVHD reaction is Step 2, where donor T cells proliferate and

Organ or site	More common in Acute GVHD	Chronic GVHD
Skin	Erythema Maculo-papular rash Pruritus	Poikiloderma Lichen planus-like features Sclerotic features; Morphea-like features, Lichen sclerosus-like features Often areas of depigmentation, hypo pigmentation or hyper pigmentation
Nails		Dystrophy; longitudinal ridging, splitting, or brittle nails Onycholysis; pterygium unguis; nail loss
Hair		Alopecia (after recovery from induction chemotherapy or radiotherapy); scaling, papulosquamous lesions
Oral	Mucositis Erythema Pain	Lichen-planus like features Xerostomia Hyperkeratotic plaques Mucocele Restriction of mouth opening from sclerosis Mucosal atrophy Pseudo membranes and ulcers
Eyes		New onset dry, gritty, or painful eyes Cicatricial conjunctivitis Keratoconjunctivitis sicca
Gastro-intestinal	Anorexia: nausea, vomiting Diarrhoea: weight loss, failure to thrive	Oesophageal web Strictures or stenosis in the upper to mid third of the oesophagus
Genitalia		Lichen-planus like features with possible scarring
Liver	Elevation of total bilirubin, alkaline phosphatase, alanine aminotransferase or aspartate aminotransferase to >2 times the upper limit of normal with no other cause	Same features as acute
Lung		Bronchiolitis obliterans
Muscles, joints		Fasciitis; joint stiffness or contractures secondary to sclerosis, sometimes myositis or polymyositis
Other		Thrombocytopenia; eosinophilia; lymphopenia; hypo- or hyper-gammopathy; auto antibodies

GVHD, graft-versus-host disease. Adapted from [5].

Table 2.
Predominant features of acute and chronic graft-versus-host disease (adapted from Filipovich et al, 2005[5])

differentiate in response to host histoincompatible antigens presented by the APCs[12]. The damage-associated signals generated in Phase I promote donor T cell responses to host antigens by increasing the expression of the 'secondary signal', the co-stimulatory molecules and by the secretion of various 'tertiary signals', the proinflammatory cyokines[13,14]. The effector phase of this process is a complex cascade of both cellular mediators such as cytotoxic T lymphocytes and NK cells. Soluble and cellular mediators synergize to amplify local tissue injury and further promote inflammation and target tissue destruction[11]. Although there is no doubt that T-cells also play a role in cGVHD, cGVHD resembles more autoimmune diseases such as systemic sclerosis. In addition auto-antibodies have been described as providing a lead to the possible role of B-cells in the pathogenesis of cGVHD[15].

Systemic GVHD: Treatment

Immunosupressive therapy for the prevention of GVHD is based on mechanisms that interfere with T-cell activation and function. Cyclosporin A (CsA) is considered to be the most important drug in the prevention of GVHD and it acts by inhibiting T-cell proliferation and IL-2 production. Combinations with mycophenolate mofetil (MMF) and methotrexate (MTX) are commonly used[16,17]. Adding corticosteroids to the first-line immunosuppressive regimen has not been found to confer any beneficial effects[18]. Standard first-line therapy for aGVHD usually consists of steroids 2mg/kg/day. Patients require secondary salvage therapy if aGVHD progresses, if there are no clinical or biochemical changes in 7 days, or if there is incomplete response after 14 days of steroid treatment[19,20]. Several agents are used such as methylprednisolone, anti-thymocyte globulin (ATG), Tacrolimus (FK506) and infliximab with moderate success, and more recently mesenchymal stroma cells[21]. The most frequently mentioned agents are also used for the treatment of cGHVD[22], but in cGVHD the depletion of B-cells[15] and the application of tyrosine kinase inhibitors have been found to improve cGHVD by reducing fibrosis of the tissue[23] (Table 3).

Ocular GVHD: Background

Ocular GVHD develops in 40-60% of patients after allo-SCT and can cause extremely severe ocular surface disease (OSD)[24]. Ocular involvement is quite rare during acute systemic GVHD and develops in about 10% of patients with aGVHD. It is usually considered a poor prognostic factor for mortality caused by systemic aGVHD[22,25].

Ocular GVHD does not generally lead to permanent visual loss but often impairs the quality of life and decreases the activities of associated with daily living[24,26,27].

First-line treatment	
(Methyl-)prednisolone (MP)	Gold standard with 50–60% long-term survival in responders; metabolic side-effects, increased incidence of aspergillosis and osteonecrosis/cataracts Dose: 2 mg/kg may induce fewer infections than higher doses (10 mg/kg) combined immunosuppression; any agent added to MP increases infectious complications (except of oral beclomethasone in early intestinal GvHD)
Second line treatment	
Mycophenolate mofetil (MMF)	May have activity, no randomized trials
Switch CsA-FK506	Minor activity on GvHD, may reduce neurological toxicity
Sirolimus	Increases endothelial toxicity
Antithymocyte globulin (ATG), anti-CD3	Activity in small trials but increased risk of infections
Anti-IL2-R antibodies	Activity in small and phase-II trials, phase-III trials lacking
Tumour necrosis factor- (TNF)-blocking agents	High activity, but strong increase in aspergillus infections for infliximab
Extracorporeal photopheresis (ECP)	High efficacy without an obvious increase in infectious complications; patients may require central lines for venous access
Regimens with specific activity in chronic GvHD	
Pentostatin	Cytostatic side-effects (bone-marrow suppression)
Thalidomide	No additional effect in randomized trials, substantial neurotoxicity
B-cell depletion	Improve cGHVD by reducing fibrosis of the tissue
Tyrosine kinase inhibitors	Improve cGHVD by reducing fibrosis of the tissue

cGVHD, chronic GVHD; GVHD, graft-versus-host disease. Adapted from [22].

Table 3.
Specific characteristics of regimens used for treatment of acute and chronic graft-versus-host disease (GvHD) (adapted from Holler et al.[22]).

Ocular GVHD: Pathophysiology

The hypothesis concerning the pathophysiology of GVHD describes the direct attack by donor lymphocytes on host histocompatibility antigens[28]. The histopathological changes in ocular GVHD are mainly seen in conjunctival and lacrimal gland tissue and predominantly consist of keratinisation[29-32].

As mentioned, aGVHD is mainly a T cell-mediated process and in the conjunctival tissue of patients with aGVHD-related pseudomembranous conjunctivitis, donor-derived mononuclear T lymphocytes have

consequently been detected[25]. Fibrinoid material with cellular debris and inflammatory cells was found by Jack *et al.* in the conjunctival tissue of patients with pseudomembranous conjunctivitis in aGVHD[33]. Pas-positive material in the acini and ductules with stasis was observed in 11 of 26 patients with acute GVHD[30].

Chronic ocular GVHD shows inflammatory destruction of the conjunctivae and lacrimal gland with fibrosis, resulting in tear deficiency and often a damaged ocular surface[33-35]. Histology shows extensive destruction, tissue atrophy and fibrosis of the tuboalveolar glands and ducts in the lacrimal gland with an increase in stromal CD34+ fibroblasts accompanied by mild lymphocytic infiltration[26,36]. CD34+ fibroblasts seem to play an important role in the pathogenesis of lacrimal gland fibrosis. Nearly half of the CD34+ fibroblasts in the affected lacrimal gland are of donor origin and a new therapeutic approach to ocular GVHD might be to inhibit the recruitment of fibroblast precursors[26].

T cells and macrophages play a very significant role in conjunctival inflammation and keratinisation as well and CD14++ macrophages were frequently encountered in the conjunctiva of patients with dry eyes in contrast to patients without dry eyes[37].

The pathophysiology of retinal lesions in GVHD remains unclear and besides GVHD, multiple additional factors are involved including the use of chemotherapeutic agents, cyclosporine toxicity, irradiation and systemic hypertension[38].

Ocular GVHD: Prevalence and high-risk factors

The development of ocular GVHD is strongly associated with systemic cGVHD and occurs in 60% up to 90% of patients with cGVHD[1,24,28]. The most frequent manifestation consists of keratoconjunctivitis sicca (KCS) which occurs in 40-60% of patients with chronic ocular GVHD. Particularly patients with skin or mouth involvement are at risk for developing ocular GVHD[24], though ocular GVHD can also be the initial manifestation of systemic GVHD[39]. Patients with allo-SCT from related donors develop ocular GVHD more often than patients receiving their transplants from matched unrelated donors (MUD). This is probably due to the use of ATG (anti-thymocyte globulin) in the conditioning regimen of patients with MUDs. No obvious associations have been found between the source of stem cells and the development of ocular GVHD[27]. KCS can also develop due to lacrimal deficiency caused by total body irradiation or the use of immunosuppression.

Posterior segment complications were observed in 12.8% of patients after bone marrow transplantation of an unspecified type[40]. The risk of developing retinopathy in GVHD might be higher in patients with low hematocrit, radiation and other predisposing factors for developing retinal microvasculopathy[38]. Optic disc edema may develop due to systemic Cyclosporin And can be resolved by discontinuing or lowering the dose[40].

Ocular GVHD: Clinical features

Ocular GVHD may affect all parts of the eye. Ocular features of aGVHD include pseudomembranous and hemorrhagic conjunctivitis sometimes followed by secondary keratopathy.

KCS is the most common ocular manifestation of GVHD and usually develops together with inflammatory signs of the conjunctiva (conjunctival edema, chemosis, pseudomembrane formation) and chronic blepharitis (Figure 1, 2). This process may lead to secondary epithelial changes such as punctate keratopathy, the formation of filaments, painful erosions and secondary corneal infections and is sometimes even associated with the ulceration and perforation of the cornea (Figure 3). Frequently, profound atrophy of the eyelid rim develops, with keratinisation of palpebral conjunctiva, the development of entropion or ectropion, atrophy of the meibomian glands, loss of lashes and stenosis or closure of the lacrimal punctum (figure 4). The dysfunction of the meibomian glands with subsequent atrophy contributes to a poor quality of the tear film. Patients with ocular GVHD, especially when on immunosuppressive treatment may develop infectious conjunctivitis, blepharitis, keratitis and even endophthalmitis which all can lead to permanent visual loss.

Uveitis is a less common manifestation of GVHD and occurs predominantly during the exacerbation of systemic GVHD. Mostly observed is a mild anterior uveitis which reacts well to treatment with local steroids and an increase in systemic anti-GVHD therapy[41,42].

Cataract development is nowadays less frequent, as the conditioning regimen includes less radiation

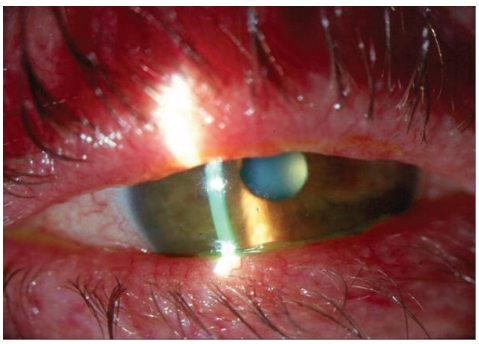


Figure 1.
Active ocular graft versus host disease in a 57 year-old male 7 months after allogeneic stem cel transplantation demonstrating blepharitis, edema and hyperamia of the lids and conjunctiva as well as keratoconjunctivitis sicca.

Figure 2.
Chronic ocular graft versus host disease documenting blepharitis, loss of lashes and keratoconjunctivitis sicca exhibiting filaments on the corneal surface in a 52-year-old male, diagnosed with chronic lymphatic leukaemia, 42 months after allogeneic stem cell transplantation from an unrelated donor

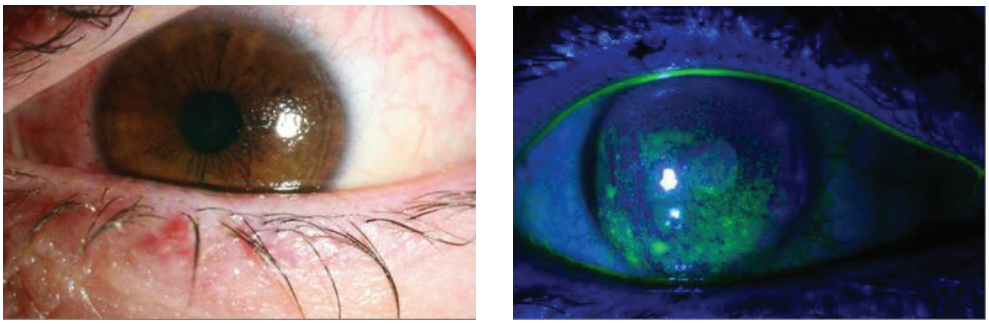


Figure 3.
Keratoconjunctivitis sicca in a 33-year-old male with ocular graft-versus-host-disease 60 months following allogeneic bone marrow transplantation from his sister. Left, irregular light reflex caused by dry corneal epithelium, right, fluorescein staining documents diffuse epithelial damage.

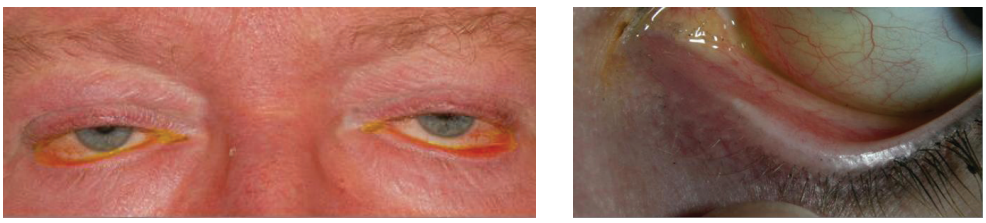


Figure 4.
Eye lid malformations due to ocular graft-versus-host-disease. Left, chronic ocular graft-versus-host-disease leading to fibrosis of eye lids with secondary ectropion and loss of lashes in a 59-year-old male with non Hodgkin lymphoma 14 months after allogeneic stem cell transplantation from his HLA-identical sister. Right, spontaneous punctal occlusion due to previous conjunctival and eye lid inflammation in ocular graft versus host disease in a 16-year-old female 58 months after allogeneic stem cell transplantation from her HLA-identical sister, causing paradoxal complaints of tearing in keratoconjunctivitis sicca with Schirmer values of over 35 mm due to lacrimal gland insufficiency.

and corticosteroids. In posterior segment retinopathy can manifest with cotton wool spots, retinal hemorrhages, lipid deposits, optic disk edema and infections.

Symptoms of ocular GVHD depend on its localization and may include dry, gritty or painful eyes, foreign body sensation, blurred vision, paradoxical excessive tearing, photophobia, and difficulty in opening the eyes in the morning due to secretions and swelling and erythema of the eyelids. Visual complaints regularly accompany these symptoms of OSD. Balaram *et al*. found severe staining in 58% of 62 patients with ocular surface disease who all had evidence of conjunctival inflammation. Sterile corneal ulcers or non-healing epithelial defects of the cornea developed in 13% (8/62) of patients and occurred in eyes with severe lacrimal deficiency[1]. Tabbara *et al*. found major ocular complications including severe OSD, corneal ulcers and perforations, and uveitis in13% (80 of 620 patients) of allo-SCT patients (bone marrow transplantation,

peripheral blood cells and cord blood cells)[27].

Although ocular complications after allo-SCT are common and may become severe and cause considerable suffering, so far no guidelines are available on screening and prevention. Early recognition of ocular GVHD and careful monitoring of patients at risk of severe ocular involvement might allow treatment to start in time before permanent tissue damage could develop.

Ocular GVHD: Diagnosis and classification

The diagnosis of ocular GVHD is based on the ocular manifestations occurring after SCT, preferably confirmed by biopsy or other relevant tests[8]. The Schirmer test values should be ≤5mm in both eyes or between 6-10 mm with KCS diagnosed by slit-lamp examination accompanied by involvement of at least 1 other organ[8]. Currently no standard classification system for ocular GVHD is accepted; however, the most generally used classification system is given in Table 4[8,24,31,43].

Ocular GVHD: Treatment options

Treatment options for ocular GVHD consist of systemic and topical ocular treatment regimens.

In short, treatment possibilities include immunosuppressive agents and symptomatic medication such as artificial tears (Table 5).

Systemic treatments for GVHD are also effective for ocular GVHD, which is illustrated by the onset of most cases of ocular GVHD during the tapering off of systemic therapy or thereafter. Very few data are available on the efficacy of specific systemic agents on ocular GVHD. The baseline topical treatment for ocular GVHD usually consists of artificial tears as well as local anti-inflammatory medications, of which topical corticosteroid or Cyclosporin A (CsA) is most commonly used. Preservative-free medications are preferred in all forms of topical treatment. One of the reasons for the limited effect of treatment for ocular GVHD may be that the therapy is usually initiated at a late stage of the disorder, with permanent tissue damage already present[1]. The most commonly used topical treatment options will be discussed here.

Topical lubricants

The mainstay of treatment for KCS occurring during GVHD consists of topical lubricants. Many different brands are available and there is a large individual variability in tolerance. No data are available on the efficacy of specific artificial tears medications in ocular GVHD but in a large study of 1293 patients with dry eyes of diverse origins no differences between specific products were observed[44]. The efficacy of Hydroxypropyl cellulose ophthalmic inserts (Lacrisert) was not systematically studied in ocular GVHD.

Autologous serum eye drops

Autologous serum (AS) contains vitamin A, epidermal growth factor, fibronectin and transforming growth factor-β, which all are valuable for corneal and conjunctival integrity[45-47]. The efficacy and safety of AS drops were investigated in a small study of 14 patients with ocular GVHD. While the improvement was noted in all patients at the 4-week follow-up, maintained responses were observed in 7/14 patients[48]. In addition, several case reports noted improvement in OSD and subjective complaints[37,45]. The risk of contamination and subsequent infection forms a possible complication of AS drops.

Contact and scleral lenses

Contact lenses help to stabilize the tear film and restore normal cell turnover[34]. In patients with GVHD two different types of lenses can be used, the bandage lens (BL) and the scleral lens (SL), which is larger and creates a tear-filled vault over the corneal surface. The use of SL in two small case series totalling 17 patients with KCS due to ocular GVHD showed a subjective improvement in the majority of patients[34,35]. Since the rigid SL rests on conjunctiva, conjunctival involvement might limit its use.

Punctal occlusion

Drainage of tears can be minimized by the use of temporary punctal plugs or permanent punctal occlusion by thermal cauterization. Initially, a beneficial effect for this treatment was reported[34,48], but repeated plug loss, often associated with conjunctival punctal fibrosis, was reported in 78% (25 /32) of the patients treated[1].

Topical anti-inflammatory drugs: Topical corticosteroids

Corticosteroids are immunosuppressive agents that mainly suppress the cellular immune response by reducing cellular migration and phagocytosis. Systemic steroids represent the mainstay in the treatment of acute (exacerbations of) GVHD, but no systematic data are available on the efficacy of topical steroids in ocular GVHD. In a small study of 7 patients with conjunctival GVHD, resolution or an improvement of the conjunctival signs was achieved using topical corticosteroids, but the signs of KCS remained unchanged[43]. Obviously, patients receiving topical corticosteroids should be monitored for adverse effects. In patients with corneal epithelial defects, stromal thinning, or infiltrates, topical corticosteroids are contraindicated.

Topical anti-inflammatory drugs: Topical Cyclosporin A

Cyclosporin A (CsA) is a cyclic polypeptide produced by the fungus Tolypocladium inflatum gams. Topical CsA inhibits the proliferation of T-lymphocytes and the production and the release of lymphokines from activated T-cells in the conjunctiva. CsA also increases the goblet cell density of the conjunctiva and decreases epithelial cell turnover. Topical CsA has been shown to be safe and effective in the treatment of moderate to severe dry eyes in Sjögren's syndrome (increased Schirmer scores, decreased surface apoptosis)[49-51], though limited data are available on its use in ocular GVHD.

Classification of Dry Eyes in GvHD adapted from Filipovich et al.[8]	
0	No dry eye symptoms
1	Dry eye symptoms not affecting ADL (eye drops ≤3 per day) or asymptomatic signs of keratoconjunctivitis sicca
2	Dry eye symptoms partially affecting ADL (eye drops >3 per day or punctal plugs) without vision impairment
3	Dry eye symptoms, significantly affecting ADL (special eyewear to relieve pain) or unable to work because of ocular symptoms or loss of vision caused by keratoconjunctivitis sicca
Classification of Conjunctivitis in Acute GvHD, Adapted From Jabs et al.[31]	
0	None
1	Hyperemia
2	Hyperemia with serosanguinous chemosis
3	Pseudo membranous conjunctivitis
4	Pseudo membranous conjunctivitis with corneal epithelial sloughing
Classification of Conjunctivitis in Chronic GvHD, Adapted From Robinson et al.[43]	
0	None
1	Hyperemia
2	Palpebral conjunctival fibrovascular changes with or without epithelial sloughing
3	Palpebral conjunctival fibrovascular changes involving 25%–75% of total surface area
4	Involvement of 75% of total surface area with or without cicatricial entropion

GVHD, graft-versus-host disease; ADI, activities of daily living.

Table 4.
Classifications of Ocular Symptoms in GvHD

Topical administration of 0.05% CsA produced significant improvement in KCS parameters in a prospective study with 14 patients refractory to conventional treatments, but it had to be stopped in 2 patients because of intolerable irritation[52]. Although a beneficial effect of topical CsA on ocular GVHD has been documented in several small studies, there is at present no large randomized study[53-55].

Topical anti-inflammatory drugs: Tacrolimus

FK506 (tacrolimus) is a macrolide antibiotic extracted from the soil fungus Streptomyces tsukubaensis

and its mechanisms of action and pharmacokinetics are similar to CsA[56], though the immunosuppressive potency of tacrolimus in vitro is 50 to 200 times greater. Although the beneficial effect of systemic tacrolimus on ocular GVHD has been observed[57], topical administration might form a better treatment option because of the adverse effects associated with its long-term systemic administration. Except for one case report in which ocular GVHD was successfully treated, no data are available on the use of topical tacrolimus[58].

Topical anti-inflammatory drugs: Tranilast

Tranilast (rizaben) is an anti-allergic drug, which inhibits the production and/or release of various inflammatory mediators and cytokines and interferes with the proliferation and migration of vascular medial smooth muscle cells, and inhibits collagen synthesis. Tranilast might theoretically prevent lacrimal gland fibrosis, but further studies are required to study its efficacy in ocular GVHD. A small non-randomized study reports on improvement in reflex tearing and Rose Bengal scores in 8 GVHD patients with topical tranilast[58]. These findings warrant further investigations.

Additional treatment options

Topical treatment modalities used for ocular GVHD included retinoic acid, moisture chamber glasses, collagen shields and transplantation of autologous limbal epithelial cells[7]. In severe cases, amniotic membrane grafting and penetrating keratoplasty were also performed[48].

Systemic therapy with selective muscarinic agonists such as cevimeline or pilocarpine may be used to increase aqueous tear flow but no specific data are available on their use in GVHD[7]. Doxycycline was recommended for treatment of KCS of various origins[7,60], but its precise effect remains unknown and no data is available on its use in ocular GVHD.

Prognosis

The long-term impact of ocular GVHD on quality of life has not yet been systematically studied. Balaram *et al*. followed 114 patients for more than 1 year after SCT and concluded that late-onset OSD can occur and points out the need for long-term monitoring[1]. Data on the long-term clinical course and visual outcomes of patients with ocular GVHD are required in order to develop the appropriate measures and therapeutic guidelines.

Conclusions and perspectives

Allo-SCT represents a curative treatment option for a variety of hematological disorders and it is more frequently indicated as SCT methods advance and make this procedure more efficacious and safer. However a large number of patients develop GVHD, which has a great impact on morbidity and mortality after SCT. Ocular GVHD develops frequently after allo-SCT and is mainly seen in cGVHD.

Available data show a prevalence of ocular GVHD in more than 50% of patients after allo-SCT and severe complications in 13% of patients[27]. The information on long-term ocular complications and outcome is lacking. Novel improvements in allo-SCT, such as HLA matching, reduction in conditioning regimen intensity and T cell depletion, might help to diminish the development and severity of (ocular) GVHD. At present, there are no curative medications for ocular GVHD and none of the available treatment options

Therapy	Indication
Topical	Mild Artificial Tears, preservative free Viscous ointment at bedtime, viscous tears during the day Moderate/severe Cyclosporine eye drops Topical steroid drops Lacriserts for patients who use artificial tears more frequently than hourly
Oral	Moderate/severe Cevimeline Pilocarpine Doxycycline
Surgical	Moderate/severe Punctal occlusion (temporary or permanent occlusion, using silicone plugs or thermal cautery) Superficial debridement of filamentary keratitis Partial tarsorrhaphy
Eyewear/environmental strategy	Moderate/severe Occlusive eye wear Lid care/warm compress/humidified environment Bandage contact lens (used with extreme caution)
Treatment not widely available	Moderate/severe Autologous serum eye drops Gas-permeable contact lens

GVHD, graft-versus-host disease. Adapted from [7].

Table 5.
Treatment options Ocular GVHD, adapted from Couriel et al. [7]

for ocular GVHD are completely satisfactory. There are few therapeutic studies with a sufficient number of patients with promising topical agents such as CsA and tacrolimus. Treatment is usually initiated during the symptomatic stage and by that time the damage to the lacrimal gland and conjunctival tissue might be permanent. So far, prophylactic treatment strategies for ocular GVHD have not been investigated. Data on the long-term development and outcome of ocular GVHD needs to be collected in order to support the development of therapeutic and pre-emptive strategies.

References

1. Balaram M, Rashid S, Dana R. Chronic ocular surface disease after allogeneic bone marrow transplantation. Ocul Surf 2005; 3: 203-211.

2. Ferrara JL, Levine JE, Reddy P, Holler E. Graft-versus-host disease. Lancet 2-5-2009; 373: 1550-1561.

3. Vigorito AC, Campregher PV, Storer BE, *et al*. Evaluation of NIH consensus criteria for classification of late acute and chronic GVHD. Blood 16-7-2009; 114: 702-708.

4. Shulman HM, Sullivan KM, Weiden PL, *et al*. Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. Am J Med 1980; 69: 204-217.

5. Filipovich AH, Weisdorf D, Pavletic S, *et al*. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant 2005; 11: 945-956.

6. Lee SJ, Flowers ME. Recognizing and managing chronic graft-versus-host disease. Hematology Am Soc Hematol Educ Program 2008; 1: 134-141.

7. Couriel DR. Ancillary and supportive care in chronic graft-versus-host disease. Best Pract Res Clin Haematol 2008; 21: 291-307.

8. Filipovich AH. Diagnosis and manifestations of chronic graft-versus-host disease. Best Pract Res Clin Haematol 2008; 21: 251-257.

9. Beatty PG, Clift RA, Mickelson EM, *et al*. Marrow transplantation from related donors other than HLA-identical siblings. N Engl J Med 26-9-1985; 313: 765-771.

10. Hale G, Slavin S, Goldman JM, *et al*. Alemtuzumab (Campath-1H) for treatment of lymphoid malignancies in the age of nonmyeloablative conditioning? Bone Marrow Transplant 2002; 30: 797-804.

11. Welniak LA, Blazar BR, Murphy WJ. Immunobiology of allogeneic hematopoietic stem cell transplantation. Annu Rev Immunol 2007; 25: 139-170.

12. Shlomchik WD. Graft-versus-host disease. Nat Rev Immunol 2007; 7: 340-352.

13. Ferrara JL, Reddy P. Pathophysiology of graft-versus-host disease. Semin Hematol 2006; 43: 3-10.

14. Dustin ML. Role of adhesion molecules in activation signaling in T lymphocytes. J Clin Immunol 2001; 21: 258-263.

15. Shimabukuro-Vornhagen A, Hallek MJ, Storb RF, von Bergwelt-Baildon MS. The role of B cells in the pathogenesis of graft-versus-host disease. Blood 3-12-2009; 114: 4919-4927.

16. Bacigalupo A, Van Lint MT, Occhini D, *et al*. Increased risk of leukemia relapse with high-dose Cyclosporin A after allogeneic marrow transplantation for acute leukemia. Blood 1-4-1991; 77: 1423-1428.

17. Bolwell B, Sobecks R, Pohlman B, *et al*. A prospective randomized trial comparing Cyclosporin And short course methotrexate with Cyclosporin And mycophenolate mofetil for GVHD prophylaxis in myeloablative allogeneic bone marrow transplantation. Bone Marrow Transplant 2004; 34: 621-625.

18. Storb R, Pepe M, Anasetti C, *et al*. What role for prednisone in prevention of acute graft-versus-host disease in patients undergoing marrow transplants? Blood 1-9-1990; 76: 1037-1045.

19. Antin JH. Approaches to graft-vs-host disease. Pediatr Transplant 2005; 9 Suppl 7: 71-75.

20. Antin JH, Chen AR, Couriel DR, *et al*. Novel approaches to the therapy of steroid-resistant acute graft-versus-host disease. Biol Blood Marrow Transplant 2004; 10: 655-668.

21. Le BK, Rasmusson I, Sundberg B,*et al*. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet 1-5-2004; 363: 1439-1441.

22. Holler E. Risk assessment in haematopoietic stem cell transplantation: GvHD prevention and treatment. Best Pract Res Clin Haematol 2007; 20: 281-294.

23. Olivieri A, Locatelli F, Zecca M, *et al*. Imatinib for refractory chronic graft-versus-host disease with fibrotic features. Blood 16-7-2009; 114: 709-718.

24. Westeneng AC, Hettinga Y, Lokhorst H, *et al*. Ocular Graft-Versus-Host Disease After Allogeneic Stem Cell Transplantation. Cornea 20-5-2010; 7: 758-63.

25. Saito T, Shinagawa K, Takenaka K, *et al*. Ocular manifestation of acute graft-versus-host disease after allogeneic peripheral blood stem cell transplantation. Int J Hematol 2002; 75: 332-334.

26. Ogawa Y, Kuwana M. Dry eye as a major complication associated with chronic graft-versus-host disease after hematopoietic stem cell transplantation. Cornea 2003; 22: S19-S27.

27. Tabbara KF, Al-Ghamdi A, Al-Mohareb F, *et al*. Ocular findings after allogeneic hematopoietic stem cell transplantation. Ophthalmology 2009; 116: 1624-1629.

28. Franklin RM, Kenyon KR, Tutschka PJ, *et al*. Ocular manifestations of graft-vs-host disease. Ophthalmology 1983; 90: 4-13.

29. Hirst LW, Jabs DA, Tutschka PJ, *et al*. The eye in bone marrow transplantation. I. Clinical study. Arch Ophthalmol 1983; 101: 580-584.

30. Jabs DA, Hirst LW, Green WR, *et al*. The eye in bone marrow transplantation. II. Histopathology. Arch Ophthalmol 1983; 101: 585-590.

31. Jabs DA, Wingard J, Green WR, *et al*. The eye in bone marrow transplantation. III. Conjunctival graft-vs-host disease. Arch Ophthalmol 1989; 107: 1343-1348.

32. Westekemper H, Meller S, Citak S, *et al*. Differntial chemokine expression in chronic GVHD of the conjunctiva. Bone Marrow Transplant 2010; e-pub ahead of print.

33. Jack MK, Hicks JD. Ocular complications in high-dose chemoradiotherapy and marrow transplantation. Ann Ophthalmol 1981; 13: 709-711.

34. Russo PA, Bouchard CS, Galasso JM. Extended-wear silicone hydrogel soft contact lenses in the management of moderate to severe dry eye signs and symptoms secondary to graft-versus-host disease. Eye Contact Lens 2007; 33: 144-147.

35. Takahide K, Parker PM, Wu M, *et al*.Use of fluid-ventilated, gas-permeable scleral lens for management of severe keratoconjunctivitis sicca secondary to chronic graft-versus-host disease. Biol Blood Marrow Transplant 2007; 13: 1016-1021.

36. Ogawa Y, Yamazaki K, Kuwana M, *et al*.A significant role of stromal fibroblasts in rapidly progressive dry eye in patients with chronic GVHD. Invest Ophthalmol Vis Sci 2001; 42: 111-119.

37. Rojas B, Cuhna R, Zafirakis P, *et al*. Cell populations and adhesion molecules expression in conjunctiva before and after bone marrow transplantation. Exp Eye Res 2005; 81: 313-325.

38. Moon SJ, Mieler WF. Retinal complications of bone marrow and solid organ transplantation. Curr Opin Ophthalmol 2003; 14: 433-442.

39. Kim SK. Update on ocular graft versus host disease. Curr Opin Ophthalmol 2006; 17: 344-348.

40. Coskuncan NM, Jabs DA, Dunn JP, *et al*. The eye in bone marrow transplantation. VI. Retinal complications. Arch Ophthalmol 1994; 112: 372-379.

41. Hettinga YM, Verdonck LF, Fijnheer R, *et al*. Anterior uveitis: a manifestation of graft-versus-host disease. Ophthalmology 2007; 114: 794-797.

42. Wertheim M, Rosenbaum JT. Bilateral uveitis manifesting as a complication of chronic graft-versus-host disease after allogeneic bone marrow transplantation. Ocul Immunol Inflamm 2005; 13: 403-404.

43. Robinson MR, Lee SS, Rubin BI, *et al*. Topical corticosteroid therapy for cicatricial conjunctivitis associated with chronic graft-versus-host disease. Bone Marrow Transplant 2004; 33: 1031-1035.

44. Doughty MJ, Glavin S. Efficacy of different dry eye treatments with artificial tears or ocular lubricants: a systematic review. Ophthalmic Physiol Opt 2009; 29: 573-583.

45. Chiang CC, Lin JM, Chen WL, Tsai YY. Allogeneic serum eye drops for the treatment of severe dry eye in patients with chronic graft-versus-host disease. Cornea 2007; 26: 861-863.

46. Fox RI, Chan R, Michelson JB, *et al*. Beneficial effect of artificial tears made with autologous serum in patients with keratoconjunctivitis sicca. Arthritis Rheum 1984; 27: 459-461.

47. Van Setten GB, Viinikka L, Tervo T, *et al*. Epidermal growth factor is a constant component of normal human tear fluid. Graefes Arch Clin Exp Ophthalmol 1989; 227: 184-187.

48. Ogawa Y, Okamoto S, Mori T, *et al*. Autologous serum eye drops for the treatment of severe dry eye in patients with chronic graft-versus-host disease. Bone Marrow Transplant 2003; 31: 579-583.

49. Barber LD, Pflugfelder SC, Tauber J, Foulks GN. Phase III safety evaluation of cyclosporine 0.1% ophthalmic emulsion administered twice daily to dry eye disease patients for up to 3 years. Ophthalmology 2005; 112: 1790-1794.

50. Sall K, Stevenson OD, Mundorf TK, Reis BL. Two multicenter, randomized studies of the efficacy and safety of cyclosporine ophthalmic emulsion in moderate to severe dry eye disease. CsA Phase 3 Study Group. Ophthalmology 2000; 107: 631-639.

51. Stevenson D, Tauber J, Reis BL. Efficacy and safety of Cyclosporin A ophthalmic emulsion in the treatment of moderate-to-severe dry eye disease: a dose-ranging, randomized trial. The Cyclosporin A Phase 2 Study Group. Ophthalmology 2000; 107: 967-974.

52. Wang, Y., Ogawa, Y., Dogru, M., Kawai, M., Tatematsu, Y., Uchino, M., … Tsubota, K. (2008). Ocular surface and tear functions after topical cyclosporine treatment in dry eye patients with chronic graft-versus-host disease. Bone marrow transplantation, 41 (3), 293–302.

53. Kiang E, Tesavibul N, Yee R, *et al*. The use of topical Cyclosporin A in ocular graft-versus-host-disease. Bone Marrow Transplant 1998; 22: 147-151.

54. Lelli GJ, Jr., Musch DC, Gupta A, *et al*. Ophthalmic cyclosporine use in ocular GVHD. Cornea 2006; 25: 635-638.

55. Rao SN, Rao RD. Efficacy of topical cyclosporine 0.05% in the treatment of dry eye associated with graft versus host disease. Cornea 2006; 25: 674-678.

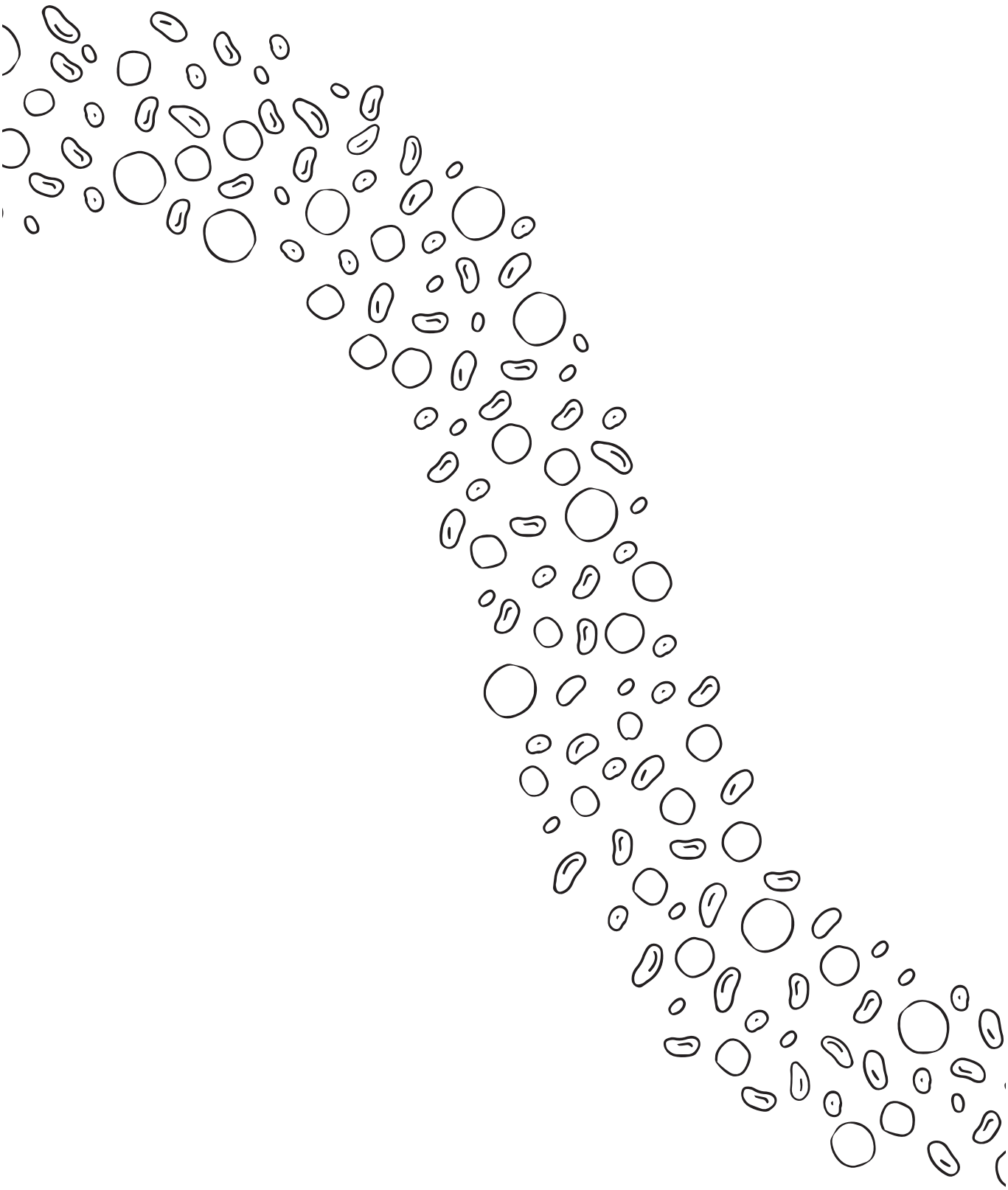
56. Kino T, Hatanaka H, Hashimoto M, *et al*. FK-506, a novel immunosuppressant isolated from a Streptomyces. I. Fermentation, isolation, and physico-chemical and biological characteristics. J Antibiot (Tokyo) 1987; 40: 1249-1255.

57. Ogawa Y, Okamoto S, Kuwana M, *et al*. Successful treatment of dry eye in two patients with chronic graft-versus-host disease with systemic administration of FK506 and corticosteroids. Cornea 2001; 20: 430-434.

58. Tam PM, Young AL, Cheng LL, Lam PT. Topical 0.03% tacrolimus ointment in the management of ocular surface inflammation in chronic GVHD. Bone Marrow Transplant 2010; 45: 957-958.

59. Ogawa Y, Dogru M, Uchino M, *et al*. Topical tranilast for treatment of the early stage of mild dry eye associated with chronic GVHD. Bone Marrow Transplant 2010; 45: 565-569.

60. Smith VA, Khan-Lim D, Anderson L, Cook SD, Dick AD: Does orally administered doxycycline reach the tear film? Br J Ophthalmol 2008; 92: 856-859.



Chapter 5

International chronic ocular graft-versus-host disease (GVHD) consensus group: Proposed diagnostic criteria for chronic GVHD (Part I)

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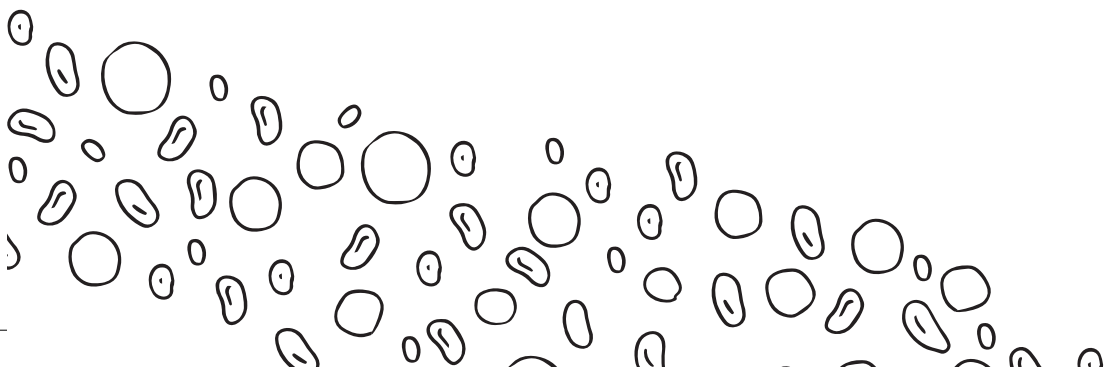


Introduction

Dry eye disease (DED) is one of the most common manifestations of chronic graft vs. host disease (cGVHD) and has been recognized as an important complication after allogeneic hematopoietic stem cell transplantation (HSCT) [1-12]. Since visual function and ocular symptoms are largely related to patient's quality of life [13], ocular complications have a great impact on morbidity after successful HSCT. More than 25000 HSCT procedures are performed annually and the number is increasing worldwide[14]. Historically, cGVHD was classified as limited or extensive based on the clinical findings in a small cohort of patients[1]. Recently, diagnostic criteria for cGVHD have been proposed by the National Institutes of Health (NIH)[15]. As per the NIH consensus criteria definition, "diagnosis of cGVHD requires the presence of at least 1 diagnostic clinical sign of cGVHD (e.g., polikiloderma or esophageal web) or the presence of at least 1 distinctive manifestation (e.g., keratoconjunctivitis sicca) confirmed by pertinent biopsy or other relevant tests (e.g., Schirmer test) in the same or another organ." The NIH Consensus therefore notes that dry eye is a distinctive sign seen in cGVHD but insufficient in itself to establish a diagnosis of cGVHD. However, this precludes the early diagnosis of systemic GVHD in the presence of new onset DED after HSCT. In this paper, we compile recently emerging evidence that supports the classification of dry eye syndrome as a diagnostic clinical entity for cGVHD.

The purpose of international workshops on chronic ocular GVHD were:

1. To provide a consensus overview of chronic ocular GVHD.
2. To refine the definition and classification of chronic ocular GVHD.
3. To assess methods of diagnosis, evaluation, and grading of chronic ocular GVHD by reviewing previously reported literature.
4. To generate a proposal to change dry eye syndrome as a sufficient clinical entity for the diagnosis of cGVHD, address the diagnostic criteria, discuss the severity scores, and recommend an amendment to the NIH Consensus diagnostic criteria for the stem cell transplantation community.

First, we would like to establish the consensus diagnostic criteria and classification for chronic ocular GVHD. Next, after validating a multicenter and prospective study using these criteria, we would like to propose the new diagnostic criteria for chronic GVHD based on ocular GVHD assessment. Our ultimate goal is to add ocular GVHD as a diagnostic sign for chronic GVHD.

Methods

The International Ophthalmology Consensus Conference on Chronic Ocular GVHD was held comprising nine ophthalmologists from the USA, the Netherlands and Japan. The workshop occurred with the purpose of reviewing the published data, gathering a consensus from those in the field, with clinical practice and research interests in chronic ocular GVHD, and providing guidelines for diagnostic criteria.

Four working meetings by the International Chronic Ocular GVHD Consensus Group were held. Each investigator participated in reviewing the data and the collective suggestions for the significance of dry eye in the diagnosis for systemic GVHD. The metric for diagnostic criteria, the severity of dry eye and relevance to systemic GVHD were discussed to identify currently used diagnostic variables in patients' history and examination. Subjective and objective factors were considered and scores were assigned to each variable to reflect severity of the disease. Consideration was given to the presence or absence of systemic GVHD.

Lacrimal gland histology

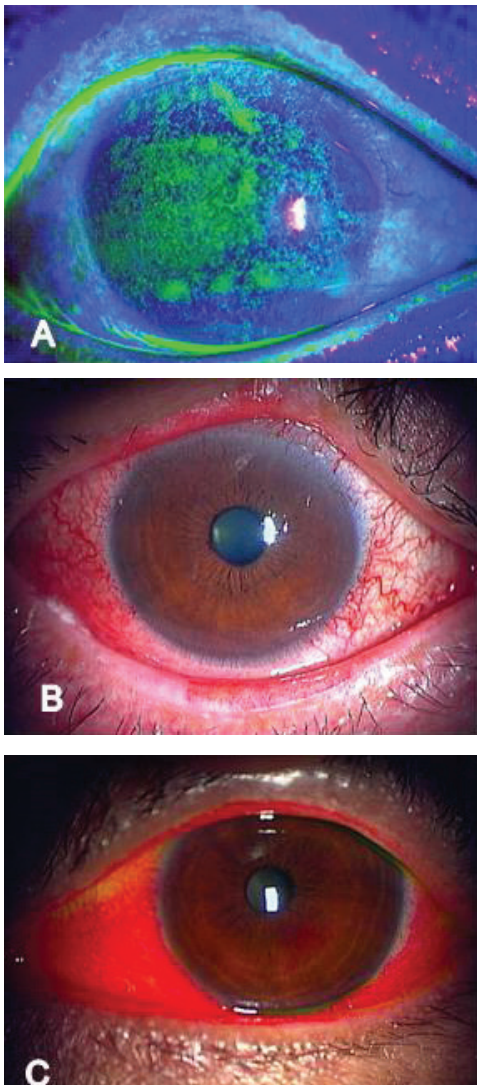
Lacrimal gland biopsy specimens that had been obtained for diagnostic purposes were used for histological analysis. This study was approved by the Keio University Institutional Review Boards (#2009-0277). Written informed consent was obtained in advance from the patients in accordance with the principles expressed in the Declaration of Helsinki for human subjects.

Results

Dry eye as a diagnostic sign of chronic GVHD

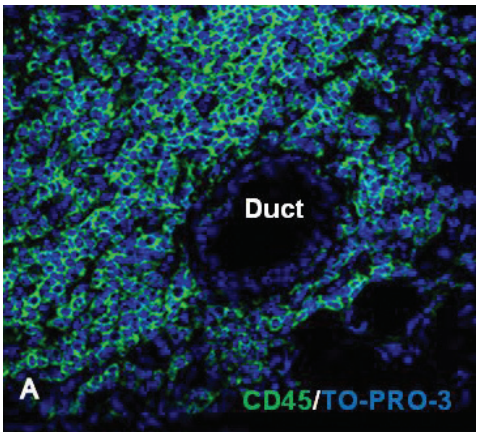
Dry eye is commonly seen in patients with chronic GVHD [6,9,16-19], and may be considered a hallmark of chronic GVHD[10,11,20-28]. The proposed criteria for diagnosis and scoring of chronic systemic as well as ocular GVHD need to be validated in prospective studies[15,29]. A prospective evaluation of patients undergoing HSCT between 1995 and 1998, pre- and post-transplantation showed that dry eye was observed with greater frequency in patients with systemic cGVHD (70.4%), than in patients without (17.7%; P<.005)[3]. Using Schirmer score for staging of ocular GVHD as proposed by the NIH consensus criteria, the false positive rate of diagnosis of ocular GVHD in patients with systemic GVHD was 19.4% and false negative rate was 22.7%. Jacobs *et al* have reported similar results [10]. In terms of temporal association of onset of ocular and systemic GVHD, Balaram *et al*[4] have reported ocular involvement in 62% patients with chronic GVHD. Twenty two percent of newly onset dry eye patients after HSCT presented with severe dry eye and conjunctival inflammation without features of systemic GVHD. They have also reported ocular involvement as a predecessor of systemic chronic GVHD[3,4]. Early diagnosis of chronic ocular GVHD enables adequate therapeutic intervention for ocular and systemic signs and symptoms. There are several risk factors

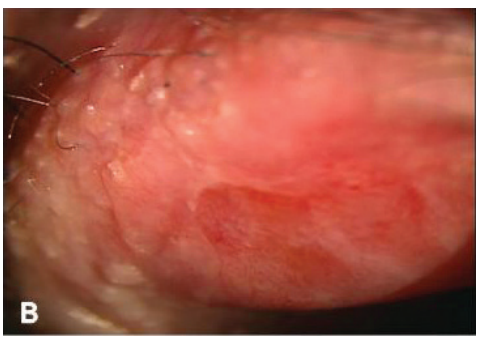
that predispose to the development of chronic GVHD. These include an episode of acute GVHD, age of donor or recipient, and donor-recipient gender disparity[5]. Female donor to male recipient transplants were shown to result in an increased ratio of severe/total dry eye than in male donor to female recipient[30]. It is well recognized that the stem cell source can impact GVHD risk [31], and Uchino, M., *et al*. reported increased frequency of chronic ocular GVHD post peripheral blood stem cell transplantation (PBSCT) compared to bone marrow (BMT) or cord blood transplantation (CBT)[32]. They demonstrated the relationship between the development of systemic GVHD and dry eye after HSCT. Patients with GVHD had a higher Odds Ratio for dry eye post-BMT (OR 12.28, 95% CI 2.48 to 60.5) and post-CBT (OR 13.8, 95% CI 0.4 to 448.6) compared to post-PBSCT (OR 3, 95% CI 0.4 to 22.7). These findings suggest that dry eye in patients post-BMT/CBT was a predictor for occurrence of systemic GVHD. In PBSCT, dry eye may be masked by intensive systemic immunosuppression. It was also noted that late onset severe dry eye can precede non-ocular systemic GVHD. This leads us to suggest that dry eye is an important sign for diagnosing or treating systemic chronic GVHD. GVHD has the potential to affect all mucosal surfaces, including ocular, oral, vaginal, and gastrointestinal areas[15]. Ocular surface mucosa is a representative target organ of chronic GVHD (Figure 1A, B, C)[2,33-37]. In addition, the inner surface of ductal area is composed of mucosal membrane which is frequently targeted by T cells or other inflammatory cells (Figure 2A), ductal area is significantly affected in target organs such as lacrimal gland, meibomian gland, nasolacrimal ducts and salivary gland, as well as hepatic bile



(A) A representative biomicroscopic image of fluorescein staining (Severity score 3) is shown. Note diffuse punctate epitheliopathy on the cornea of a patient with chronic GVHD related dry eye. (B) Note severe conjunctival injection on damaged ocular surface. (Severity score 2). (C) Note Rose Bengal staining of conjunctiva on severely damaged ocular surface.

Figure 1.
Typical ocular surface findings of chronic ocular GVHD





(A) Typical histological findings of the lacrimal gland in a 39 year old woman at 2 months after onset of chronic GVHD related dry eye. An abundant CD45+ inflammatory cell infiltration at periductal areas of the medium sized ducts. Original magnification: X630. (B) Note total obstruction of meibomian gland and Zeis gland orifices and severely fibrotic tarsal conjunctiva in a 21-year-old male suffering from chronic GVHD. Upper eyelid, Right eye.

Figure 2.
Histological findings of lacrimal gland ducts and clinical findings of orifices of meibomian gland and Zeiss gland on eye lid margin

ducts, and lung ducts (Figure 2A, B)[2,38-42].

Conjunctival involvement in chronic GVHD has been reported as a sign of severe chronic GVHD[2]. Kaplan-Meier analysis of survival demonstrated a decreased survival of patients with GVHD presenting with conjunctival involvement[2]. It has been proposed by Jabs *et al* that the conjunctiva may mirror systemic mucosal membrane such as intestine, lung, and oral mucosa. They described that conjunctival involvement by GVHD may represent "a distinct clinical finding"[2]. The description might leads to dry eye (keratoconjunctivitis sicca) being recognized as a "distinct sign" in chronic GVHD, particularly in reference to the NIH consensus criteria. Conjunctivitis may present concurrently or precede the onset of dry eye in chronic GVHD. Conjunctival inflammation may be a primary manifestation of chronic GVHD or secondary to severe dry eye. The role of conjunctival biopsy for ocular GVHD remains unclear. A recent report studied conjunctival biopsy specimen in symptomatic patients suspected of ocular GVHD[34]. Using a modified Lerner classification designed for skin GVHD histopathology, Auw-Haedrich and colleagues found that the modified grade III-IV bulbar conjunctival inflammation cohort compared to modified grade I-II showed shorter survival in Kaplan-Meier analysis. This study supports the earlier observation made by Jabs *et al* that conjunctival involvement may confer a prognostic value[2].

We can detect the ocular surface change of chronic GVHD directly by using a biomicroscope and comprehensive variables for diagnosing dry eye disease after HSCT. Therefore, early detection, precise diagnosis, and treatment of dry eye in ocular GVHD as well as systemic GVHD are

Severity scores	Schirmer's test	CFS	OSDI	Conj
0	15 >	0	< 13	None
1	11-15	< 2	13-22	Mild/Moderate
2	6 -10	2-3	23-32	Severe
3	≤ 5	≤ 4	> 33	

CFS; corneal fluorescein staining, OSDI; Ocular Surface Disease Index. Conj; conjunctival injection. Severity classification; Total score (Schirmer's test score+ CFS score+ OSDI score+ Conj injection score) = None; 0-4, Mild/Moderate; 5-8, Severe, 9-11

Table 1.
Severity scale in chronic ocular GVHD

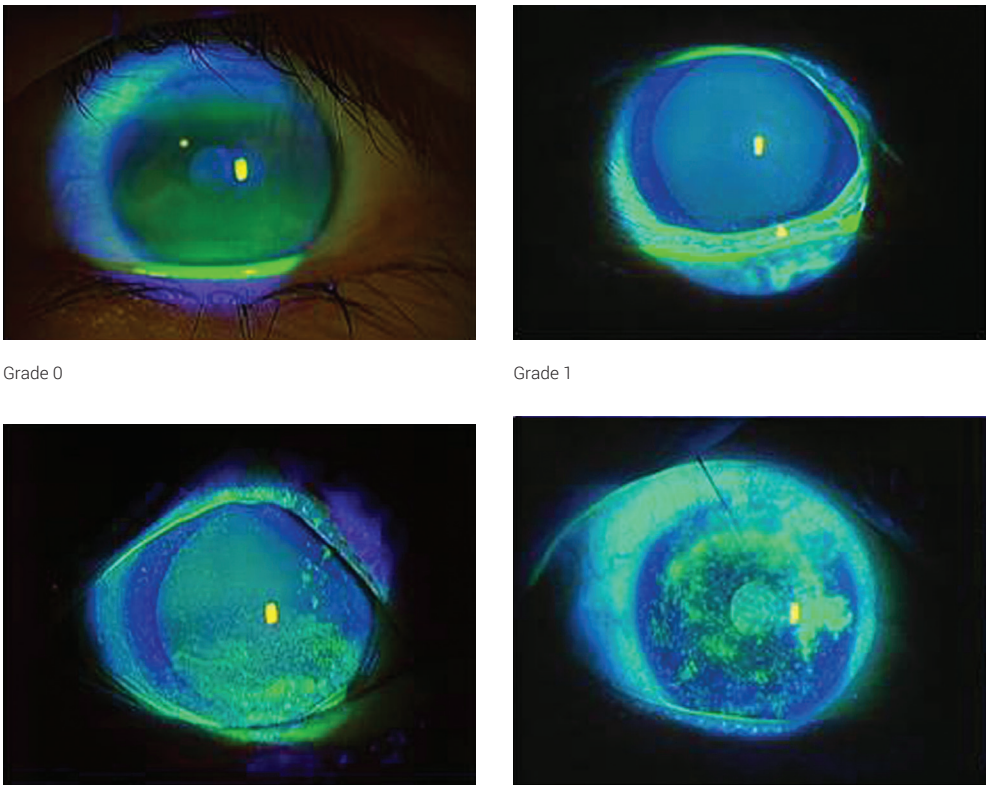
	None	Probable GVHD	Definite GVHD
Systemic GVHD (-)	0-5	6-7	≥ 8
Systemic GVHD (+)	0-3	4-5	≥ 6

Table 2.
Diagnosis of chronic ocular GVHD

necessary to prevent blindness for long term survivors. Based on these reports, dry eye post-HSCT has been established as a significant finding in the spectrum of systemic involvement in chronic GVHD. Since dry eye can precede clinical signs in other organs, we propose the inclusion of dry eye as a concrete diagnostic sign of chronic GVHD without being substantiated by involvement in other organ systems as originally recommended by the NIH working committee.

Proposed diagnostic criteria and severity of grading system

A collaborative, prospective-multicenter study from the USA, the Netherlands, and Japan on ocular GVHD has been proposed. Based on discussion and consensus at the 1st Chronic Ocular GVHD meeting, the parameters for diagnosis include 1) Ocular Surface Disease Index (OSDI)[43,44], 2) Schirmer's test score without anesthesia[45], 3) Corneal fluorescein staining (Figure 3)[45,46], and 4) conjunctival injection (Figure 4)[47]. Ocular GVHD classification will be based on the baseline examination as threshold scores for grading. The proposed grading system is shown in Tables 1 and 2. Severity scores 0, 1, 2, and 3 will be assigned to OSDI, corneal fluoresecein staining, and Schirmer's score[44-46]. Conjunctival injection will be scored 0, 1, and 2 (Table 1)[47]. The corneal fluorescein staining score ranged from 0 to 3 points (Grade 0 = no staining, Grade 1 = minimal staining, Grade 2 = mild/moderate staining, Grade 3 = severe staining) (Figure 3)[46]. The conjunctival hyperemia score of the conjunctiva ranged from 0 to 2 points (Grade 0 = none, Grade 1 = mild/



Grade 0 Grade 1

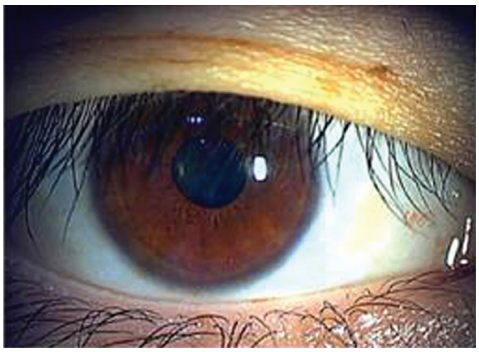
Grade 2 Grade 3

Grading scale of corneal fluorescein staining score ranged from 0 to 3 points (Grade 0 = no staining, Grade 1 = minimal staining, Grade 2 = mild/moderate staining, Grade 3 = severe staining).

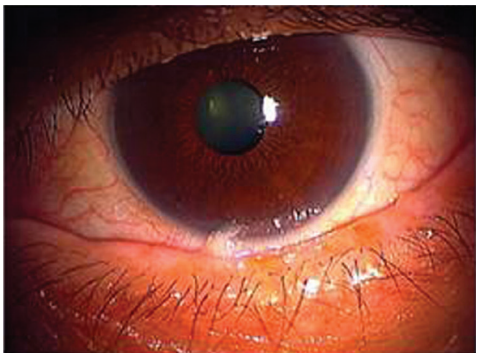
Figure 3.
Slitlamp micrograph of grading scale for corneal fluorescein staining

moderate, Grade 2 = severe) (Figure 4)[47]. Any score above 1 points is regarded as abnormal.

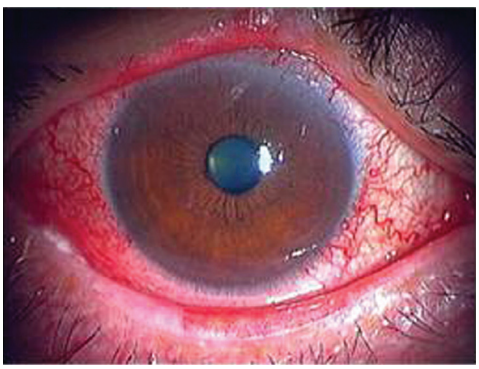
Disease severity will be graded as none, mild/moderate and severe based on an aggregate of scores for each parameter (Table 1). Based on the presence or absence of systemic GVHD and the aggregate scores assessed, a diagnosis of ocular GVHD will be made. In the presence of systemic GVHD, score 0-3 indicates absence of ocular GVHD; score 4-5 indicates "probable" ocular GVHD and score ≥6 indicates "definite" ocular GVHD. In the absence of systemic GVHD, score 0-5 indicates absence of ocular GVHD, score 6-7 indicates "probable" ocular GVHD and score ≥8 indicates "definite" ocular GVHD (Table 2). Based on the above criteria, data collected at each center will be analyzed and validated at a central location and a proposal to amend the NIH consensus criteria will be presented.



Grade 0



Grade 1



Grade 2

Grading scale of conjunctival injection score ranged from 0 to 2 points (Grade 0 = none, Grade 1= mild/moderate injection, Grade 2= severe injection).

Figure 4.
Slit lamp micrograph of grading scale for conjunctival injection

Discussion

Dry eye disease is responsible for a significant decrease in the quality of life in allogeneic transplant recipients. Dry eye disease, is currently listed as a distinctive sign, and not a diagnostic criteria sufficient alone for establishing a diagnosis of chronic GVHD by the NIH consensus criteria[15]. The NIH consensus criteria diagnosis and staging working group report defined ocular criteria for diagnosis as "new ocular sicca documented by low Schirmer test values with a mean value of both eyes ≤5 mm at 5 minutes or a new onset of keratoconjunctivitis sicca by slit-lamp examination with mean values of 6 to 10 mm on the Schirmer test accompanied by distinctive manifestations in at least 1 other organ"[15]. While recognizing that the NIH criteria were developed for the use of transplant physicians in prospective clinical trials for chronic GVHD, the ocular criteria need to be evaluated by ophthalmologists. Decreased Schirmer's score and signs and symptoms of dry eye disease must be assessed simultaneously in correlation with the clinical findings, since infection, trauma, and side effects from prolonged use of topical medications may also present with ocular surface disease. Using the Schirmer's score as a sole defining criterion for the diagnosis of chronic ocular GVHD would be inadequate. The high false positive and negative rates of diagnosis of ocular GVHD if based solely on the Schirmer's score have been reported by various groups[10]. In addition, the importance of a comprehensive ocular evaluation has been stressed. It is commonly accepted that 5 mm of wetting denotes tear deficiency when the test is performed without anesthesia.

However, a comparable diagnostic cut-off value for

Schirmer's test with anesthesia is not as clear. Other limitations include the errors in measurement induced by reflex tearing and the influence of external environmental factors brings its infallibility into question[48]. Patient symptom scores, ocular surface staining, tear film dynamics, are all important in assessing a patient with dry eye in ocular GVHD, especially in assessing systemic chronic GVHD. Diagnostic procedures for dry eye have been reported by the International Dry Eye Workshop (DEWS) report[13]. Modification of the proposed criteria may allow for standardization of dry eye assessment in chronic GVHD because patient evaluation, follow-up and treatment regimens differ at various centers.

Irrespective, a careful examination by an ophthalmologist perceptive to the diagnosis of ocular GVHD serves a crucial role. A collaborative dialogue between the ophthalmologists and BMT specialists can lead to improved management of chronic GVHD in general[3,6].

Considering conjunctival staining as parameter to assess ocular GVHD, there is no reliable, standardized, and readily adopted way to assess the conjunctival staining element of ocular GVHD at present time. Conjunctival staining is more variable even than corneal staining. In addition, while there are patients with conjunctival staining that don't stain corneas, the diagnostic criteria are ill-defined. Therefore, we exclude the conjunctival staining as the consensus parameter for the diagnosis of ocular chronic GVHD at present time. The NIH proposed scoring system for ocular GVHD (Table 3)[15] is easy to understand and can be performed without a clinical examination even by a non-ophthalmologist. However, it may not be able to accurately assess the severity of the disease. There is an inherent limitation in a scale that relies on behavior rather than objective findings.

The current grading systems available for ocular GVHD rely on clinical findings only. Jabs' proposed stages of conjunctival ocular GVHD are applicable to acute GVHD[2]; Robinson *et al* proposed a grading system for

Eyes	Score 0	Score 1	Score 2	Score 3
Mean tear test (mm): >10 6-10 <5 Not done	No symptoms	Mild dry eye symptoms not affecting ADL (requiring eyedrops ≤ 3x per day) OR asymptomatic signs of keratoconjunctivitis sicca	Moderate dry eye symptoms partially affecting ADL (requiring drops > 3 x per day or punctal plugs), WITHOUT vision impairment	Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) OR unable to work because of ocular symptoms OR loss of vision caused by keratoconjunctivitis sicca

Table 3. Eye scoring of chronic GvHD by NIH consensus development project[15]

chronic ocular GVHD. Both are based on clinical findings[36]. Baseline profiles of ocular surface and tear film dynamics, in patients post-HSCT with or without cGVHD related dry eye have been reported. In this report, baseline values indicative of dry eye, using modern diagnostic techniques were demonstrated[49]. These data may be useful for an additional detailed evaluation of chronic ocular GVHD. Analysis of the efficacy of topical and systemic treatment for chronic ocular GVHD must take into account the baseline clinical findings and their severity[49]. It is our opinion, that a metric inclusive of patient symptom evaluation by the ocular surface disease index (OSDI) could be used to evaluate the effect on activities of daily living rather than the frequency of instillation of eye drops.

The recommendation of the current Chronic Ocular GVHD Workshop is to propose changes in the NIH Consensus Diagnostic Criteria to include chronic ocular GVHD, as a diagnostic criteria sufficient alone to establish a diagnosis of chronic GVHD. We also propose and amendment of the severity scoring criteria chronic ocular GVHD by validating the metric proposed by our group. This will be done using the data generated at the centers enrolled in this study. Once validated, chronic ocular GVHD-specific metric, may be a valuable tool for future clinical trials and may help patients with ocular and systemic cGVHD. Early prophylaxis, diagnosis and treatment can be accomplished by examining the eye in detail. In this regard, a prospective study evaluating a comprehensive ophthalmic evaluation pre- and post-transplantation to diagnose new onset dry eye or other ocular cGVHD-related and non-GVHD complications is needed. It is necessary to communicate in detail concerning the dose of systemic and local immunosuppressant and the time of commencement and cessation for treatment between transplant internists and ophthalmologists.

References

1. Shulman, H. M. *et al*. Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. Am J Med 69, 204-217 (1980).

2. Jabs, D. A. *et al*. The eye in bone marrow transplantation. III. Conjunctival graft-vs-host disease. Arch Ophthalmol 107, 1343-1348 (1989).

3. Ogawa, Y. *et al*. Dry eye after haematopoietic stem cell transplantation. Br J Ophthalmol 83, 1125-1130 (1999).

4. Balaram, M. & Dana, M. R. Phacoemulsification in patients after allogeneic bone marrow transplantation. Ophthalmology 108, 1682-1687 (2001).

5. Couriel, D. R. *et al*. Extracorporeal photochemotherapy for the treatment of steroid-resistant chronic GVHD. Blood 107, 3074-3080 (2006).

6. Kim, S. K. Update on ocular graft versus host disease. Curr Opin Ophthalmol 17, 344-348 (2006).

7. Lew, J. & Smith, J. A. Mucosal graft-vs-host disease. Oral Dis 13, 519-529 (2007).

8. Riemens, A., te Boome, L., Imhof, S., Kuball, J. & Rothova, A. Current insights into ocular graft-versus-host disease. Curr Opin Ophthalmol 21, 485-494 (2010).

9. Townley, J. R., Dana, R. & Jacobs, D. S. Keratoconjunctivitis sicca manifestations in ocular graft versus host disease: pathogenesis, presentation, prevention, and treatment. Semin Ophthalmol 26, 251-260 (2011).

10. Jacobs, R. *et al*. Prevalence and risk factors associated with development of ocular GVHD defined by NIH consensus criteria. Bone Marrow Transplant 47, 1470-1473 (2012).

11. Hessen, M. & Akpek, E. K. Ocular graft-versus-host disease. Curr Opin Allergy Clin Immunol 12, 540-547 (2012).

12. Shikari, H., Antin, J. H. & Dana, R. Ocular Graft-versus-Host Disease: A Review. Surv Ophthalmol (2013).

13. The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye WorkShop (2007). Ocul Surf 5, 75-92 (2007).

14. Ferrara, J. L., Levine, J. E., Reddy, P. & Holler, E. Graft-versus-host disease. Lancet 373, 1550-1561 (2009).

15. Filipovich, A. H. *et al*. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant 11, 945-956 (2005).

16. Dietrich-Ntoukas, T. *et al*. Diagnosis and treatment of ocular chronic graft-versus-host disease: report from the German-Austrian-Swiss Consensus Conference on Clinical Practice in chronic GVHD. Cornea 31, 299-310 (2012).

17. Dignan, F. L. *et al*. Organ-specific management and supportive care in chronic graft-versus-host disease. Br J Haematol 158, 62-78 (2012).

18. Westeneng, A. C. et al. Ocular graft-versus-host disease after allogeneic stem cell transplantation. Cornea 29, 758-763 (2010).

19. Ogawa, Y., Shimmura, S., Dogru, M. & Tsubota, K. Immune processes and pathogenic fibrosis in ocular chronic graft-versus-host disease and clinical manifestations after allogeneic hematopoietic stem cell transplantation. Cornea 29, S68-S77 (2010).

20. Riemens, A., Stoyanova, E., Rothova, A. & Kuiper, J. Cytokines in tear fluid of patients with ocular graft-versus-host disease after allogeneic stem cell transplantation. Mol Vis 18, 797-802 (2012).

21. Inamoto, Y. *et al*. Validation of measurement scales in ocular graft-versus-host disease. Ophthalmology 119, 487-493 (2012).

22. Wang, Y. *et al*. Baseline profiles of ocular surface and tear dynamics after allogeneic hematopoietic stem cell transplantation in patients with or without chronic GVHD-related dry eye. Bone Marrow Transplant (2009).

23. Mencucci, R. *et al*. Ophthalmological aspects in allogenic bone marrow transplantation: Sjogren-like syndrome in graft-versus-host disease. Eur J Ophthalmol 7, 13-18 (1997).

24. Tichelli, A. Late ocular complications after bone marrow transplantation. Nouv Rev Fr Hematol 36 Suppl 1, S79-82 (1994).

25. Jack, M. K., Jack, G. M., Sale, G. E., Shulman, H. M. & Sullivan, K. M. Ocular manifestations of graft-v-host disease. Arch Ophthalmol 101, 1080-1084 (1983).

26. Jabs, D. A. *et al*. The eye in bone marrow transplantation. II. Histopathology. Arch Ophthalmol 101, 585-590 (1983).

27. Hirst, L. W., Jabs, D. A., Tutschka, P. J., Green, W. R. & Santos, G. W. The eye in bone marrow transplantation. I. Clinical study. Arch Ophthalmol 101, 580-584 (1983).

28. Franklin, R. M. *et al*. Ocular manifestations of graft-vs-host disease. Ophthalmology 90, 4-13 (1983).

29. Greinix, H. T. *et al*. Diagnosis and staging of chronic graft-versus-host disease in the clinical practice. Biol Blood Marrow Transplant 17, 167-175 (2011).

30. Kamoi, M. *et al*. Donor-recipient gender difference affects severity of dry eye after hematopoietic stem cell transplantation. Eye (Lond) 25, 860-865 (2011).

31. Takahashi, S. *et al*. Comparative single-institute analysis of cord blood transplantation from unrelated donors with bone marrow or peripheral blood stem-cell transplants from related donors in adult patients with hematologic malignancies after myeloablative conditioning regimen. Blood 109, 1322-1330 (2007).

32. Uchino, M. *et al*. Comparison of stem cell sources in the severity of dry eye after allogeneic haematopoietic stem cell transplantation. Br J Ophthalmol 96, 34-37 (2012).

33. Rojas, B. *et al*. Cell populations and adhesion molecules expression in conjunctiva before and after bone marrow transplantation. Exp Eye Res 81, 313-325 (2005).

34. Auw-Haedrich, C. *et al*. Histological and immunohistochemical characterization of conjunctival graft vs host disease following haematopoietic stem cell transplantation. Graefes Arch Clin Exp Ophthalmol 245, 1001-1007 (2007).

35. Ogawa, Y. *et al*. Epithelial mesenchymal transition in human ocular chronic graft-versus-host disease. Am J Pathol 175, 2372-2381 (2009).

36. Robinson, M. R. *et al*. Topical corticosteroid therapy for cicatricial conjunctivitis associated with chronic graft-versus-host disease. Bone Marrow Transplant 33, 1031-1035 (2004).

37. Tatematsu, Y. *et al*. Mucosal microvilli in dry eye patients with chronic GVHD. Bone Marrow Transplant 47, 416-425 (2012).

38. Hanada, R. & Ueoka, Y. Obstruction of nasolacrimal ducts closely related to graft-versus-host disease after bone marrow transplantation. Bone Marrow Transplant 4, 125-126 (1989).

39. Ogawa, Y. *et al*. A significant role of stromal fibroblasts in rapidly progressive dry eye in patients with chronic GVHD. Invest Ophthalmol Vis Sci 42, 111-119 (2001).

40. Kamoi, M. *et al*. Spontaneous lacrimal punctal occlusion associated with ocular chronic graft-versus-host disease. Curr Eye Res 32, 837-842 (2007).

41. Ban, Y. *et al*. Morphologic evaluation of meibomian glands in chronic graft-versus-host disease using in vivo laser confocal microscopy. Mol Vis 17, 2533-2543 (2011).

42. Espana, E. M., Shah, S., Santhiago, M. R. & Singh, A. D. Graft versus host disease: clinical evaluation, diagnosis and management. Graefes Arch Clin Exp Ophthalmol (2013).

43. Schiffman, R. M., Christianson, M. D., Jacobsen, G., Hirsch, J. D. & Reis, B. L. Reliability and validity of the Ocular Surface Disease Index. Arch Ophthalmol 118, 615-621 (2000).

44. Miller, K. L. *et al*. Minimal clinically important difference for the ocular surface disease index. Arch Ophthalmol 128, 94-101 (2010).

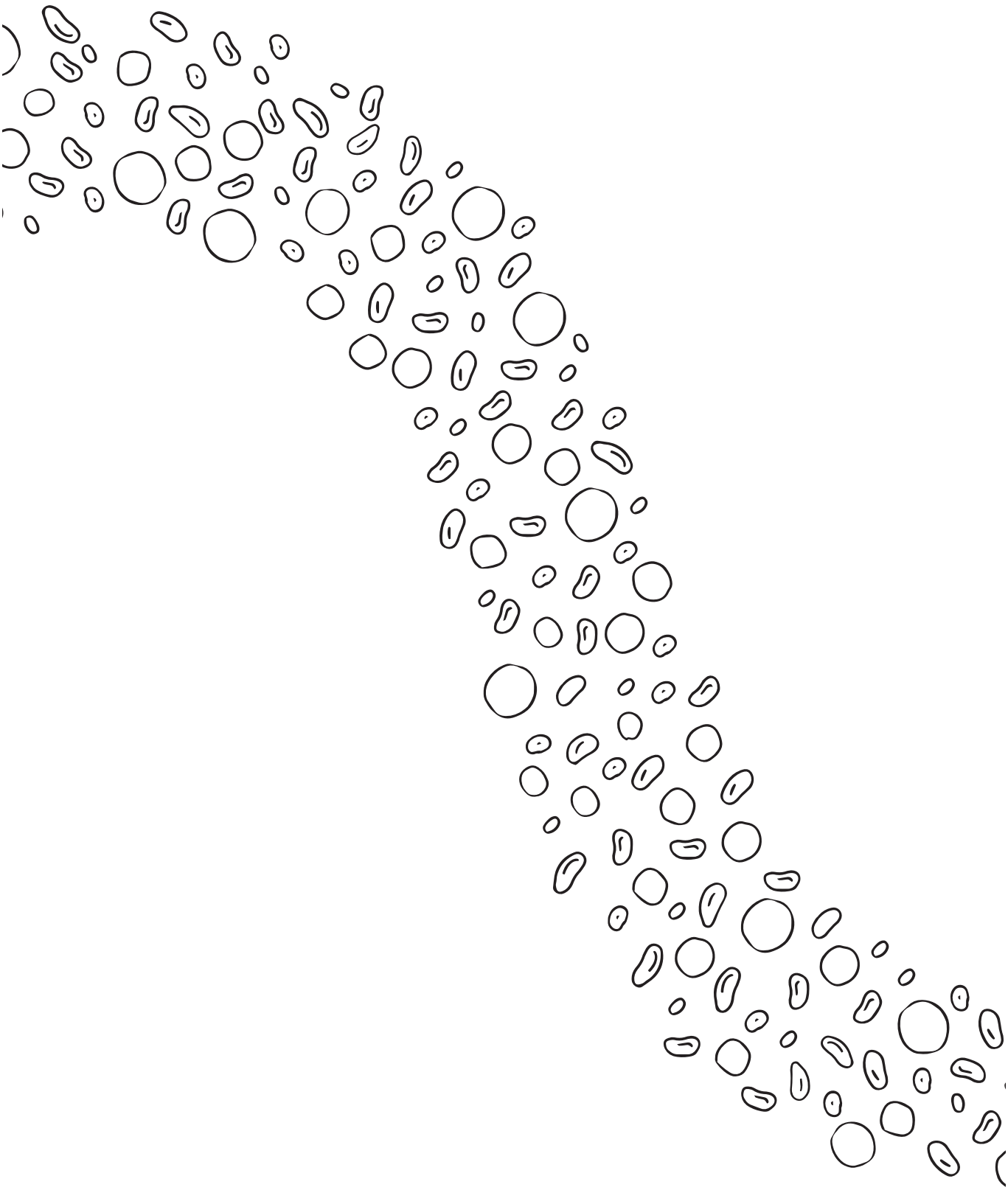
45. Sullivan, B. D. *et al*. An objective approach to dry eye disease severity. Invest Ophthalmol Vis Sci 51, 6125-6130 (2010).

46. Bron, A. J., Evans, V. E. & Smith, J. A. Grading of corneal and conjunctival staining in the context of other dry eye tests. Cornea 22, 640-650 (2003).

47. Efron, N. Grading scales for contact lens complications. Ophthalmic & physiological optics : the journal of the British College of Ophthalmic Opticians 18, 182-186 (1998).

48. Dogru, M. *et al*. Changing trends in the definition and diagnosis of dry eyes. Am J Ophthalmol 140, 507-508 (2005).

49. Wang, Y. *et al*. Baseline profiles of ocular surface and tear dynamics after allogeneic hematopoietic stem cell transplantation in patients with or without chronic GVHD-related dry eye. Bone Marrow Transplant 45, 1077-1083 (2010).



Chapter 6

Impact of ocular graft-versus-host disease on visual quality of life in patients after allogeneic stem cell transplantation: questionnaire study.

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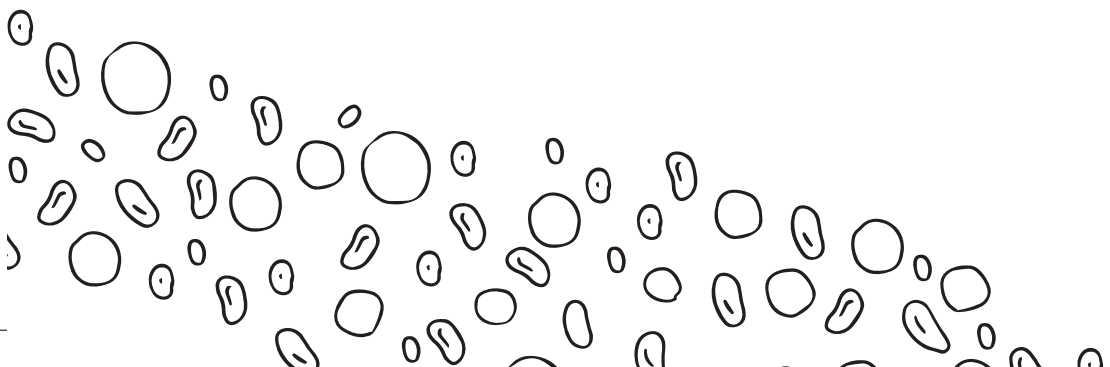


Abstract

Purpose

To determine the influence of ocular complications on quality of life (QoL) three years after allogeneic stem cell transplantation (allo-SCT).

Methods

All 54 adult patients that underwent and survived allo-SCT in 2006/2007 in our centre received two questionnaires (VFQ-25: visual function questionnaire-25 and OSDI: ocular surface disease index). In addition the following data were included: gender, age, underlying disease, presence of chronic and/or ocular Graft-versus-Host Disease (GvHD), number of visits to an ophthalmologist, manifestations of dry eye disease, the duration of follow up and treatment for ocular GvHD.

Results

Ocular GvHD developed in 26% (14/54) of patients and 71% (10/14) received treatment for ocular GvHD. The presence of ocular GvHD correlated with the severity of systemic GvHD (correlation coefficient: 0.52, P=0.00). The Karnofsky scores were significantly lower in the patients with ocular GvHD compared to the patients with no ocular GvHD (p=0.001). Karnofsky scores were weakly correlated with the severity of systemic GvHD (correlation coefficient: 0.25, P=0.03). Three years after the allo-SCT, OSDI and VFQ-25 scores were significantly impaired in patients with ocular GvHD (mean: 76.5; range: (46.1-100) and mean: 31.1; range: (0-72.9)) compared to patients with no ocular GvHD (mean: 89.4; range: (45.2-100) and mean: 12.9; range: (0-58 .3)) (P0.02). The scores of the VFQ-25 were significantly lower in the domains of general health, ocular pain, social functioning and role difficulties.

Conclusion

The long-term vision-related QoL measured by the OSDI and VFQ-25 was impaired in patients with ocular GvHD.

Introduction

Graft-versus-Host-Disease (GvHD) forms the major reason for non-relapse mortality and morbidity in patients who underwent allogeneic hematological stem cell transplantation (allo-SCT) for hematological malignancies and bone marrow disorders. GvHD is mainly caused by donor T-cells which attack various tissues of the recipient. Approximately 40% of Human Leukocyte Antigen -matched patients develop acute GvHD and 30-70% of patients develop extensive chronic GvHD [1,2]. Chronic GvHD is known to determine the quality of life (QoL) in this population[3].

Ocular GvHD develops in 40-60% of patients after allo-SCT and causes predominantly ocular surface disease (OSD) due to lacrimal and conjunctival damage[4,5]. Ocular GvHD does not generally lead to permanent visual loss but leads to a large variety of ocular complaints including dry eyes, pain and photophobia as well as blurring of vision[5-7]. Ocular GvHD might lead to frequent visits to ophthalmologists, requires frequent application of various topical medications, and can temporary (and occasionally permanently) decrease useful vision. The impact of ocular GvHD on the vision related QoL has not yet been systematically studied. In the present study we assess the vision-related QoL three years after allo-SCT using the visual functioning questionnaire-25 (VFQ-25)[8] and the ocular surface disease index (OSDI)[9-11].

Methods

Hundred and thirty-four consecutive patients who underwent allo-SCT in 2006 and 2007 at the hematology department of the University Medical Centre Utrecht (UMCU) in The Netherlands were selected. Of these patients 63/134 (47%) died within four years after transplantation, the remaining 71 patients were approached (November 2010) for this study. Sixty-three patients were willing to participate and informed consent was obtained. However four patients were excluded due to significant (ocular) co-morbidity (ankylosing spondylitis-associated uveitis, serous retinal detachment, macular hole and cystoid macular edema) and one additional patient was excluded because data on ophthalmologic examination were missing. Another four patients developed ocular disorders possibly related to allo-SCT but other than standard ocular GvHD features. Specifically two patients developed uveitis during the exacerbation of their chronic GvHD, one patient exhibited radiation retinopathy and one patient developed an endogenous Scedosporium endophthalmitis during Scedosporium sepsis. All four patients were excluded and the remaining 54 patients were included in the study.

Patients received a non-manipulated peripheral blood stem cell transplant, 48 patients after conditioning with fludarabine and low dose total body irradiation (TBI) (two Gray) and six patients after conditioning with cyclophosphamide and TBI (six times two Gray). For patients with an unrelated donor, anti-thymocyte-globulin (ATG) was added to the conditioning regimen. Post transplant GvHD prophylaxis consisted of Cyclosporin-A and Mycophenolate Mofetil.

The study was performed in accordance with the Declaration of Helsinki[12] and with the approval of the local Institutional Review Board. After obtaining written informed consent patients were asked to fill in two questionnaires: the VFQ-25 and the OSDI. The VFQ-25 and the OSDI are validated questionnaires to examine the vision-related QoL[8,11,13]. The VFQ-25 has previously been used for diabetic retinopathy, Behçet uveitis, as well as other ocular conditions[14-20]. The OSDI has been previously used in many studies assessing the impact on vision related QoL in dry eye syndromes.

The following data were registered: gender, age, reason for allo-SCT, presence of chronic GvHD (three, six, 12 or 24 months after SCT), Karnofsky scores, presence of ocular GvHD, clinical manifestations of dry eye disease (visual acuity, Schirmer test, tear break up time, corneal fluorescein staining) and duration of follow up. In addition, the number of visits to an ophthalmologist and the treatment for ocular GvHD was assessed.

Ocular GvHD was diagnosed according to the criteria of Filipovich *et al* which includes two groups[21]. The first group are patients with a maximal mean value of tear Schirmer test of both eyes of five mm at five minutes and the second group include all patients with a new onset of keratoconjunctivitis sicca (KCS) documented by slit-lamp examination exhibiting mean values of six to 10 mm on the Schirmer test if accompanied by distinctive manifestations of chronic GvHD in at least one other organ. We used the NIH scoring system (0-three points) to asses the severity of ocular GvHD[21].

Questionnaires

The VFQ-25 (National Eye Institute (NEI) Dutch consensus translation version 2001) is a questionnaire which assesses the vision related QoL and consists of 11 vision-specific domains and one question concerning general health. Each subscale is scored from 0 to 100, with 0 being the worst possible function and 100 being the best possible function. The 11 subscales are averaged to produce a VFQ total score ranging from 0 to 100, the general health subscale is not included in generating the composite score[8]. The VFQ-25 was developed at the RAND Corporation under the sponsorship of the National Eye Institute.

The OSDI (Dutch translation) is a 12-item validated questionnaire to assess OSD symptoms and its impact on vision related QoL. The OSDI overall and subscale scores range from 0 to 100. The scoring of the OSDI and the VFQ-25 was performed according to the published guidelines[9,10].

Data Analysis

SPSS 15.0.1 (SPSS Inc., Chicago, IL) was used for data analysis. The Kolmogorov-Smirnov test was used as the test of normality. The t-test was used to calculate the p value of normally distributed variables and the Mann-Whitney test and Kruskal-Wallis test were used to assess differences between questionnaire scores, clinical manifestations of dry eye disease and Karnofsky scores between patients with and without GvHD. The Pearson chi-square test or Fischer exact test was used for univariate analysis of categorical variables. Spearman's rho was used to calculate with ordinal variables. A p-value less than 0.05 was regarded as statistically significant.

Results

General data of the patients are given in Table 1. The median age of the patients at the moment of allo-SCT was 48 years (range 18-68 years, SD 12.9). Chronic GvHD was diagnosed in 74% (40/54) of patients between three and 36 months after SCT. Ocular GvHD was diagnosed in 26% (14/54) of all survivors three to four years after SCT. According to the NIH scoring system (Filipovich *et al*. 2008)[21] five patients had mild (score one) ocular GvHD, three moderate (score two) and six (score three) severe ocular GvHD. We could not correlate these scores to visual QoL due to the low number of patients.

Of all 14 patients with ocular GvHD at three years after SCT 71% (10/14) received treatment for this condition and 57% (8/14) of these patients were treated with frequent applications of multiple (> two different types) medications including lubricant drops, topical steroids, topical Cyclosporin A, scleral and bandage lenses, serum drops and systemic doxycycline.

The mean number of visits to the ophthalmology outpatient department in patients with ocular GvHD was 13 visits during the three to four years follow up. Three out of all 14 patients with ocular GvHD had to apply their medications at least every two hours three years after SCT and two additional patients had to use eye drops at the same frequency in the past but were now treated with scleral lenses so they could use their eye drops less frequently.

Results VFQ-25 and OSDI questionnaires

Both VFQ-25 and OSDI scores were significantly impaired in patients with ocular GvHD (mean: 76.5; range: (46.1-100) and mean: 31.1; range: (0-72.9)) compared to patients without ocular GvHD (mean: 89.4; range: (45.2-100) and mean: 12.9; range: (0-58.3)) (P0.02). The results of the VFQ-25 subscales are given in Table 2. The scores of the VFQ-25 questionnaire were significantly lower in the domains of general health, ocular pain, social functioning and role difficulties, whereas the domains such as driving, color vision and peripheral vision showed no substantial differences when compared to patients without ocular GvHD (Table 2). Two patients were not able to practice their hobbies and/or sporting activities and three patients occasionally preferred not to leave their house due to extremely painful eyes outdoors. Patients without ocular GvHD scored more than 80 points in all 11 domains of the VFQ and the OSDI score was 12.9 (within normal limits). We correlated the results of the significantly impaired (domains of) the questionnaires with the clinical findings of dry eye disease, the number of medications used and the Karnofsky scores (Table 3). Mainly BCVA and Schirmer values correlated with the impaired (domains of) the questionnaires (Table 3). Because general health can have influence on the other domains, we compared the scores on general health in patients with chronic GvHD with and without ocular GvHD (median: 75.0; range: (50-100) and median: 93.8; range (50-100), P=0.005) and between patients with no ocular GvHD with and without systemic disease (median: 93.8; range: (50-100) and median: 93.8; range (56.3-100), P=0.2). In addition, we compared the Karnofsky scores between the patients with and without ocular GvHD, and found significantly impaired score in the ocular GvHD group (p=0.001). The severity of systemic GvHD

was weakly correlated with the Karnofsky scores (correlation coefficient: 0.25, P=0.03). The severity of systemic GvHD did correlate with the presence of ocular GvHD (correlation coefficient: 0.52, P=0.00).

	Ocular GvHD n=14	No ocular GvHD n=40	Total n=54	P Value ocular GvHD vs no ocular GvHD
Age in years median (range)	52 (36-68)	45 (18-68)	48 (18-68)	0.06
Sex, N (%)				
Male	10 (71)	24 (60)	34 (63)	0.46
Underlying disease, N (%)				
AML	2 (14)	14 (35)	16 (30)	ND
ALL	0 (0)	4 (10)	4 (7)	ND
CML	1 (7)	3 (8)	4 (7)	ND
CLL	1 (7)	2 (5)	3 (6)	ND
Hodgkin's disease	0 (0)	1 (3)	1 (2)	ND
Non Hodgkin lymphoma	3 (21)	8 (20)	11 (20)	ND
MM	7 (50)	5 (13)	12 (22)	ND
MDS	0 (0)	2 (5)	2 (4)	ND
AA	1 (7)	0 (0)	1 (2)	ND
Systemic chronic GVHD, N (%)	14 (100)	27 (68)	41 (76)	NA
Relapse of underlying disease, N (%)	3 (21)	7 (18)	10 (18)	0.75
Visits outpatient department median (range)	13 (1-40)	1 (0- 3)	1 (0-40)	<0.01
Duration of follow-up in years mean ± SD	2.9±1.2	2.4±1.2	2.5±1.2	0.2
Karnofsky score, N (%)				
90-100	6 (40)	22 (58)	28 (53)	0.001
70-80	7 (47)	13 (34)	20 (38)	
50-60	2 (13)	2 (5)	4 (8)	
30-40	0 (0)	1 (3)	1 (2)	

AA: aplastic anemia; AML: Acute Myeloid Leukemia; ALL: Acute Lymphoid Leukemia; CML: Chronic Myeloid Leukemia; CLL: Chronic Lymphoid Leukemia; GvHD: Graft-Versus-Host-Disease; MDS: myelodysplastic syndrome; MM: Multiple Myeloma; NA: not applicable; ND: not determined; OPD: outpatient department.

Table 1.
Allogeneic stem cell transplantation patient characteristics

VFQ subscale	Ocular GvHD n=14 Median (range)	No ocular GvHD n=40 Median (range)	Ocular GvHD vs no ocular GvHD Crude P value*	Total n=54 Median (range)
General health	75.0 (25-100)	93.8 (50-100)	0.005	89.1 (31.3-100)
General vision	60.0 (40-80)	80.0 (25-100)	0.053	80.0 (25-100)
Ocular pain	50.0 (12.5-100)	87.5 (25-100)	0.001	87.5 (12.5-100)
Near vision	75.0 (50-100)	91.7 (41.7-100)	0.082	87.5 (41.7-100)
Distant vision	83.3 (41.7-100)	91.7 (50-100)	0.101	91.7 (41.7-100)
Vision Specific				
Soc. functioning	100.0 (50-100)	100.0 (62.5-100)	0.021	100.0 (50-100)
Mental health	84.4 (25-100)	93.8 (25-100)	0.126	92.2 (31.3-100)
Role difficulties	62.5 (25-100)	100.0 (12.5-100)	0.002	87.5 (12.5-100)
Dependency	100.0 (41.7-100)	100.0 (41.7-100)	0.084	100.0 (50-100)
Driving	83.3 (0-100)	91.7 (0-100)	0.096	91.7 (0-100)
Color vision	100.0 (25-100)	100.0 (75-100)	0.612	100.0 (25-100)
Peripheral vision	87.5 (50-100)	100.0 (25-100)	0.215	100.0 (25-100)

GvHD: Graft-Versus-Host-Disease; SD: Standard Deviation; VFQ-25: Visual Functioning Questionnaire 25.
* P-value calculated using the Mann-Whitney test

Table 2.
VFQ-25 subscales in patients after allogeneic stem cell transplantation

	VFQ-25 Median (range)	General health Median (range)	Ocular pain Median (range)	Role difficulties Median (range)	Social functioning Median (range)	OSDI score Median (range)
Schirmer test value						
> 5 mm	92 (45-100)	94 (25-100)	88 (25-100)	88 (13-100)	100 (63-100)	6 (0-58)
≤5 mm	79 (46-96)	78 (31-100)	63 (13-100)	63 (25-100)	100 (50-100)	32 (2-73)
	P=0.04[a]	P= 0.01[a]	P=0.02[a]	P=0.01[a]	P=0.3[a]	P=0.0
Tear break up time						
Normal	91 (45-100)	94 (25-100)	88 (25-100)	88 (13-100)	100 (63-100)	7 (0-58)
Low	81 (46-100)	69 (31-88)	63 (13-100)	69 (25-100)	100 (50-100)	28 (0-73)
	P=0.05[a]	P=0.00[a]	P=0.03[a]	P=0.08[a]	P=0.16[a]	P=0.20
Fluorescein staining						
None	91 (45-100)	94 (25-100)	88 (25-100)	88 (13-100)	100 (63-100)	6 (0-60)
Positive	85 (46-100)	75 (31-100)	75 (13-100)	75 (25-100)	100 (50-100)	19 (0-73)
	P=0.60[a]	P=0.001[a]	P=0.04[a]	P=0.21[a]	P=0.02[a]	P=0.20[a]
Mean Visual acuity (logMAR)						
	-0.31b	-0.37[b]	-0.38[b]	-0.50[b]	-0.50[b]	0.24[b]
	P=0.03[c]	P=0.01[c]	P=0.01[c]	P=0.00[c]	P=0.001[c]	P=0.10[c]
Number of medications						
No therapy	92 (45-100)	94 (50-100)	88 (25-100)	88 (13-100)	100 (63-100)	6.3 (0-58)
Only tear drops	82 (48-98)	84 (25-100)	63 (25-100)	69 (25-100)	100 (63-100)	22 (0-35)
More types of treatment	85 (46-100)	69 (31-94)	50 (13-100)	69 (25-100)	100 (50-100)	40 (0-73)
	P=0.08[d]	P=0.001[d]	P=0.003[d]	P=0.13[d]	P=0.17[d]	P=0.20[d]
Karnofsky score						
90-100	93 (45-100)	94 (56-100)	88 (25-100)	88 (13-100)	100 (63-100)	8.3 (0-60)
70-80	89 (46-100)	88 (25-100)	81 (13-100)	81 (25-100)	100 (50-100)	7.3 (0-73)
50-60	84 (55-96)	70 (50-100)	81 (38-88)	56 (13-100)	100 (75-100)	35 (6-54)
	P=0.75[d]	P=0.07[d]	P=0.74[d]	P=0.42[d]	P=0.96[d]	P=0.27[d]

VFQ-25: Visual Functioning Questionnaire 25; OSDI : ocular surface disease index ;
[a]Mann Whitney test, significance level is p=0.05; [b]Correlation coefficient (Spearman's rho); [c]Spearman's rho, significance level p=0.05; [d]Kruskal-Wallis test, significance level is p=0.05.

Table 3.
Associations between visual functioning questionnaire-25 scores and clinical manifestations of dry eye

VFQ subscale	Diabetic retinopathy (Warrian et al, 2010)[14] n=91 Median (range)	BRVO (Awdeh et al, 2010)[20] n=51 Mean±SD	Behçet uveitis (Onal et al, 2010)[16] n=46 Median (range)	Ocular GvHD n=14 Median (range)
General health	ND	61.4±20.9	55.0 (18-83)	75.0 (25-100)
General vision	60.0 (20-100)	67.8 ±15.5	65.0 (5-85)	60.0 (40-80)
Ocular pain	87.5 (13-100)	76.6± 20.2	62.5 (25-100)	50.0 (12.5-100)
Near vision	58.3 (8-100)	72.8± 18.9	80.0 (4-100)	75.0 (50-100)
Distant vision	66.7 (17-100)	77.2 ± 18.8	85.0 (8-100)	83.3 (41.7-100)
Vision Specific				
Soc. functioning	87.5 (13-100)	94.0 ± 12.8	100.0 (17-100)	100.0 (50-100)
Mental health	68.8 (0-100)	73.8± 20.9	65.0 (0-100)	84.4 (25-100)
Role difficulties	62.5 (0-100)	75.0 ± 27.0	75.0 (0-100)	62.5 (25-100)
Dependency	75.0 (0-100)	91.7 ± 19.1	87.5 (6-100)	100.0 (41.7-100)
Driving	75.0 (0-100)	75.3 ± 23.6	91.6 (42-100)	83.3 (0-100)
Color vision	100.0 (25-100)	96.7 ± 12.5	100.0 (0-100)	100.0 (25-100)
Peripheral vision	75.0 (25-100)	83.2 ± 20.4	100.0 (25-100)	87.5 (50-100)

BRVO; Branch Retinal Vein Occlusion; GvHD: Graft-Versus-Host-Disease; ND: not determined; SD: Standard Deviation; VFQ-25: Visual Functioning Questionnaire [25].

Table 4.
VFQ-25 scores in various ocular disorders

Discussion

Our results show that patients who develop ocular GvHD can suffer from impaired visual QoL on the long term after allo-SCT. Specific domains affected included general health, ocular pain, social functioning and role difficulties. In particular, patients with ocular GVHD exhibited scores of more than 80 points in four out of all 11 domains examined, while the patients without ocular GvHD exhibited such results in all domains (Table 2).

In the last decades, the pattern of ocular disorders following allo-SCT has profoundly changed. In the past, patients who underwent classical myeloablative bone marrow transplants needed intensive conditioning before the transplantation including chemotherapy and TBI together with short and long-term high dosages of systemic corticosteroids. Therefore the main ocular complications consisted of development of cataract and radiation retinopathy[22,23]. The conditioning regimens of allo-SCT are nowadays characterized by less intensive conditioning regimens and frequent development of graft-versus-host reactions. GvHD forms the major reason for morbidity and mortality after a successful allo-SCT. However, the graft versus tumor (GvT) effect forms an important beneficial aspect of the allo-SCT.

All our patients received an unmanipulated stem cell graft after non-myeloabaltive conditioning. In consequence, the prevalence of cataract and radiation retinopathy decreased, while ocular complications of GvHD have increased. Effective preventive treatment and intensive follow-up regimens have diminished the prevalence of ocular infections. Currently, ocular disorders encountered after allo-SCT include predominantly manifestations of ocular GvHD characterized by chronic OSD and can be accompanied by profound secondary changes in conjunctiva, cornea and eyelids[24]. These ocular disorders might not seem severe in patients surviving malignant disease, but can be associated with severe ocular discomfort and pain, require frequent application of medications, and visits to an ophthalmologist. In addition, this study validates that patients with ocular GvHD have difficulties performing their daily tasks and work. More importantly, ocular GvHD also diminishes direct social functioning up to three or four years after allo-SCT. For example, some patients are not able to practice their hobbies and/or sporting activities or even preferred not to leave their house due to extremely painful eyes outdoors.

We compared the results of the VFQ-25 in ocular GvHD to the results in other ocular diseases such as type-one diabetes mellitus, branch retinal vascular occlusions (BRVO) and Behçet uveitis[14,16,20] (Table 4). The results in the domains of general vision and role difficulties were similarly impaired in all disorders compared. In addition, our results on QoL are similar to previous findings in Sjögren's syndrome. Both Sjögren's syndrome and ocular GvHD, exhibited particularly impaired domain of ocular pain[18]. These findings underline the severity and impact of ocular GvHD.

A major point of discussion of this current study is the fact the patients after allo-SCT are severely ill and their systemic disease might have had influenced the results of the VFQ-25. However, the presence of chronic GvHD without ocular involvement did not influence the results on visual QoL according to the domain of general health of the VFQ-25, but the Karnofsky scores were significantly lower in the ocular

GvHD group, it is however not possible to discern how much their systemic disease contributed to this finding.

It is important to realize that ocular GvHD is a chronic disease and the complaints and symptoms may vary in time and patients have variable responses to treatment. Some domains of the VFQ-25 might also be affected by variables not taken into account in this present study. Possible confounders not taken into account include underlying disease and systemic medications.

Balaram *et al*. followed 114 patients for over one year after SCT and concluded that late-onset ocular surface disease may occur, and points out the need for long-term monitoring[1]. Our results confirm the presence of long term morbidity in patients post allo-SCT, and that even three to four years after transplantation patients may suffer from impaired vision related QoL due to ocular GvHD. The draw backs or our study include the limited number of patients with ocular GvHD. However even with these limited numbers, we point out the severe consequences of this neglected ocular disorder and clearly show impaired vision-related QoL, even on the long term after allo-SCT. A future larger study might document the full impact of ocular GvHD on QoL.

At the present there are no effective preventive strategies available for ocular GvHD and current therapeutic options consists of entirely symptomatic treatment regimens aimed at alleviation of symptoms. In our view, the results of our study show the serious impact of ocular GvHD on visual related QoL of patients surviving hematological malignancies and underline the need for the future studies on prevention and treatment of ocular GvHD occurring in allo-SCT survivors.

References

1. Balaram M, Rashid S, Dana R (2005) Chronic ocular surface disease after allogeneic bone marrow transplantation. Ocul Surf 3 (4): 203-211.

2. Ferrara JL, Levine JE, Reddy P, Holler E (2009) Graft-versus-host disease. Lancet 373: 1550-1561.

3. Pallua S, Giesinger J, Oberguggenberger A, Kemmler G, Nachbaur D, Clausen J, Kopp M, Sperner-Unterweger B, Holzner B (2010): Impact of GvHD on quality of life in long-term survivors of haematopoietic transplantation. Bone Marrow Transplant 45: 1534-1539.

4. Rojas B, Cuhna R, Zafirakis P, J.M. Ramirezc, M. Lizan-garcii´ad, T. Zhaoa, C.S. Fostera *et al*. Cell populations and adhesion molecules expression in conjunctiva before and after bone marrow transplantation. Experimental eye research 2005; 81: 313-25.

5. Ogawa Y, Kuwana M (2003): Dry eye as a major complication associated with chronic graft-versus-host disease after hematopoietic stem cell transplantation. Cornea 22: S19-S27.

6. Tabbara KF, Al-Ghamdi A, Al-Mohareb F, Ayas M, Chaudhri N, Al-Sharif F, Al-Zahrani H, Mohammed SY, Nassar A, Aljurf M (2009): Ocular findings after allogeneic hematopoietic stem cell transplantation. Ophthalmology 116: 1624-1629.

7. Westeneng AC, Hettinga Y, Lokhorst H, Verdonck L, van DS, Rothova A (2010) Ocular graft-versus-host disease after allogeneic stem cell transplantation. Cornea 29: 758-763.

8. Mangione CM, Lee PP, Gutierrez PR, Spritzer K, Berry S, Hays RD (2001): Development of the 25-item National Eye Institute Visual Function Questionnaire. Arch Ophthalmol 119: 1050-1058.

9. Miller KL, Walt JG, Mink DR, Satram-Hoang S, Wilson SE, Perry HD, Asbell PA, Pflugfelder SC (2010): Minimal clinically important difference for the ocular surface disease index. Arch Ophthalmol 128: 94-101.

10. Walt J (2004): Ocular surface disease index (OSDI) administration and scoring manual. Irvine, CA: Allergan, Inc.

11. Schiffman, R. M., Christianson, M. D., Jacobsen, G., Hirsch, J. D., & Reis, B. L. (2000): Reliability and validity of the Ocular Surface Disease Index. Archives of ophthalmology, 118 (5), 615-21.

12. WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects (2008): 59th WMA General Assembly, Seoul, Korea, October

13. Schiffman RM, Walt JG, Jacobsen G, Doyle JJ, Lebovics G, Sumner W (2003): Utility assessment among patients with dry eye disease. Ophthalmology 110: 1412-1419.

14. Warrian KJ, Lorenzana LL, Lankaranian D, Dugar J, Wizov SS, Spaeth GL (2010): Thassessment of disability related to vision performance-based measure in diabetic retinopathy. Am J Ophthalmol 149: 852-860.

15. van Gestel GA, Webers CA, Beckers HJ, van Dongen MC, Severens JL, Hendrikse F, Schouten JS (2010): The relationship between visual field loss in glaucoma and health-related quality-of-life. Eye (Lond) 24: 1759-1769.

16. Onal S, Savar F, Akman M, Kazokoglu H (2010): Vision- and health-related quality of life in patients with Behcet uveitis. Arch Ophthalmol 128: 1265-1271.

17. Mazhar K, Varma R, Choudhury F, Kean-Cowdin R, Shtir CJ, Azen SP (2010): Severity of Diabetic Retinopathy and Health-Related Quality of Life The Los Angeles Latino Eye Study. Ophthalmology 118: 649-655.

18. Mizuno Y, Yamada M, Miyake Y (2010): Association between clinical diagnostic tests and health-related quality of life surveys in patients with dry eye syndrome. Jpn J Ophthalmol 54: 259-265.

19. Seland JH, Vingerling JR, Augood CA, Bentham G, Chakravarthy U, Paulus TVM. deJong, Rahu M, Soubrane G, Tomazzoli L, Topouzis F, Fletcher A.E (2011): Visual impairment and quality of life in the older European population, the EUREYE study. Acta ophthalmologica 89: 608-13.

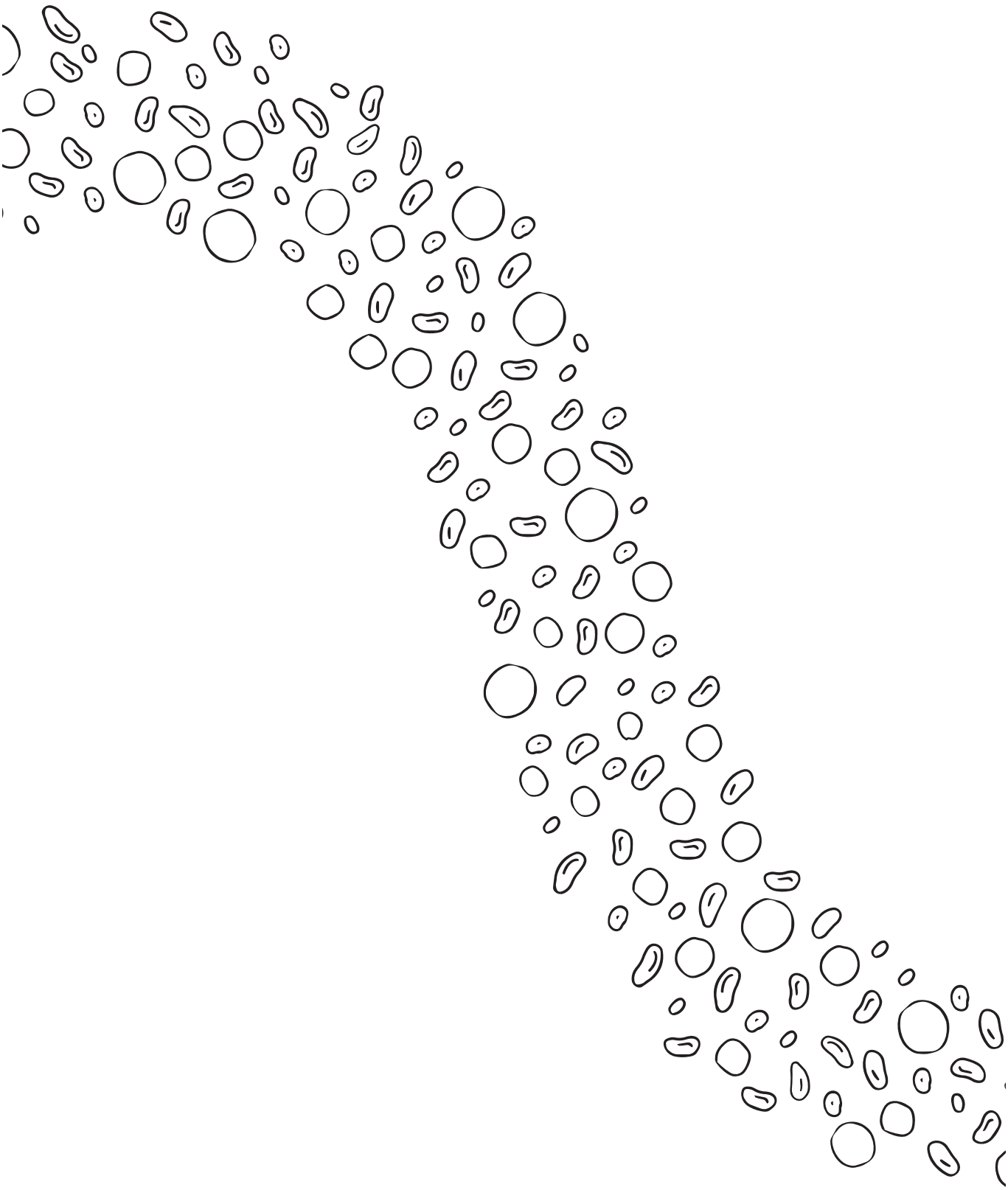
20. Awdeh RM, Elsing SH, Deramo VA, Stinnett S, Lee PP, Fekrat S (2010): Vision-related quality of life in persons with unilateral branch retinal vein occlusion using the 25-item National Eye Institute Visual Function Questionnaire. Br J Ophthalmol 94: 319-323.

21. Filipovich AH (2008): Diagnosis and manifestations of chronic graft-versus-host disease. Best Pract Res Clin. Haematol 21: 251-257.

22. Coskuncan NM, Jabs DA, Dunn JP, Haller JA, Green WR, Vogelsang GB, Santos GW (1994): The eye in bone marrow transplantation. VI. Retinal complications. Arch Ophthalmol 112: 372-379.

23. Dunn JP, Jabs DA, Wingard J, Enger C, Vogelsang G, Santos G (1993): Bone marrow transplantation and cataract development. Arch Ophthalmol 111: 1367-1373.

24. Riemens A, te Boome L, Imhof S, Kuball J, Rothova A (2010): Current insights into ocular graft-versus-host disease. Curr Opin Ophthalmol 21: 485-494.



Chapter 7

Age Related Risk of ocular Graft-versus-Host-Disease (GvHD) after allogeneic hematopoietic stem cell transplantation in children and adults

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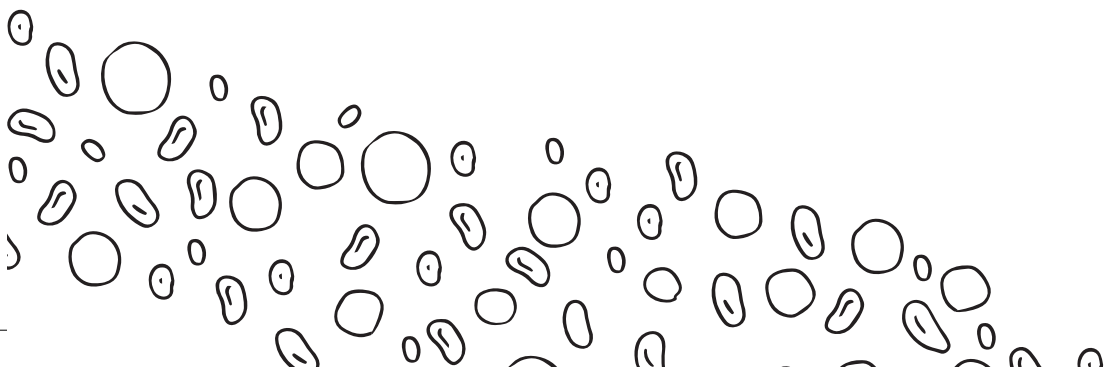


Abstract

Importance

Development of ocular Graft-versus-Host-Disease (GvHD) after allogeneic hematopoietic stem cell transplantation (HSCT) can lead to debilitating dry eye syndrome (DES) and therefore it is important to identify its risk factors.

Objective

To study the risk factors for ocular GvHD in both adults and children after allogeneic HSCT.

Design and setting

This retrospective study includes all consecutive patients who had undergone an HSCT at the University Medical Center Utrecht (in the adult and pediatric HSCT program), Utrecht, the Netherlands, in 2009 and 2010.

Participants

One hundred seventy consecutive patients underwent systematic ophthalmologic evaluations after HSCT and were evaluated for possible risk factors for the development of ocular GvHD.

Main outcome measure

Frequency of ocular GvHD and predictors of its development.

Results

Ocular GvHD developed in 29/126 (23%) of adult patients within one year after HSCT and in 9 % of children (4/44) (p=0.04). In children, treatment with artificial tear drops only was sufficient in all patients while in adults the majority required additional immunosuppressive eye treatment and had to use multiple local medications. Children who developed ocular GvHD were significantly older than children without ocular GVHD (median age 13 vs. 5 years). Incidence of systemic manifestations in chronic or acute GvHD did not differ between adults and children (47% versus 27%, p=0.4 and 44% versus 32%, p=0.16, respectively). Development of ocular GvHD was associated with older age (p=0.002) in the entire study population. In a multivariate Cox survival analysis adjusted for gender, malignancy as HSCT indication, HLA-disparity, source of stem cells and conditioning regimen, age was the only significant factor associated with development of ocular GVHD in the whole group of adults and children (p=0.007).

Conclusions and relevance

We found that rising age is a major predictor for the development of ocular GvHD.

Introduction

Chronic Graft-versus-Host-Disease (GvHD) is a potentially life threatening condition that can develop in adults and children after allogeneic hematopoietic stem cell transplantation (HSCT) for haematological malignancies and disorders. Chronic GvHD develops in 60-70% of in adult patients after HSCT, however children were reported to develop chronic GvHD less often (6-65%)[1-3]. Ocular GvHD is a manifestation of chronic GvHD which may have a severe impact on the quality of life of these patients[4]. Clinical manifestations of ocular GvHD include keratoconjunctivitis sicca (KCS) which is being often accompanied by (chronic) inflammatory signs of the conjunctiva and blepharitis[5-7]. Ocular GvHD develops in 10-62% of adult patients after HSCT[5-6, 8-9]. Reported incidence rates of dry eye syndrome (DES) after HSCT in children vary from as low as 12.5% to as high as 89%, however it is not always clear whether the DES reported in these studies could be addressed to ocular GvHD[10-13].

Risk factors for developing systemic manifestations of chronic GvHD include precedent acute GvHD, unrelated donor, Human Leucocyte Antigen (HLA) mismatched donor, Peripheral Blood Stem Cells (PBSC) as donor source, female donor to male recipient, grafting with mobilized blood cells and malignant disease[1,14]. Factors that tend to be associated with low rates of GvHD include the use of cord-blood (CB) stem cells and use of methotrexate and cyclosporin-A as prophylaxis[1, 15-16].

In this study we evaluate the development of ocular GvHD and identify predictors that are responsible for the differences in development of ocular GvHD in adults and children.

Materials and Methods

All consecutive adult (>18 years) (N=126) and pediatric patients (<18 years) (N=44) that underwent an allogeneic HSCT in 2009 and 2010 in the UMC Utrecht within the adult and pediatric HSCT program and had one or more ophthalmic examinations were included. The study was performed in accordance with the Declaration of Helsinki and with the approval of the local Institutional Review Board.

Data on age, gender, underlying condition, type of SCT, HLA matching, conditioning regimen, development of acute and chronic-GvHD, prophylaxis, and development and signs of ocular GvHD were collected.

Adult patients received a non-manipulated peripheral blood stem cell transplant, 99 patients after conditioning with fludarabine and low dose total body irradiation (TBI) (two Gray) and 19 patients after conditioning with cyclophosphamide and TBI (six x two Gray), 8 patients received other regimens or data were missing. In patients with an unrelated donor, anti-thymocyte-globulin ((ATG) Thymoglobuline, Sanofi)) was added to the conditioning regimen.

Pediatric patients' transplantation, conditioning regimens, supportive care, graft-versus-host disease (GvHD) prophylaxis and infection monitoring were performed according to the "pediatric blood and marrow transplantation program" of the UMC Utrecht (Wilhelmina Children's Hospital). All patients received

conditioning according to applicable international and national protocols. The conditioning consisted of Fludarabine and Busulfan (Busilvex; Pierre Fabre Medicament, Boulogne, France) for all inborn errors, immune deficiencies (including hemophagocytic lympho-histiocytotis), myeloid malignancies, acute lymphoblastic leukemia (ALL) who had a contraindication for TBI and patients younger than 3 years of age. In pediatric patients receiving an unrelated donor graft, ATG was added to the conditioning regimen. Patients with an ALL received TBI (6 x 2 Gray) / VP16 (etoposide).

Post transplant GvHD prophylaxis in adults consisted of cyclosporin-A with addition of mycophenolate mofetil. GvHD prophylaxis in the pediatric population consisted of cyclosporin-A (aiming for a trough level of 100-250 µg/L, based on national protocol guidelines), supplemented with methylprednisolone in patients receiving a cord blood (CB) transplant, or short course of methotrexate in patients receiving an unrelated bone marrow (BM) or peripheral blood stem cell (PBSC) transplant.

Standard ophthalmologic examination of both adults and children included visual acuity measurement (if feasible), slit lamp examination (including fluorescein staining of the cornea and measurement of tear break-up time (BUT)) and fundus examination with dilated pupil. The Schirmer test (after local anaesthesia with Oxybuprocain 0.4%) was only performed in adults due to limited cooperation of young children. Additional registered signs of ocular GvHD included blepharitis, chemosis, Meibomian gland dysfunction and low tear break-up time (TBUT). Adult patients had an ophthalmologic screening 3 months after HSCT and in case of ocular complaints or signs. All included children had an ophthalmologic evaluation before and after HSCT (before leaving the HSCT-unit with a median time of 3 weeks after HSCT, and later on at 3, 6 and 12 months after the SCT). The examinations were adapted to the age and co-operability of every child. Examination at the HSCT-unit contained full ophthalmic examination with hand slit lamp and fundoscopy. Duration of follow-up was the time to last ophthalmologic examination for survivors or the time until death. In case of multiple ocular examinations, we registered the most severe ocular signs of GvHD.

The diagnosis of ocular GvHD in adults was made based on the criteria of Filipovich *et al*[17]. In short, the Schirmer test values ≤5mm in both eyes or between 6-10 mm with KCS diagnosed by slit-lamp examination accompanied by involvement of at least 1 other organ with GvHD. In children the diagnosis was based on the presence of KCS with corneal epithelial staining in combination with lower TBUT at slit-lamp examination and involvement of at least 1 other organ with GvHD. Only (ocular GvHD) that developed within one year after HSCT were included in the statistical analysis because the last ocular examination in children was performed 12 months after HSCT.

For statistical analysis the most affected eye was included. The data were entered and analysed using SPSS software version 20.0 (IBM Inc, Chicago, Illinois, USA). Kolmogorov-Smirnov test was used to analyze the normality of data distribution. Fisher's Exact test and Pearson Chi square were used for analysis of categorical data. Mann-Whitney U-test was used for non-parametric analysis of differences between the groups. To analyze predictors for development of ocular GvHD the following recipient associated variables

were taken into account: age at HSCT, gender and malignancy as indication for HSCT. Transplantation associated variables included source of stem cells, human leukocyte antigen (HLA) disparity and the basis of the conditioning regimen (chemotherapy or total body irradiation). Cox proportional hazard model was used to analyze associations between variables and outcome. P-values of < 0.05 were considered significant.

Results

Indications for HSCT in all patients are listed in Table 1 and other general characteristics are listed in Table 2. Briefly, the median age at the moment of HSCT of all included patients was 43 years (range 0-67 years). Sixty percent (101/170) of patients were male, the gender ratio did not differ significantly between adults and children (p= 0.72). Thirty-two percent of all patients died after HSCT during follow up, 39/126 (31%) of adults and 15/44 (34%) of children (P=0.71). HSCT related factors including malignancy as indication (p<0.01), HLA-mismatch (p<0.01), type of HSCT (CB versus peripheral/BM cells) (p<0.01) and TBI (p<0.01) did significantly differ between adults and children.

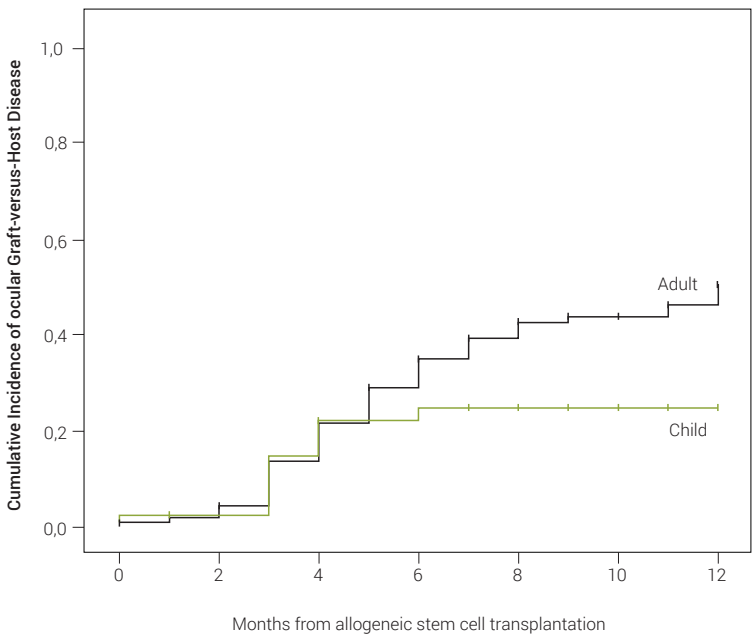


Figure 1.
Cumulative incidence of systemic chronic Graft-versus-Host Disease (GvHD) after allogeneic stem cell transplantation (HSCT)

Acute systemic GvHD developed within 3 months after HSCT in 44% (56/126) of adults and 32% (14/44) of children (p=0.16). Chronic systemic GvHD developed in 47% (59/126) of adults versus 27% (12/44) of children within one year after HSCT (p=0.4). In adults that developed only chronic GvHD (not preceded by acute GvHD) the median time to develop chronic GvHD was 125 days (range 95-190 days) versus 124 days (range 62-363 days) in children and 180 days (range 62-363 days) in the total study population. Figure 1 shows the cumulative incidence curve of chronic systemic GvHD for adults and children, which was significantly different (p=0.03).

Ocular-GvHD within one year after HSCT developed in 23% (29/126) of adults and in 9% (4/44) of children (p=0.04). The median time of development of ocular GVHD was 113 days (range 27-365 days) in adults and 72 days (range 27-142) in children (p=0.5). The median age of patients who developed ocular GvHD was significantly higher than that of patients without ocular GvHD in both adult and pediatric groups (59 vs. 49 years in adults (p=0.05) and 13 vs. 5 years (p=0.002) in children; Table 2). In 3/4 (75%) children with ocular GVHD chronic GVHD in other organs was preceded by acute GvHD and in adults in 14/29 (48%). Figure 2 shows the cumulative incidence curve of ocular GvHD for adults and children, which was significantly different (p=0.04).

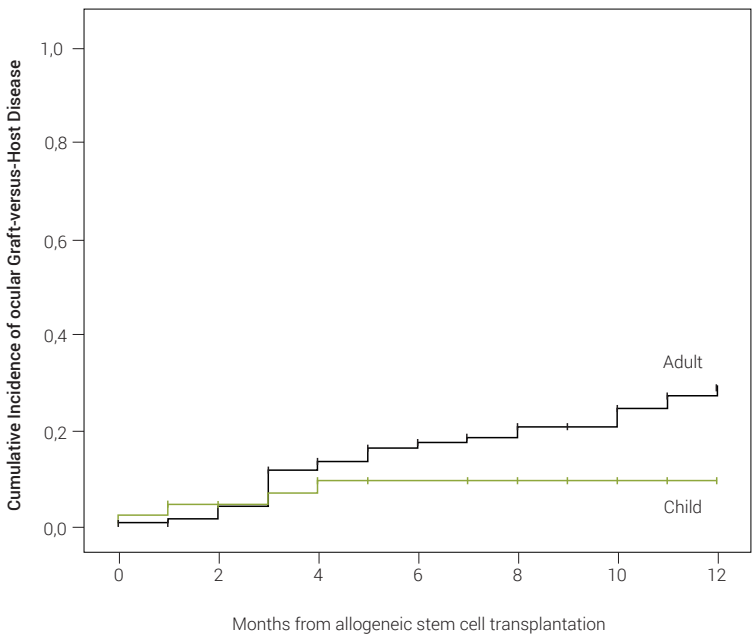


Figure 2.
Cumulative incidence of ocular Graft-versus-Host Disease (GvHD) after allogeneic stem cell transplantation (HSCT)

Underlying condition	Adults n =126 (%)	Children n = 44 (%)	Total n = 170 (%)
Malignancy, n (%)	110 (87%)	24 (55%)	134 (79%)
Non-malignant disorders, n (%)	16 (13%)	20 (45%)	36 (21%)
Specific diagnoses, n (%)			
AML	35 (28)	6 (14)	41 (24)
MDS	6 (5)	2 (5)	8 (5)
Myelofibrosis	6 (5)	-	6 (4)
Multiple myeloma	18 (14)	-	18 (11)
CLL	7 (6)	-	7 (4)
NHL	18 (14)	2 (5)	20 (12)
Fanconi anemia	-	4 (9)	4 (2)
ALL	17 (13)	11 (25)	28 (16)
Aplastic anemia	6 (5)	-	6 (4)
Biliniar acute leukemia	1 (1)	-	1 (1)
CML	3 (2)	-	3 (2)
Metachromatic leukodystrophy	3 (2)	2 (5)	5 (3)
CMML	1 (1)	-	1 (1)
Mucositis fungoides	1 (1)	-	1 (1)
JMML	-	3 (7)	3 (2)
Hurler Syndrome	-	2 (5)	2 (1)
ALL + fanconie anemia	-	1 (2)	1 (1)
Beta-thalassemia	-	1 (2)	1 (1)
Chronic granulomatous disease	1 (1)	1 (2)	2 (1)
Common variable immune deficiency	-	2 (5)	2 (1)
Hemophagocytic lymphohistiocytosis	-	1 (2)	1 (1)
Miscellaneous	3 (2)	6 (14)	9 (5)

* Miscellaneous included Kostmann syndrome, Omenn syndrome, osteopetrosis, CD40 ligand deficiency, Hodgkin lymphoma, Burkitt lymphoma, alpha-thalassemia and mitochondrial neurogastrointestinal encephalopathy syndrome (MNGIE).
AML: Acute Myeloid Leukemia, MDS: Mylodysplastic Syndrome, CLL: Chronic Lymphoid Leukemia, NHL: Non Hodgkin Lymphoma, ALL: Acute Lymphoid Leukemia, CML: Chronic Myeloid Leukemia, CMML: Chronic myelomonocytic leukemia, JMML: Juvenile myelomonocytic leukemia

Table 1.
Indications for allogeneic haematopoietic stem cell transplantation (HSCT)

Patient characteristics	Adults, n=126				Children, n=44
	All adults (100%)	Ocular GvHD Within one year after HSCT N=29 (23%)	No ocular GvHD N=97 (77%)	p-value	All children (100%)
Gender, n (%)					
Male	76 (60%)	19 (66%)	57 (59%)	P=0.6*	25 (57%)
Age at allo-SCT, years (median-range)					
	51 (18-67)	59 (18-67)	49 (18-67)	P=0.05**	5 (0-17)
Indication, n (%)					
Malignant	110 (87%)	25 (86%)	85 (88%)		24 (55%)
Non-Malignant	16 (13%)	4 (14%)	12 (13%)	P=1.0*	20 (45%)
Source stem cells, n (%)					
BM/PBSCT	116 (92%)	26 90%)	90 (93%)	P=0.7*	15 (34%)
HLA matched	107	24	83		15
HLA mismatched	9	2	7		0
Cord blood	10 (8%)	3 (10%)	7 (7%)		29 (66%)
Conditioning, n (%)					
TBI-based	108 (86%)	22 (74%)	86 (89%)		9 (20%)
Chemotherapy-based	18 (14%)	7 (24%)	11 (11%)	P=0.2*	35 (80%)
GvHD, n (%)					
Acute	23 (18%)	1 (3%)	22 (23%)		8 (18%)
Chronic	26 (21%)	10 (35%)	16 (17%)		6 (14%)
Both	33 (26%)	13 (45%)	20 (21%)		6 (14%)
No GvHD	44 (35%)	5 (15%)	39 (41%)	P=0.001*	24 (55%)
Death, n (%)					
	39 (31%)	10 (35%)	29 (30%)	P=0.7*	15 (34%)

* Pearson Chi-square/ Fischer exact test ** Mann-Whitney U Test HSCT: allogeneic hematopoietic stem cell transplantation; BM: bone marrow; PBSCT: peripheral blood stem cell transplantation

Table 2.
General patient characteristics of 126 adults and 44 children with allogeneic haematopoietic stem cell transplantation (HSCT)

Children, n=44			Total, n=170			
Ocular GvHD within one year after HSCT N=4 (9%)	No ocular GvHD N=40 (91%)	p-value	All patients (100%)	Ocular GvHD Within one year after HSCT N=33 (19%)	No ocular GvHD N=137 (81%)	p-value
2 (50%)	23 (58%)	P=1.0*	101 (60%)	21 (64%)	80 (58%)	P=0.7*
13 (11-17)	5 (0-17)	P=0.002**	43 (0-67)	55 (11-67)	40 (0-67)	P=0.002**
4 (100%)	20 (50%)		134 (79%)	29 (88%)	105	
0	20 (50%)	P=0.1*	36 (21%)	4 (12%)	32	P=0.2*
3 (75%)	12 (30%)	P=0.1*	131 (77%)	29 (88%)	102 (74%)	
3	12		122	27	95	P=0.1*
10	0		9	2	7	
1 (25%)	28 (70%)		39 (23%)	4 (12%)	35 (26%)	
1 (25%)	8 (20%)		117 (69%)	23 (70%)	94 (69%)	
3 (75%)	32 (80%)	P=1.0*	53 (31%)	10 (30%)	43 (31%)	P=0.9*
0	8 (20%)		31 (18%)	1 (3%)	30 (22%)	
1 (25%)	5 (13%)		32 (19%)	11 (33%)	21 (15%)	
3 (75%)	3 (8%)		39 (23%)	16 (48%)	23 (17%)	
0	24 (60%)	P=0.003*	68 (40%)	5 (15%)	63 (46%)	P=0.0*
2 (50%)	13 (33%)	P=0.6*	54 (32%)	12 (36%)	42 (31%)	P=0.5*

Variable	Adults N=126	Children N=44	p-value
Ocular GvHD, n (%)	29 (23%)	4 (9%)	P=0.04
Schirmer value, median (IQR)	4 mm (3mm)	NA	
Treatment ocular GvHD, n (%)	26 (90%)	4 (100%)	P=0.14
Tear drops, n (%)	10	4	
Cyclosporine drops/ointment, n	3	-	
Combination of 2 therapies, n	6	-	
Combination of 3 therapies, n	4	-	
Combination of >3 therapies, n	3	-	
Period allo-SCT until ocular GvHD in days (range)	113 (27-359)	72 (27-142)	P=0.5
Dry eyes, n (%)	11 (9%)	1 (2%)	P=0.2
Blepharitis/Conjunctivitis, N (%)	7 (6%)	-	

*Multivariate Cox proportional hazards regression analysis
NA: not applicable
Allo-SCT: allogeneic stem cell transplantation; GvHD: Graft-versus-Host-Disease; IQR= interquartile range

Table 3.
Ocular Graft-versus-Host-Disease in children and adults after allogeneic hematopoietic stem cell transplantation (HSCT)

Some additional patients developed signs of dry eye without signs of GVHD (11/126 adults (9%) and 1/44 children (2%)). Additionally 6% (7/126) of adult patients developed blepharitis and/or conjunctivitis. Ocular GvHD was treated in 90% (26/29) of adults and in all children (4/4); however the treatment regimens were far more intensive in adults. Table 3 presents the used treatment regimes: tear drops only in 38% of adults and 100% of children, cyclosporine drops or ointment in 12% of adults, combination of 2 therapies in 23% of adults, 3 therapies in 15% of adults and more than 3 therapies in 7% of adults; 14% of adults (4/26) received no treatment.

Overall, development of ocular GvHD was associated with older age (p=0.002) in the entire study population. No association was found between the development of ocular GvHD and gender (p=0.7), malignancy as HSCT indication (p=0.2) HLA-disparity (p=0.1), source of stem cells (p=0.3), conditioning regimen (p=0.9), previous acute systemic GvHD (p=0.24) and death (p=0.5) (Table 2) for all patients included.

In a multivariate Cox survival analysis age was in the whole study population the only significant factor associated with development of ocular GVHD (p=0.007), adjusted for HLA disparity, type of SCT, gender, conditioning regimen and malignancy as HSCT indication.

Discussion

Our study shows that age at HSCT is an important predictor for the development of ocular GvHD. It has previously been reported that ocular GvHD develops in up to 62% of adult patients after HSCT and reports on DES in children rang from 12.5% after HSCT up to 89% in children, however the definitions in different studies are variable and probably not all these cases can be accounted for ocular GVHD[5-12]. In our study, within one year after HSCT, fewer children developed ocular GvHD when compared to adults. It is known that children generally develop less systemic manifestations of GvHD which is associated with more CB as stem cell source, less frequent use of TBI in conditioning regimen and less malignancies as transplantation indication[13-15,17].

In our study children also underwent HSCT more often for non-malignant disorders in which they were treated for a longer time in the post transplant period with immunosuppressive agents (up to 1 year). Twenty-four out of 44 (55%) children received HSCT with an indication of malignancy whereas in adults this was 87% (110/126). It is expected that patients develop GvHD more often with malignancy as indication because the post transplant immunosuppressive therapy is given for a shorter period of time in these patients to induce the so-called Graft-versus-Tumor effect. Our group had previously reported that in patients transplanted in the pediatric HSCT program, malignancy as indication for HSCT was associated with the development of DES after adjustment for age and other potential confounders[12]. However in this combined patient population this relationship was not found. The reason that this association lost its significance in the current study can be probably declared by the allocation of patients older than 18 years of age who has been transplanted in the pediatric unit according to pediatric protocols to the adult group in the current analysis.
Ocular GvHD in adults required more complex treatment modalities which suggests that the symptoms of ocular GvHD were more severe in the adult group. Within the group of children, age dependence was also noted as ocular GVHD was less frequent in younger children in contrast to teenagers. This has been earlier noted for DES in children and young adults who have been transplanted according to pediatric HSCT protocol in Wilhelmina Children's Hospital[12]. In this study, the patients who developed DES were significantly older than patients without DES (median age 13.7 vs. 5.5 year, p=0.003).

Different hypotheses might explain why ocular GvHD manifests less frequently and is less severe at younger age. Young patients have a lower prior exposure to infections and hereby have a less aggressive immune response[13-20]. Younger patients have a better thymus function than elderly patients which allows a better regulation of the immune system. Further, younger patients have better tear film function with a higher tear volume and better tear flow and osmolarity[21].

One might argue that some cases of ocular GvHD might be missed in children because the ocular

examination is more difficult in (young) children and that Schirmer test was not performed in this young age group. However the development of ocular GvHD leads to significant complaints and visible signs. In addition, children in our study underwent regular and intensive ocular examinations, and ocular GvHD would be detected even without subjective complaints.

In conclusion we found that rising age is a major predictor for the development of ocular GvHD in both children and adults. Awareness of ocular GvHD, especially in older children and adults undergoing HSCT is required to minimize the damage to the lacrimal gland and conjunctival tissue by early treatment.

References

1. Baird K, Cook K, Schultz K. Chronic Graft Versus Host Disease (GVHD) in Children. pediatr clin north am. 2011; 57 (1): 297–322.

2. Meisel R, Laws H-J, Balzer S, *et al*. Comparable long-term survival after bone marrow versus peripheral blood progenitor cell transplantation from matched unrelated donors in children with hematologic malignancies. Biology of blood and marrow transplantation.. 2007; 13 (11): 1338–45.

3. Wagner JE, Kernan N a, Steinbuch M, Broxmeyer HE, Gluckman E. Allogeneic sibling umbilical-cord-blood transplantation in children with malignant and non-malignant disease. Lancet. 1995; 346 (8969): 214–9.

4. Riemens A, Te Boome LCJ, Kalinina Ayuso V, *et al*. Impact of ocular graft-versus-host disease on visual quality of life in patients after allogeneic stem cell transplantation: questionnaire study. Acta ophthalmologica. 2013: 1–6.

5. Balaram M, Rashid S, Dana R. Chronic ocular surface disease after allogeneic bone marrow transplantation. Ocul Surf. 2005 Oct; 3 (4): 203-11.

6. Tabbara KF, Al-Ghamdi A, Al-Mohareb F, *et al*. Ocular findings after allogeneic hematopoietic stem cell transplantation. Ophthalmology. 2009; 116 (9): 1624–9.

7. Riemens A, te Boome L, Imhof S, Kuball J, Rothova A. Current insights into ocular graft-versus-host disease. Current opinion in ophthalmology. 2010; 21 (6): 485–94.

8. Westeneng AC, Hettinga Y, Lokhorst H, Verdonck L, van Dorp S, Rothova A. Ocular graft-versus-host disease after allogeneic stem cell transplantation. Cornea. 2010; 29 (7): 758–63.

9. Ogawa Y, Okamoto S, Wakui M, *et al*. Dry eye after haematopoietic stem cell transplantation. British Journal of Ophthalmology. 1999; 83 (10): 1125–1130.

10. Fahnehjelm KT, Törnquist A-L, Winiarski J. Dry-eye syndrome after allogeneic stem-cell transplantation in children. Acta ophthalmologica. 2008; 86 (3): 253–8.

11. Suh DW, Ruttum MS, Stuckenschneider BJ, Mieler WF, Kivlin JD. Ocular findings after bone marrow transplantation in a pediatric population. Ophthalmology. 1999; 106 (8): 1564–70.

12. Kalinina Ayuso V, Hettinga Y, van der Does P, Boelens JJ, Rothova A, de Boer J. Ocular complications in children within 1 year after hematopoietic stem cell transplantation. JAMA ophthalmology. 2013; 131 (4): 470–5.

13. Ng JS, Lam DS, Li CK, *et al.* Ocular complications of pediatric bone marrow transplantation. Ophthalmology. 1999; 106 (1): 160–4.

14. Flowers MED, Inamoto Y, Carpenter P a, *et al*. Comparative analysis of risk factors for acute graft-versus-host disease and for chronic graft-versus-host disease according to National Institutes of Health consensus criteria. Blood. 2011; 117 (11): 3214–9.

15. Rocha V, Jr JW. Graft-versus-host disease in children who have received a cord-blood or bone marrow transplant from an HLA-identical sibling. N Engl J Med. 2000 Jun 22; 342 (25): 1846-54.

16. Zecca M, Prete A, Rondelli R, *et al*. Chronic graft-versus-host disease in children: incidence, risk factors, and impact on outcome. Blood. 2002; 100 (4): 1192–200.

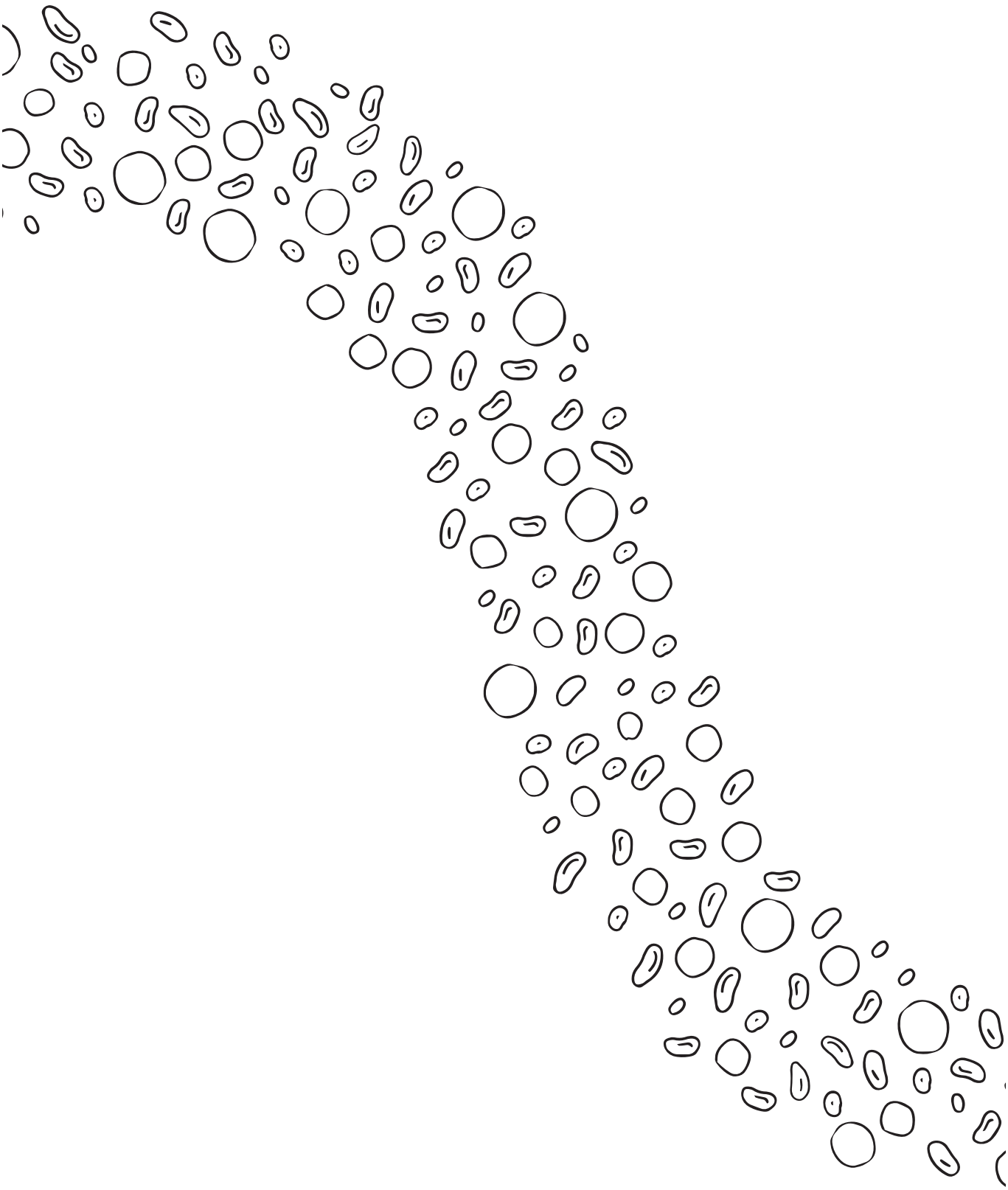
17. Filipovich AH, Weisdorf D, Pavletic S, *et al*. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biology of blood and marrow transplantation. 2005; 11 (12): 945–56.

18. Kondo M, Kojima S, Horibe K, Kato K, Matsuyama T. Graft-versus-host disease Risk factors for chronic graft-versus-host disease after allogeneic stem cell transplantation in children. Bone Marrow Transplant. 2001 Apr; 27 (7): 727-30.

19. Langrish CL, Buddle JC, Thrasher a J, Goldblatt D. Neonatal dendritic cells are intrinsically biased against Th-1 immune responses. Clinical and experimental immunology. 2002; 128 (1): 118–23.

20. Tasker L, Marshall-Clarke S. Functional responses of human neonatal B lymphocytes to antigen receptor cross-linking and CpG DNA. Clin Exp Immunol. 2003 Dec; 134 (3): 409-19.

21. Mathers W, Lane J, Zimmerman M. Tear film changes associated with normal aging. Cornea. 1996 May; 15 (3): 229-34.



Chapter 8

Absence of intraocular infections after hematopoietic stem cell transplantation at a single center: the experience with current preventive regimens

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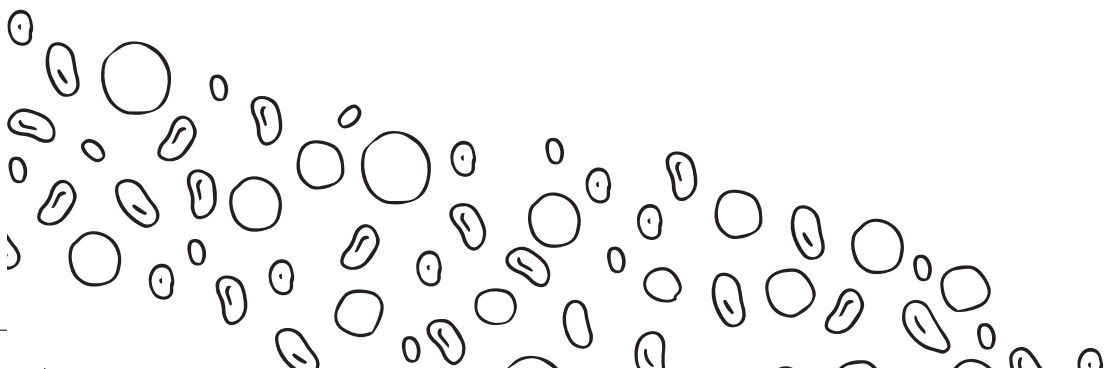


Abstract

Purpose

To investigate the prevalence of intraocular infections after allogeneic stem cell transplantation (allo-SCT).

Methods

The study design was a single institutional retrospective non-comparative cohort of 135 consecutive patients in 2006 and 2007 who underwent allo-SCT for hematological malignancy. The primary outcome was the development of intraocular infections after allo-SCT and secondary outcome consisted of development of other ocular disorders during follow-up.

Results

The most frequent ocular sequel of allo-SCT included ocular graft-versus-host disease (GvHD) which developed in 37/135 patients (27%). Intraocular infection occurred in one of 135 patients (0.7%). This patient developed infectious chorioretinitis together with osteomyelitis, endocarditis and brain abscess with fungus Scedosporium and was successfully treated with a combination of voriconazole, amphotericine B and surgical interventions. Viral and /or bacterial intraocular infections were not observed at all.

Conclusions

Intraocular infections after allo-SCT are currently uncommon due to systematic use of current pre-emptive treatment regimens, frequent controls and early treatment of systemic infections.

Introduction

Severe sight threatening ocular infections can occur after allogeneic stem cell transplantation (allo-SCT)[1]. The use of conditioning regimens and immunosuppressive drugs improves the overall success rate and increases the chance of survival after allo-SCT, but also increases the risk of developing bacterial, viral and fungal infections[2,3]. After allo-SCT, all patients receive preventive antibiotic treatment during the period of immune insufficiency[4] and are frequently assessed for systemic infections and/or re-activations. There is a lack of available data on the prevalence of ocular infections after allo-SCT in patients who received current prophylactic regimens for prevention of infections. The objective of the present study is to report on the up-to-date prevalence of intraocular infections after SCT in adult population in terms of the efficacy of the tailored prophylactic regimen.

Subjects and Methods

This study was approved by the institutional review board and is in compliance with the Declaration of Helsinki.

Patients

In this retrospective cohort study all adult patients who received allo-SCT between January 2006 and December 2007 at the university medical centre in Utrecht (UMCU), the Netherlands, were included. All patients received ophthalmic examination as part of an active screening protocol starting 3 months post-SCT or earlier in case of ocular complaints or increased risk of developing an intraocular infection in case of systemic infection. The examination consisted of registration of ocular and medical history, evaluation of current eye complaints, visual acuity test, slit-lamp examination with additional fluorescein staining, intra-ocular pressure measurement, followed by Schirmer test with local anesthetic and dilated fundus examination.

Medical data

Data collection included demographic characteristics; cytomegalovirus (CMV) and Epstein-Barr virus (EBV) serologic status of donors and recipients; type of immunosuppressive therapy; type of post-SCT prophylaxis; onset, type and treatment of systemic and intraocular infections; visual acuity (VA) during and if applicable after (intra)ocular infection; additional ocular complications and the presence of systemic and ocular GvHD. If multiple SCTs were performed, the follow-up time was considered the time between the last allo-SCT and the last medical assessment at the UMCU.

Subtype of allo-SCT	Immunosuppressive treatment	Durations (days)
NMA MUD	1. cyclosporine 4.5 mg/kg 2/day p.o.	1. D-3 to D+180[a]
	2. mycophenolate 15 mg/kg 3/day p.o.	2. D0 to D+84[b]
NMA Matched Sibling	1. cyclosporine 6.25 mg/kg 2/day p.o.	1. D-3 to D+180[a]
	2. mycophenolate 15 mg/kg 3/day p.o.	2. D0 to D+84[b]
MA MUD and MA Matched Sibling	1. cyclosporine 1.5 mg/kg/24hours 2/day i.v.	1. D-3 to D+20
	cyclosporine 2/day p.o.[c]	D+21 to D+180[a]
	2. mycophenolate 15 mg/kg 3/day i.v.	2. D-0 to D+20
	mycophenolate 15 mg/kg 3/day p.o.[d]	D+21 to D+84[b]

[a] After D+180 10% weekly dose reduction, if until D+120 no GvHD, reduced in 2 weeks
[b] Dose reduction in 2 weeks after D+84
[c] Oral dose adapted according to referential blood values of 0.2-0.4 mg/L.
[d] Maximal dose mycophenolate 1gram 3/day p.o.
D, day; NMA, non-myeloablative; MA, myeloablative; MUD, HLA- matched unrelated donor; p.o., per os; i.v., intravenous.

Table 1.
Immunosuppressive treatment after allogeneic stem cell transplantation (allo-SCT).

Post-transplantation procedure

The post-SCT immunosuppressive protocol is described in Table 1. Furthermore, all patients received co-trimoxazol 480mg qd and valaciclovir 500mg bid for 18 months. Ciproxin 500mg bid p.o. and fluconazole 150 mg qd p.o. were administered during the post-SCT neutropenic state. Additional antiviral (valganciclovir) and antifungal (voriconazol) treatment was used in case of infection/ reactivation with CMV and Aspergillus, respectively.

Results

Subjects

One hundred forty patients were included in the study. Five patients were excluded from the study due to incomplete medical records. The basic characteristics of the study population are described in Table 2. The median follow-up time was 15 months, ranging from one to 74 months. The cause of limited follow-up included mostly death and in one case referral to another hospital for follow-up. All patients had ocular examination three months after allo-SCT, 61/135 (45%) patients were ophthalmic examination repeated at one year follow-up and 74/135 (55%) at two year follow-up. Seventy patients (52%) were alive at the moment of data analysis. The median follow-up time of all patients who have died was 6 months (range 1-50 months) in comparison to all living patients whose median follow-up time was 51 months (range 2-74 months).

Males, n (%)	82/135 (61%)
Age, median (range)	56.0 (21-73)
Diagnosis, n (%)	
ALL	13/135 (10%)
CLL	9/135 (7%)
AML	35/135 (26%)
CML	9/135 (7%)
NHL	22/135 (16%)
HL	7/135 (5%)
MM	25/135 (19%)
Other	15/135 (11%)
Systemic opportunistic infection/ reactivation, n (%)	59/135 (44%)
One agent	37/135 (27%)
Two or more agents	22/135 (16%)
Follow-up to systemic infection/ reactivation, median months (range)	2.49 (0.03-56.52)
Donor, n (%)	
MUD	77/135 (57%)
Matched Sibling	52/135 (39%)
Other	6/135 (5%)

N, number; ALL, acute lymphatic leukemia; CLL, chronic lymphatic leukemia, AML, acute myeloid leukemia; CML, chronic myeloid leukemia; NHL, non-Hodgkin lymphoma; HL, Hodgkin lymphoma; MM, multiple myeloma; MUD, HLA-matched unrelated donor;

Table 2.
Demographic characteristics of allogeneic stem cell transplantation (allo-SCT) patients.

Opportunistic agent, n (%)	Median in months (minimum - maximum)
CMV reactivation and/or infection, n=36/135 (27%)	1.46 (0.03-56.52)
EBV reactivation and/or infection, n=15/135 (11%)	1.77 (0.49-22.43)
Aspergillus pneumoniae, n=12/135 (9%)	4.46 (0.26-31.44)

CMV, Cytomegalovirus; EBV, Epstein-Barr virus

Table 3.
Median interval between last allo-SCT and systemic reactivation and/or infection(s).

Systemic infectious sequelae

Fifty-nine patients (44%) developed systemic infection(s) or reactivations of which 22 (16%) had simultaneously two or more infectious agents (see Table 2). The median time of development of systemic infection or reactivation since last-SCT was two months (range, 0.03-56.52 months). The most numerous were patients with CMV reactivation occurring in 27% of the patients, followed by EBV reactivation and/or infection and Aspergillus pneumoniae, detected in 11% and 9% respectively. One patient (1%) with CMV reactivation was co-infected with human herpes virus type 6 (HHV6). The median reactivation times are described in Table 3. In 14 patients (10%) the specific origin of infection was either unknown or of a non-opportunistic origin. None of the patients has developed infection with Toxoplasma. One hundred and five patients developed systemic GvHD (78%), of which 45 (33%) had acute GvHD and 60 chronic GvHD (44%). Of the patients with systemic opportunistic reactivations or infections, 84% had concurrent systemic GvHD and 22% had a concurrent ocular GvHD.

Ocular infectious sequelae

Only one patient (0.7%) developed an intraocular infection. The infectious agent was fungus (Scedosporium apiospermum) and this infection developed six months after allo-SCT (see selected case report later in the results section). None of the patients developed bacterial or viral intraocular infections. Anterior segment complications included ocular GvHD in 37/135 (27%) consisting of dry eye syndrome in 22 (16%) and conjunctival involvement in 15/135 (11%).

Two patients developed uveitis, both with negative intraocular fluid analyses for infectious agents. The first case of anterior uveitis (0.7%) developed concurrently with an exacerbation of chronic GvHD and diminished quickly with anti-GvHD therapy and in the other case the patient developed uveitis after radiation therapy. Optic disc edema (ODE) was observed in three patients (2%) and was associated with the Scedosporium apiospermum chorioretinitis in one patient. In the second case ODE was accompanied by perimacular puckering OD and was diagnosed using optical coherence tomography (OCT) and fluorescein angiography (FAG). During pars plana vitrectomy (PPV) no infectious agent was found and it was hypothesized that ODE might be related to cyclosporine medication in this patient. The final VA slightly improved and remained stable at VA 0.4. In the third case, the patient's decreasing VA could not be explained by the ophthalmic examination findings. My means of OCT and FAG, ODE and mild unilateral papillitis were diagnosed. The patient has developed severe systemic GvHD, successfully treated with high dose prednisone p.o. Despite the clinical improvement of optic disc edema, the vision remained very low (VA 0.1). The ODE in this patient was considered idiopathic as we could not identify any cause of ODE by neurologic examination and imaging and the patient did not use cyclosporine medication.

Selected case report: Intraocular infection with Scedosporium apiospermum.

A 59-year-old female suffered from non-Hodgkin lymphoma for which she underwent myeloablative

matched unrelated donor allo-SCT, secondary to total body irradiation and chemotherapy. One year later she was diagnosed with a right elbow abscess and showed osteomyelitis signs, and subsequently developed Scedosporium aspiospermum endocarditis and parieto-occipital abscess in the left hemisphere. Simultaneously, the patient developed painless loss of vision in her right eye and slit-lamp examination revealed normal anterior segment while active chorioretinal lesion was observed during ophthalmoscopy. The Scedosporium infection further progressed into a retinal abscess for which she underwent vitrectomy, lensectomy and retinectomy. The final diagnosis of Scedosporium apiospermum chorioretinitis was confirmed from vitreous cultures and the patient was treated with amfotericine B and voriconazol. Three years later her vision was 0.1 in the affected eye due to an inactive retinal scar.

Discussion

Our study documents solely one case of intraocular infection during a 2-year follow-up of 135 patients after allo-SCT; this infection was caused by fungus Scedosporium apiospermum. Intraocular bacterial and viral infections were not observed, nor was intraocular toxoplasmosis diagnosed. The most frequent ocular sequel of allo-SCT included ocular graft-versus-host disease (GvHD) and developed in 37/135 patients (27%).

Intraocular infections in immunosuppressed patients and their dramatic manifestations may lead to severe visual loss[5]. The prevalence of intraocular infections after solid organ transplants was previously reported to range from 3 up to 15%[2,6,7]. No recent systematic studies are available on the incidence of intraocular infections following allo-SCT. One study reported on a 0.8% incidence of intraocular infections after allo-SCT; however this study focused mainly on ocular GvHD and furthermore its precise follow-up pattern is not clear[5]. CMV infections after allo-SCT represented the most frequent intraocular infection (2.2%), followed by EBV (2%) and Toxoplasma gondii (0.97%)[5,8-12]. Originally, intraocular CMV retinitis after allo-SCT was reported to represent a rare complication with low incidence[13], however Xhaard *et al*. reported that implementation of mismatched donorship in allo-SCT has increased the CMV retinitis incidence more than 10 times[11]. The reported cases also suffered from chronic GvHD, a disease with a drastically increasing incidence as a result of the matched unrelated donorship techniques[14]. Other reports also suggest that there is a higher chance of CMV infection to occur among CMV seronegative recipients from CMV seropositive donors[11].

Up to date, transplantation centers show a great variety of immunosuppressive and antimicrobial regimes in keeping with type, doses and duration of the administered medication resulting in discrepant incidence reports[4,5,15-17]. In our study, we report a single case of disseminated Scedosporium apiospermum infection complicated by endocarditis, brain abscess and chorioretinitis. From a recently conducted literature study such filamentous fungi have been related to poor vision outcomes and low survival rate[18]. Previously, McKelvie *et al.* described two patients with disseminated post-SCT Scedosporium sp. endophthalmitis

and fungemia, nonresponsive to antifungal therapy with amphotericin B and fluconazole that resulted in death[19]. Husain *et al*. conducted a study in which voriconazol was related to a lower mortality rate than amfotericine B or itraconazol[20]. Our patient was successfully treated with a combination of voriconazole, amphotericine B combined with surgical abscess drainage.

Our study reports no intraocular infections due to viruses or bacteria, which is consistent with an earlier report which points out that the major cause of post-transplant systemic infections is due to fungal infections[15]. Although this study was designed in a retrospective fashion, the strict follow-up procedures at the hematology and ophthalmology departments secure a detailed and reliable medical file data collection in regard to complaints registration and detection of intraocular infections. The median follow-up in our study of 15 months allowed an optimal time for intraocular infections development, excluding acute retinal necrosis and progressive outer retinal necrosis, which have been documented to develop in majority of cases later than five years after transplantation[8]. In this study, 19 patients had a post-SCT follow-up time longer than five years and none of them developed these viral intraocular manifestations.

Our findings of lack of intraocular infections are consistent with a previous, but independently conducted study by Westeneng *et al*. in the UMCU[21] with a prospectively kept database, in which the incidence of intraocular infection of unknown origin was 1.1%.

Our results point out when preventive antibacterial and antiviral treatment regimens are combined together with regular controls for reactivations of systemic infections and early treatments they effectively decrease the occurrence of intraocular infections. Although the modern prophylactic protocol is successful in reducing the probability of bacterial and viral infections, the awareness of a possible fungal intraocular infection and its timely recognition are of high importance for visual prognosis of post allo-SCT patients.

References

1. Moss P. Developments in the treatment of post-transplant viral disease. Best Pract Res Clin Haematol. 2001; 14: 777-792.

2. Moon SJ, Mieler WF. Retinal complications of bone marrow and solid organ transplantation. Curr Opin Ophthalmol. 2003; 14: 433-442.

3. Coskuncan NM, Jabs DA, Dunn JP, *et al*. The eye in bone marrow transplantation. VI. Retinal complications. Arch Ophthalmol. 1994; 112: 372-379.

4. Subramanian AK. Antimicrobial prophylaxis regimens following transplantation. Curr Opin Infect Dis. 2011; 24: 344-349.

5. Tabbara KF, Al-Ghamdi A, Al-Mohareb F, *et al*. Ocular findings after allogeneic hematopoietic stem cell transplantation. Ophthalmology. 2009; 116: 1624-1629.

6. Shimmyo A, Miyazaki S, Onoe S, Nojima M, Ihara H, Ikoma F. Ocular complications after renal transplantation. Nihon Ganka Gakkai Zasshi. 1997; 101: 220-226.

7. Fishburne BC, Mitrani AA, Davis JL. Cytomegalovirus retinitis after cardiac transplantation. Am J Ophthalmol. 1998; 125: 104-106.

8. Chung H, Kim KH, Kim JG, Lee SY, Yoon YH. Retinal complications in patients with solid organ or bone marrow transplantations. Transplantation. 2007; 83: 694-699.

9. Cohen J, Gandhi M, Naik P, *et al*. Increased incidence of EBV-related disease following paediatric stem cell transplantation with reduced-intensity conditioning. Br J Haematol. 2005; 129: 229-239.

10. Douglas RS, Goldstein SM, Katowitz JA, *et al*. Orbital presentation of posttransplantation lymphoproliferative disorder: a small case series. Ophthalmology. 2002; 109: 2351-2355.

11. Xhaard A, Robin M, Scieux C, *et al*. Increased incidence of cytomegalovirus retinitis after allogeneic hematopoietic stem cell transplantation. Transplantation. 2007; 83: 80-83.

12. van Esser JW, van der Holt B, Meijer E, *et al*. Epstein-Barr virus (EBV) reactivation is a frequent event after allogeneic stem cell transplantation (SCT) and quantitatively predicts EBV-lymphoproliferative disease following T-cell--depleted SCT. Blood. 2001; 98: 972-978.

13. Crippa F, Holmberg L, Carter RA, *et al*. Infectious complications after autologous CD34-selected peripheral blood stem cell transplantation. Biol Blood Marrow Transplant. 2002; 8: 281-289.

14. Riemens A, Te Boome L, Imhof S, Kuball J, Rothova A. Current insights into ocular graft-versus-host disease. Curr Opin Ophthalmol. 2010.

15. Coskuncan NM, Jabs DA, Dunn JP, *et al*. The eye in bone marrow transplantation. VI. Retinal complications. Arch Ophthalmol. 1994; 112: 372-379.

16. Brinkman K, Debast S, Sauerwein R, Ooyman F, Hiel J, Raemaekers J. Toxoplasma retinitis/encephalitis 9 months after allogeneic bone marrow transplantation. Bone Marrow Transplant. 1998; 21: 635-636.

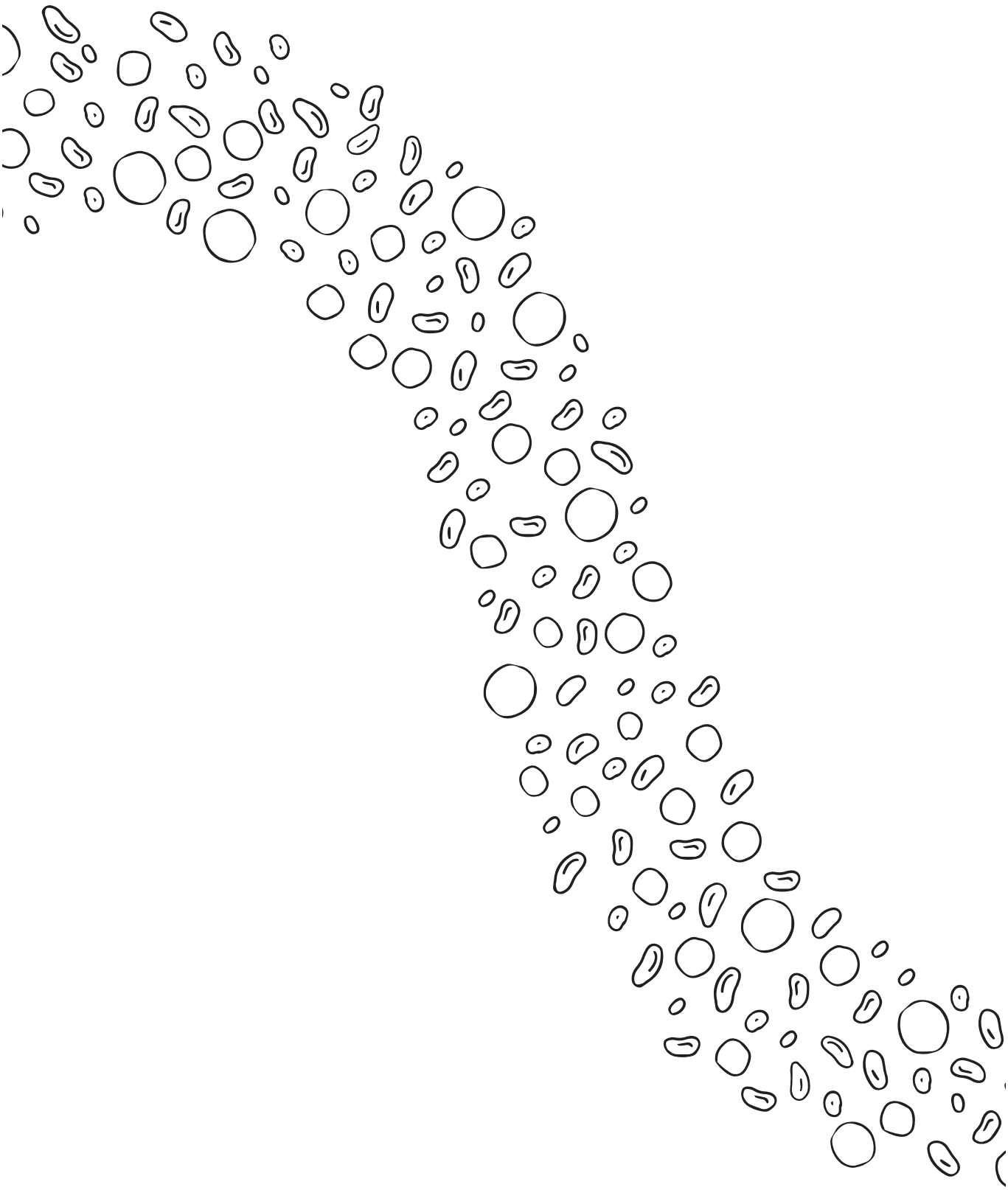
17. Voigt S, Michel D, Kershaw O, *et al*. Fatal reactivation of postnatal cytomegalovirus infection with rapid emergence of ganciclovir resistance in an infant after allogeneic stem cell transplantation. J Clin Microbiol. 2005; 43: 3551-3554.

18. Vergoulidou M, Krause L, Foerster MH, Thiel E, Schwartz S. Endogenous filamentous fungal endophthalmitis--single-centre survey in patients with acute leukaemia or postallogeneic stem cell transplantation and review of the literature. Mycoses. 2011; 54: e704-11.

19. McKelvie PA, Wong EY, Chow LP, Hall AJ. Scedosporium endophthalmitis: two fatal disseminated cases of Scedosporium infection presenting with endophthalmitis. Clin Experiment Ophthalmol. 2001; 29: 330-334.

20. Husain S, Munoz P, Forrest G, *et al*. Infections due to Scedosporium apiospermum and Scedosporium prolificans in transplant recipients: clinical characteristics and impact of antifungal agent therapy on outcome. Clin Infect Dis. 2005; 40: 89-99.

21. Westeneng AC, Hettinga Y, Lokhorst H, Verdonck L, van Dorp S, Rothova A. Ocular graft-versus-host disease after allogeneic stem cell transplantation. Cornea. 2010 Jul; 29 (7): 758-63.



Chapter 9

Cytokines in tear fluid of patients with ocular graft-versus-host disease after allogeneic stem cell transplantation

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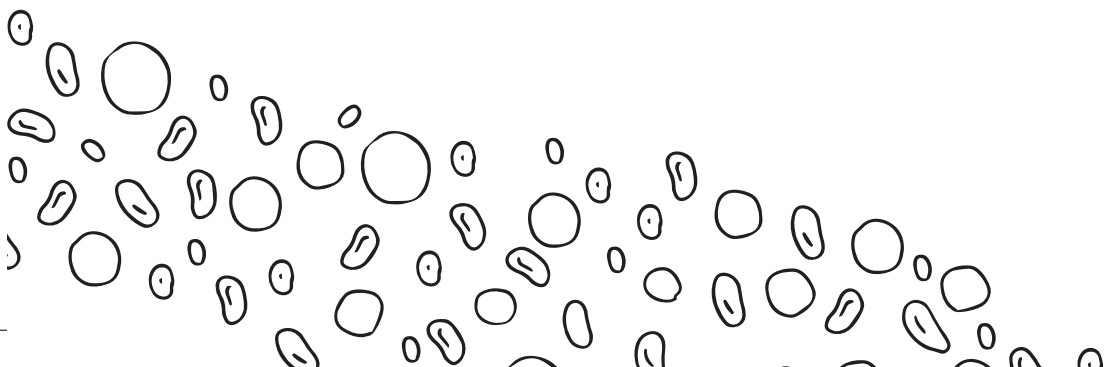


Abstract

Purpose

To investigate the profile of cytokines in tear fluid of patients after allogeneic stem cell transplantation (allo-SCT) and determine their relation to the presence and manifestations of ocular graft-versus-host disease (GvHD).

Methods

In this cross sectional study tear fluid was collected in 34 consecutive adult patients that previously underwent allo-SCT (16 with ocular GvHD and 18 without) and 16 age- and gender-matched healthy controls using Schirmer test under local anaesthesia. Tear fluid was analysed by multiplex immunoassay for the presence of interleukin (IL)-2, IL-4, IL-6, IL-10, IL-17, tumor necrosis factor (TNF)-α and interferon (IFN)-γ. Levels of measured cytokines were correlated with the findings in slit lamp examination and the Ocular Surface Disease Index (OSDI).

Results

The levels of IL-6 and IFN-γ in tear fluid in ocular GvHD patients were significantly elevated in comparison to patients without ocular GvHD and healthy controls ($P<0.005$ for each) The levels of IFN-γ correlated with the Schirmer score ($r = -0.48$. $P < 0.0001$) and tear break up time (TBUT) ($r = -0.38$, $P = 0.03$).Tear IL-6 levels correlated with complaints of dry eyes ($r = 0.39$, $P = 0.02$), tear production ($r = -0.59$, $P < 0.0001$), fluorescent staining of the cornea ($r =0.42$, $P = 0.01$), and with the OSDI score ($r =0.40$, $P = 0.005$).

Conclusion

IL-6 and IFN-γ were elevated in tear fluid of patients with ocular GvHD and correlated with different symptoms of dry eye disease, suggesting that IFN-y is elevated during the early stages and IL-6 is involved in later stages of ocular GVHD and exhibits moreover an association with its severity.

Introduction

Hematopoietic stem cell transplantation (SCT) is the treatment of choice for many life-threatening malignant and non-malignant hematologic diseases. Allogeneic SCT (allo-SCT) is commonly accompanied by graft-versus-host disease (GvHD), a multi-organ systemic disease associated with high morbidity and mortality. Ocular GvHD predominantly affects the anterior ocular segment and manifestations include conjunctival or corneal epithelial changes, Meibomian gland dysfunction and dry eye disease (DED) manifesting as keratoconjunctivitis sicca (KCS)[1,2]. KCS is the most frequently occurring symptom of ocular GvHD and until now its presence is used to diagnose ocular GvHD[3]. The pathogenesis of ocular GvHD has not yet been clarified. Cytokines have been suggested to play a major role in development of systemic and ocular GvHD and increased levels of interleukin (IL)-6 and interferon gamma (IFN-γ) have been observed in serum in patients with systemic acute GvHD[4].

Increased levels of pro-inflammatory cytokines in tear fluid, including interleukin (IL)-6 and IFN-γ were noted in DED of various origins, nonetheless their role in DED is not yet clarified[5,6].

The purpose of this study was to determine the cytokine levels in tear fluid in patients after allo-SCT and to identify their relation to the presence ocular GvHD and clinical symptoms of DED.

Materials and methods

The study was performed in accordance with the Declaration of Helsinki and with the approval of the local Institutional Review Board.

Patients

In this cross-sectional pilot study, we included 34 consecutive allo-SCT patients referred to the Department of Ophthalmology of the University Medical Centre of Utrecht (UMCU). Patients with ocular comorbidity and patients with a mean Schirmer of ≤ 1 mm in 5 minutes were excluded (n=1). Patients consulted our ophthalmology clinic 3 months after SCT for screening or at any other time if they had ocular complaints after SCT. Sixteen gender- and age-matched healthy volunteers with no history of ocular disease, current systemic and ocular infection were included as controls.

Diagnostic criteria

All patients underwent a visual acuity test, slit-lamp examination and a Schirmer test as part of the standard examination for ocular GvHD. The diagnosis of ocular GvHD was based on the National Institute for Health (NIH) consensus criteria, which combines the presence of systemic GvHD manifestations with either a mean Schirmer test value ≤ 5 mm in 5 minutes or newly developed slit-lamp-confirmed KCS with mean score of Schirmer test between 6 – 10 mm in 5 minutes (with local anaesthesia (Oxybuprocain 0.4%))[3].

The diagnosis of KCS with the slit-lamp was made based on the presence of fluorescent staining of the cornea, decreased tear break up time (TBUT) and complaints of dry eyes such as grittiness and blurring of vision. According to NIH consensus criteria (Filipovich *et al*.), blepharitis, Meibomian gland dysfunction and chemosis were not taken into account for the diagnosis of ocular GvHD[3]. Patients and controls completed the Ocular Surface Disease Index (OSDI) questionnaire to determine the subjective ocular symptoms as described previously[7,8].

Tear sample collection

The tear production was determined with a Schirmer test under local anaesthetic drops (Oxybuprocaine HCl, 0.4%) applied 3 minutes prior to applying the strip. The Schirmer strip (Schirmer Tear Test Strips, Biotech Vision Care Pvt.Ltd, Ahmedabad, Gujarat, India) was placed in the lateral lower conjunctival sac and the participants were instructed to close their eyes. After 5 minutes the strips were removed and the tear production recorded in millimetres. Each Schirmer strip was immediately placed in an Eppendorf plastic tube (Sarstedt, Numbrecht, Germany) and diluted 1: 20 with phosphate-buffered saline solution (PBS, pH=7.4). The strips were incubated at 4°C overnight and stored at –80°C until use.

Cytokine Concentration Analysis

The tear fluid of the eye with the lowest Schirmer score of each subject was analyzed. If the values in both eyes were equal, the Schirmer strip of the right eye was chosen for analysis. The levels of IL-2, IL-4, IL-6, IL-10, IL-17A, TNF-α and IFN-γ in tears were measured by multiplex immunoassay (Cytometric Bead Array, BD Biosciences, San Jose, California, USA) according to manufacturer's protocol. Detection limits for IL-2, IL-4, IL-6, IL-10, IL-17A, TNF-α and IFN-γ were 2.6, 4.9, 2.4, 4.5, 18.9, 3.8, and 3.7 pg/mL respectively. Concentrations were calculated from the generated standard curves.

Statistical analysis

Statistical analysis was performed with the SPSS software package (SPSS version 15 for Windows; Chicago, IL, USA). Analysis of variance (ANOVA), Mann-Whitney U test and Fisher's exact test were used to analyse group differences. Correlation between clinical parameters and interleukin levels was determined by Spearman rank test. P-values were adjusted for multiple comparisons using a Bonferroni's correction. A P-value less than 0.05 was regarded statistically significant.

Results

Study population

Study group demographic details are summarized in Table 1; 34 patients after allo-SCT, 16 with ocular GvHD and 18 patients without ocular GvHD were included. The healthy controls (N=16; mean age: 48.0 ±

15.1, male/female ratio: 11/5) were age- and gender- matched to the patients group. The median interval time between the last allo-SCT and clinical examination was 37.2 months (interquartile range 68) for the ocular GvHD group and 3 months (interquartile range 3) for the no ocular GvHD group (P = 0.007).

Diagnostic clinical tests

Of all patients with ocular GvHD 14/16 (88%) had complaints of dry eyes, 6/16 (38%) had a decreased TBUT and in 9/16 (56%) fluorescein staining of the cornea was seen.

The mean Schirmer score was lower in the ocular GvHD group (4.4 ± 2.0 mm) in comparison to the no ocular GvHD group (16.2 ± 7.5 mm, P < 0.0001) and the healthy control group (22.1 ± 7, P < 0.0001). The median OSDI score was significantly impaired in the ocular GvHD compared to the two other study groups (Table 2).

Cytokine tear levels

Results of the tear cytokine levels are shown in Table 2. IL-6 was detected in the tear fluid of 15/34 (44%) allo-SCT patients and in 1/16 (6%) of healthy controls (P < 0.0001). The median level of IL-6 in tear fluid was significantly elevated in the ocular GvHD group compared to the no ocular GvHD group (P = 0.005), and the control group (P < 0.0001). IL-6 levels in patients without ocular GvHD patients and healthy controls did not differ (P = 0.162). IFN-γ was detected in tear fluid in 8/16 (50%) ocular GvHD patients. IFN-γ could not be detected in the other groups. IL-2, IL-4, IL-10, IL-17 and TNF-α could not be detected in any of the samples. The mean age of patients in which IL-6 (53.8±13) or IFN-γ (53.9±12) was detected, was not different from patients in whom no IL-6 (48.5±14) or IFN-γ (43.6±16) could be detected (P = 0.28; P = 0.11).

Correlation between cytokines and clinical manifestations of ocular GVHD

Correlation analysis was performed between tear cytokine levels and symptoms of ocular GvHD for IL-6 and IFN-γ. No significant correlation could be found between the levels of IFN- γ and IL-6 (r = 0.23, P = 0.189).Tear IL-6 levels correlated with complaints of dry eyes (r = 0.39, P = 0.02), tear production (r = -0.59, P < 0.0001), fluorescent staining of the cornea (r = 0.42, P = 0.01), and OSDI score (r = 0.40, P = 0.005). IL-6 did not correlate with TBUT (r = -0.03, P = 0.88). The levels of IFN-γ correlated with the tear production measured by Schirmer test (r = -0.48. P < 0.0001) and TBUT (r = -0.38, P = 0.03). However, IFN-γ did not correlate significantly with complaints of dry eyes (r = 0.12, P = 0.48), fluorescent staining of the cornea (r = 0.10, P =0.57), or OSDI scores (r = 0.16, P = 0.61).

Patient characteristics	Total, allo-SCT Patients (n=34)	Ocular GvHD* (n=16)	No Ocular GvHD (n=18)
Mean age			
(yrs) ± SD	51.5 ± 13.8	50.1 ± 15.8	52.7 ± 12.1
Minimum, maximum	18-71	23-71	18-64
Gender, n (%)			
Male	22/34 (65%)	13/16 (81%)	9/18 (50%)
Female	12/34 (35%)	3/16 (19%)	9/18 (50%)
Follow-up last transplantation, n (%)			
≤ 100 days	15/34 (44%)	3/16 (19%)	12/18 (67%)
> 100 days	19/34 (56%)	13/16 (81%)	6/18 (33%)
Type of transplant, n (%)			
Matched related donor	11/34 (32%)	9/16 (56%)	2/18 (11%)
Matched unrelated donor	23/34 (68%)	7/16 (44%)	16/18 (89%)
TBI, n (%)			
Yes	27/34 (79%)	13/16 (81%)	14/18 (78%)
Unknown	2/34 (6%)	1/16 (6%)	1/18 (6%)
TBI and chemotherapy (ATG or FLU), n (%)			
Yes	27/34 (54%)	13/16 (81%)	14/18 (78%)
Unknown	2/34 (4%)	1/16 (6%)	1/18 (6%)
Type of disorder, n (%)			
Multiple myeloma	10/34 (29%)	5/16 (31%)	5/18 (28%)
AML	9/34 (26%)	1/16 (6%)	8/18 (44%)
NHL	5/34 (15%)	3/16 (19%)	2/18 (11%)
ALL	3/34 (9%)	1/16 (6%)	2/18 (11%)
CLL	1/34 (3%)	1/16 (6%)	0/18 (0%)
HL	1/34 (3%)	1/16 (6%)	0/18 (0%)
CML	1/34 (3%)	1/16 (6%)	0/18 (0%)
Other	4/34 (12%)	3/16 (19%)	1/18 (6%)

n, Number of patients; ATG, Anti-thymocyte globulin; ALL, Acute lymphblastic leukemia; Allo-SCT, allogeneic stem cel transplantation; AML, Acute myeloblastic leukaemia; CLL, Chronic lymphocytic leukaemia; CML, Chronic myelcytic leukemia; FLU, Fludarabine; GvHD, Graft-verus-Host-Disease; HL, Hodgkin's lymphoma; NHL, non-Hodgkin's lymphoma; SD, standard deviation; TBI, Total body irradiation.

* diagnosed according to the National Institute for Health consensus criteria[3]

Table 1.
General characteristics of patients after allogeneic stem cell transplantation (allo-SCT)

Inflammatory cytokines and ocular findings	Allo-SCT Patients (N=34)	Ocular GvHD Patients (N=16)	No Ocular GvHD Patients (N=18)	Healthy Controls (N=16)	P-value, Ocular GvHD vs. No Ocular GvHD	P-value, Ocular GvHD vs. Healthy Controls	P-value, No Ocular GvHD vs. Healthy Controls
IL-6, pg/mL, n (%)	15/34 (44%)	11/16 (69%)	4/18 (22%)	1/16 (6 %)	P =0.005	P<0.0001	NS
Median, iq range	0, 130.6	107.4, 417.1	0, 7.9	0, 0			
IFN-γ, pg/mL, n (%)	8/34 (23%)	8/16 (50%)	0/18 (0%)	0/16 (0%)	P =0.001	P=0.002	NA
Median, iq range	0, 0	572, 1372.9	0, 0				
Mean Schirmer ± SD, mm	10.7 ± 8.2 mm	4.4 ± 2.0 mm	16.2 ± 7.5 mm	22.1 ± 7 mm	P<0.0001	P<0.0001	P=0.021
Median OSDI score, iq range	11.1, 14.0	13.9, 21.2[1)]	6.0, 27.8[2)]	0, 3.7	P= 0.015	P<0.0001	P=0.026

Allo-SCT, allogeneic stem cell transplantation; n, Number of patients; GvHD, Graft-versus-Host-Disease; IFN- γ, interferon- γ; IL-6, interleukin-6; Iq range, Interquartile range; OSDI, Ocular Surface Disease Index; NS= Not statistically significant; NA, Not Applicable; SD, Standard Deviation; [1)] Data from 1 patient is missing; [2)] Data from 2 patients is missing

Table 2.
Inflammatory cytokines in tear fluid and ocular findings after allo-ACT

Discussion

In this study, significantly elevated IL-6 and IFN-γ levels in tear fluid in patients with ocular GvHD were detected. These cytokines were associated with different clinical features: IL-6 with complaints of dry eyes, OSDI scores and corneal staining whereas IFN- γ showed an association with decreased TBUT and low Schirmer test score. These results might demonstrate that IFN-γ is elevated in the early stage of ocular GvHD and that IL-6 is elevated later in the disease and is associated with the severity of ocular GvHD.

The measurements of tear production using Schirmer test might reveal variable results[9]. When applying the strips without previous anaesthesia, the results are a subject to variability due to reflex tearing and when using prior anaesthesia, the residual amount of topical anaesthetics might influence the Schirmer values. To minimize these variable measurements, we have chosen to apply anaesthetic drops to measure a basal tear production and further included a standard interval between the application of anaesthetic drops and Schirmer test to minimize the influence of their possible residual presence.

IFN-γ is a T-cell associated cytokine that plays a major role in the pathogenesis of GvHD[10]. IL-6 is known to contribute to GvHD severity[11] but has also been reported to be correlated with disease onset[12].

The current knowledge about the involvement of interleukins in ocular GvHD is very limited. The conjunctiva is known to be one of the target organs in the T-cell mediated GvHD, resembling complex autoimmune fibrotic processes[13-15].

Ogawa *et al*. reported that donor-derived Cluster of Differentiation 34+ stromal fibroblasts were present in lacrimal gland of DED patients after allo-SCT[13]. Subsets of stromal fibroblasts can function as antigen presenting cells and activate donor T-cells, which may result in the formation of pro-inflammatory cytokines like IL-6 and IFN-γ[13].

Elevated levels of IL-6 and IFN-γ in tears were previously found and were associated with DED seen in different aetiologies, including Sjorgren's disease[5,16,17]. IFN-γ is an crucial cytokine in the induction of GvHD[4]. Recently, a central role for IFN-γ-producing natural killer cells in the induction of DED was demonstrated by Chen *et al*[18], which is entirely consistent with our findings of association of IFN-γ with tear production and TBUT but not being associated with the overall surface damage.

The measured tear IL-6 concentrations in our study correlate with the subjective complaints tear production and corneal damage, suggesting that the increased IL-6 in tear fluid may be a result of the progressing DED. Lam *et al*. concluded that IL-6 correlated with the severity of symptoms and signs of tear dysfunctional syndrome, which is consistent with our findings[19]. The exact source of IL-6 production in patients with DED is not known; however IL-6 can be produced by many cells including monocytes, macrophages and fibroblasts[20]. IL-6 is a potent cytokine and mediator in local and systemic inflammation and has effect on B cells and T cells[20-22]. IL-6 can also promote the differentiation of Th17 T cells, important regulators of autoimmune responses, which occur in DED and acute GvHD[21,23,24]. Much of our understanding of IL-6 and Th17 responses in GvHD come from mice models. Th17 cells, with the characteristic production of IL-17, can mediate pathology associated with GvHD in mice[24]. In multiple mouse strains blocking of IL-6 signaling dramatically attenuates GvHD and Th17 responses[11,25]. However Th17 responses may not be prerequisite for the development of GvHD[26]. It was suggested that posttransplantational epigenetic modifications of donor Th17 cells, which alter their cytokine production, lead to loss of IL-17 and increase in IFN-γ producing cells[24]. In contrast to the mouse models, blocking of the IL-6 receptor in humans does not alter dendritic cell maturation, allogeneic T cell proliferation, or Th1/Th17 responses[27]. The role of IL-6 in ocular GvHD in humans may be beyond Th17 promoting and differ between mice models and human disease. Although a recent study with patients with dry eyes in aqueous-deficient patients suggested that elevated IL-6 levels in tear fluid had to be considered as a Th17 promoting response, the levels of IL-17 were not assessed[16]. We detect increased levels of IL-6 and IFN-γ in tear fluid of ocular GvHD, but were unable to detect IL-17 in any of the samples. It has been observed that IL-6 also promotes the differentiation of B cells and the induction of antibody production[28]. B cells play an important role in the development of GvHD which accounts for the effectiveness of B-cell depletion in the treatment of GVHD[29]. The role of IL-6 and the involvement of Th17 responses in human ocular GvHD remains to be elucidated.

In our study the group with ocular GvHD had a longer time interval between the measurements of cytokines than the group without ocular GvHD. This time difference can be explained by the fact that patients without ocular GvHD visited our OPD three months after SCT for screening purposes while patients with ocular GvHD visited our OPD for their treatment of ocular GvHD. However this time difference has no impact on the main message of this study. Confounders such as treatment with radiotherapy and chemotherapy and

other medication were not taken in account due to the small number of patients.

Ocular GvHD is a serious ocular disorder significantly affecting the quality of life of patients following allo-SCT. The pathogenesis is unknown and the current therapy includes symptomatic regimens with lubricant and anti-inflammatory medications. In order to develop preventive and/or curative treatments the further clarification of pathogenesis of this disorder is necessary. In addition, ocular GvHD offers a unique opportunity to study the early onset of DED since it develops in many individuals after allo-SCT and the moment of onset is predictable. In contrast to former studies of DED, tear fluid analyses in ocular GvHD offer a unique opportunity for studying the pathogenesis of DED by determination of specific inflammatory mediators in diverse phases of this disorder. Giving the cross-sectional nature of this pilot study and its results, future prospective studies can concentrate on the changes occurring in tear fluid over time, before and after allo-SCT (in the preclinical stages of ocular GvHD and before the onset of DED) in order to identify the initial stages of ocular GvHD and determine the value of specific cytokines in the initiation and continuation of DED in ocular GvHD.

The results of this small study seem promising and show that IL-6 and IFN-γ were elevated in tear fluid of patients with ocular GvHD and correlated with different symptoms of dry eye disease.

References

1. Khanal S, Tomlinson A (2012) Tear physiology in dry eye associated with chronic GVHD. Bone Marrow Transplant 47 (1): 115-9.

2. Ban Y, Ogawa Y, Ibrahim OM, Tatematsu Y, Kamoi M, Uchino M, Yaguchi S, Dogru M, Tsubota K (2011) Morphologic evaluation of meibomian glands in chronic graft-versus-host disease using in vivo laser confocal microscopy. Mol Vis 17: 2533-43.

3. Filipovich AH, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ, Martin P, Chien J, Przepiorka D, Couriel D, Cowen EW, Dinndorf P, Farrell A, Hartzman R, Henslee-Downey J, Jacobsohn D, McDonald G, Mittleman B, Rizzo JD, Robinson M, Schubert M, Schultz K, Shulman H, Turner M, Vogelsang G, Flowers MED (2005) National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant 11 (12): 945-56.

4. Toubai T, Tanaka J, Paczesny S, Shono Y, Reddy P, Imamura M (2011) Role of cytokines in the pathophysiology of acute graft-versus-host disease (GVHD)- Are serum/plasma cytokines potential biomarkers for diagnosis of acute GVHD following allogeneic hematopoietic cell transplantation (allo-HCT)? Curr Stem Cell Res Ther. In press 2012.

5. Massingale ML, Li X, Vallabhajosyula M, Chen D, Wei Y, Asbell PA (2009) Analysis of inflammatory cytokines in the tears of dry eye patients. Cornea 28 (9): 1023-7.

6. Yoon KC, Jeong IY, Park YG, Yang SY (2007) Interleukin-6 and tumor necrosis factor-alpha levels in tears of patients with dry eye syndrome. Cornea 26 (4): 431-7.

7. Ozcura F, Aydin S, Helvaci MR (2007) Ocular surface disease index for the diagnosis of dry eye syndrome. Ocul Immunol Inflamm 15 (5): 389-93.

8. Schiffman RM, Christianson MD, Jacobsen G, Hirsch JD, Reis BL (2000) Reliability and validity of the Ocular Surface Disease Index. Arch Ophthalmol 118 (5): 615-21.

9. Nichols KK, Mitchell GL, Zadnik K (2004) The repeatability of clinical measurements of dry eye. Cornea 23 (3): 272-85.

10. Blazar BR, Taylor PA, Panoskaltsis-Mortari A, Narula SK, Smith SR, Roncarolo MG, Vallera DA (1998) Interleukin-10 dose-dependent regulation of CD4+ and CD8+ T cell-mediated graft-versus-host disease. Transplantation 66 (9): 1220-9.

11. Tawara I, Koyama M, Liu C, Toubai T, Thomas D, Evers R, Chockley P, Nieves E, Sun Y, Lowler KP, Malter C, Nishimoto N, Hill GR, Reddy P (2011) Interleukin-6

modulates graft-versus-host responses after experimental allogeneic bone marrow transplantation. Clin Cancer Res 17 (1): 77-88.

12. Coghill JM, Sarantopoulos S, Moran TP, Murphy WJ, Blazar BR, Serody JS (2011) Effector CD4+ T cells, the cytokines they generate, and GVHD: something old and something new. Blood 117 (12): 3268-76.

13. Ogawa Y, Kuwana M (2003) Dry eye as a major complication associated with chronic graft-versus-host disease after hematopoietic stem cell transplantation. Cornea 22 (7 Suppl): S19-S27.

14. Riemens A, te BL, Imhof S, Kuball J, Rothova A (2010) Current insights into ocular graft-versus-host disease. Curr Opin Ophthalmol 21 (6): 485-94.

15. Rojas B, Cuhna R, Zafirakis P, Ramirez JM, Lizan-garciia M, Zhao T, Foster CS (2005) Cell populations and adhesion molecules expression in conjunctiva before and after bone marrow transplantation. Exp Eye Res 81 (3): 313-25.

16. Boehm N, Riechardt AI, Wiegand M, Pfeiffer N, Grus FH (2011) Proinflammatory cytokine profiling of tears from dry eye patients by means of antibody microarrays. Invest Ophthalmol Vis Sci 52 (10): 7725-30.

17. 17. Li S, Sack R, Vijmasi T, Sathe S, Beaton A, Quigley D, Gallup M, McNamara N (2008) Antibody protein array analysis of the tear film cytokines. Optom Vis Sci 85 (8): 653-60.

18. Chen Y, Chauhan SK, Saban DR, Sadrai Z, Okanobo A, Dana R (2011) Interferon gamma-secreting NK cells promote induction of dry eye disease. J Leukoc Biol 89 (6): 965-72.

19. Lam H, Bleiden L, de Paiva CS, Farley W, Stern ME, Pflugfelder SC (2009) Tear cytokine profiles in dysfunctional tear syndrome. Am J Ophthalmol 147 (2): 198-205.

20. Kishimoto T (1992) Interleukin-6 and its receptor in autoimmunity. J Autoimmun 5 Suppl A: 123-32.

21. Miossec P, Korn T, Kuchroo VK (2009) Interleukin-17 and type 17 helper T cells. N Engl J Med 361 (9): 888-98.

22. Min CK, Lee WY, Min DJ, Lee DG, Kim YJ, Park YH, Kim H J, Lee S, Kim DW, Lee JW, Min WS, Kim CC (2001) The kinetics of circulating cytokines including IL-6, TNF-alpha, IL-8 and IL-10 following allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant 28 (10): 935-40.|

23. de Paiva CS, Chotikavanich S, Pangelinan SB, Pitcher JD, III, Fang B, Zheng X, Ma P, Farley WJ, Siemasko KF, Niederkorn JY, Stern ME, Li DQ, Pflugfelder SC (2009) IL-17 disrupts corneal barrier following desiccating stress. Mucosal Immunol 2 (3): 243-53.

24. Serody JS, Hill GR (2012) The IL-17 Differentiation Pathway and its Role in Transplant Outcome. Biol Blood Marrow Transplant 18 (1 Suppl): S56-S61.

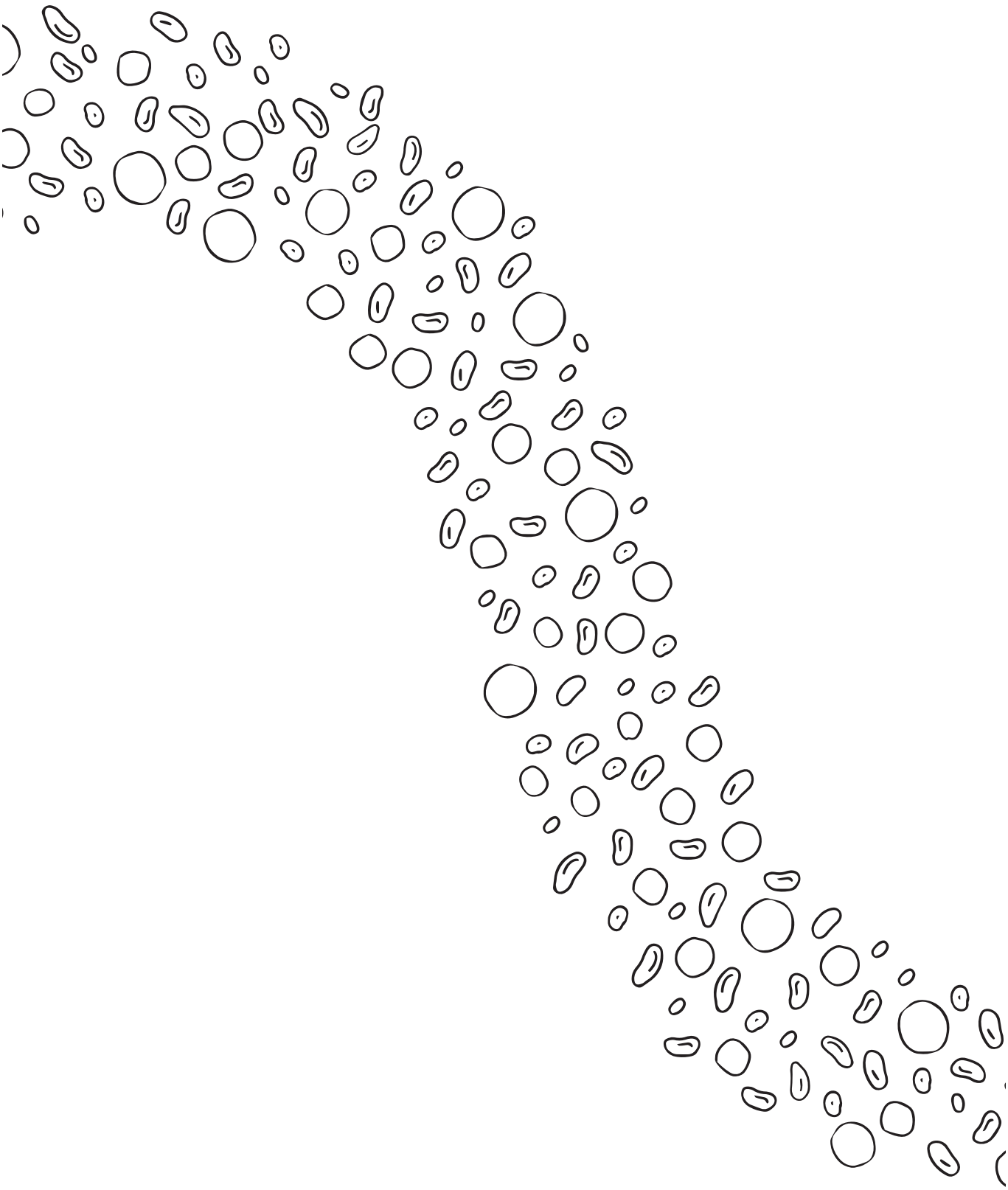
25. Chen X, Das R, Komorowski R, Beres A, Hessner MJ, Mihara M, Drobyski WR (2009) Blockade of interleukin-6 signaling augments regulatory T-cell reconstitution and attenuates the severity of graft-versus-host disease. Blood 114 (4): 891-900.

26. Broady R, Yu J, Chow V, Tantiworawit A, Kang C, Berg K, Martinka M, Ghoreishi M, Dutz J, Levings MK (2010) Cutaneous GVHD is associated with the expansion of tissue-localized Th1 and not Th17 cells. Blood 116 (25): 5748-51.

27. Betts BC, St Angelo ET, Kennedy M, Young JW (2011) Anti-IL6-receptor-alpha (tocilizumab) does not inhibit human monocyte-derived dendritic cell maturation or alloreactive T-cell responses. Blood 118 (19): 5340-3.

28. Hirano T, Yasukawa K, Harada H, Taga T, Watanabe Y, Matsuda T, Kashiwamura S, Nakajima K, Koyama K, Iwamatsu A, Tsunasawa S, Sakiyama F, Matsui H, Takahara Y, Taniguchi T, Kishimoto T (1986) Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. Nature 324 (6092): 73-6.

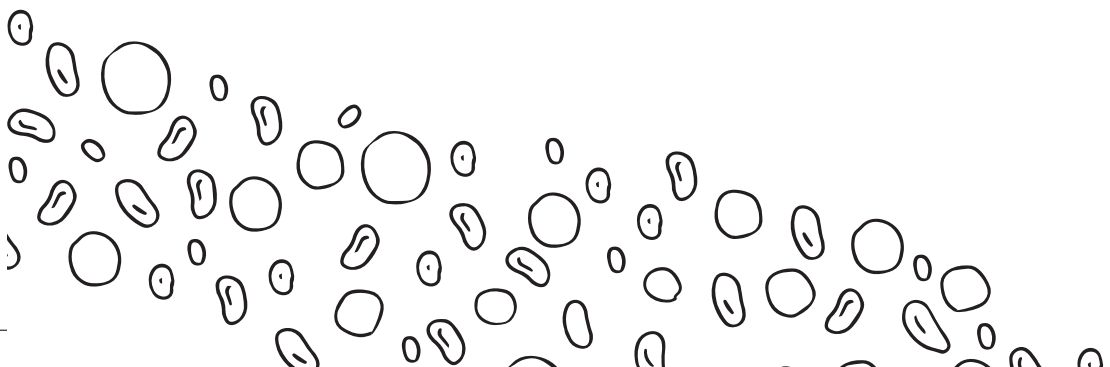
29. Shimabukuro-Vornhagen A, Hallek MJ, Storb RF, von Bergwelt-Baildon MS (2009) The role of B cells in the pathogenesis of graft-versus-host disease. Blood 114 (24): 4919-27.



Chapter 10

Modification of ocular graft-versus-host disease development by early local Cyclosporin A application in patients after donor stem cell transplantation: a one year evaluation of a randomized controlled single masked trial

Anjo Riemens, Liane C.J. te Boome, Saskia M. Imhof, Henk M. Lokhorst, Aniki Rothova



Introduction

Allogeneic haematopoietic (allo-SCT) is a commonly used treatment option for haematological malignancies and some non-malignant disorders. The most common complication of allo-SCT is graft-versus-host-disease (GvHD), which is the major cause of morbidity and mortality in patients after allo-SCT. GvHD is a T-cell mediated process in which host tissue is attacked and destroyed by donor cells. Every organ can be affected by GVHD, in the acute phase mostly skin gut and liver are affected and in the chronic phase skin, mouth,eye, liver, lung, joints, gastrointestinal and genital tract. Involvement of the eye occurs in 40–60% of the patients[1-5]. After allo-SCT, all patients are treated systemically with immunosuppressive agents to obviate GvHD. Chronic systemic GVHD frequently develops in about three to six months after transplantation during tapering or discontinuation of systemic immunosuppression, but might develop up to three years after transplantation.

Chronic ocular GVHD can cause severe ocular surface disease (OSD) including keratoconjunctivitis sicca (KCS), conjunctival inflammation, meibomian gland dysfunction, pseudomembranous and cicatricial conjuncitivitis[1-7]. KCS is the most common manifestation of ocular GVHD and usually develops together with inflammatory signs of the conjunctiva (conjunctival edema, chemosis, pseudomembrane formation) and chronic blepharitis[1]. This process may lead to secondary corneal changes such as punctate keratopathy, the formation of filaments, painful erosions and secondary corneal infections and is sometimes even associated with ulceration and perforation of the cornea. The dysfunction of the Meibomian glands with subsequent atrophy contributes to a poor quality of the tear film.

Ocular GvHD can severely impair the visual quality of life (QoL) and decrease the activities of daily living[8]. Due to new and improved transplantation techniques the survival after allo-SCT has greatly improved. In consequence more patients suffer, even on the long-term follow-up, from incapacitating ocular GvHD.

There is no satisfactory treatment option for chronic ocular GVHD. The standard systemic therapy for chronic systemic GVHD is commonly not sufficient for the treatment of ocular GvHD. The baseline topical treatment usually consists of lubricants, topical steroids, autologous serum drops and sometimes punctual plug occlusion[1]. Most treatment options only alleviate symptoms of ocular GvHD whereas local anti-inflammatory medications, of which topical corticosteroids or Cyclosporin A are most commonly used, can modify the graft versus host reaction. Topical corticosteroids are known to have significant side effects including cataract formation and raised intraocular pressure.

One of the reasons for the limited effect of treatment for ocular GVHD may be that the therapy is usually initiated at a late stage of the disorder, with permanent tissue damage already present. Since administration of topical Cyclosporin A can modify the Graft-versus-Host reaction and has little side effects when administered topically, this agent could have the potential to prevent damage caused by GvHD when administered in an early stage of the disease.

In this prospective randomised study we investigate the potential prevention of OSD in ocular GVHD in patients who have undergone allo-SCT by administration of topical Cyclosporin A application compared to application of tear drops.

Methods

This is a prospective randomized single masked study which is conducted at the University Medical Center Utrecht. The study was approved by the research committee of our centre and by the competent authority (CCMO: Central Committee on Research involving Human Subjects).

The study design

The main parameters of our study were determined at every visit to the ophthalmology department: visual acuity, slit lamp examination of the anterior segment, fluorescein staining, tear break up time (TBUT) and the Schirmer's test. Lens and fundus examination (pupillary dilatation 0.5% tropicamide and 5% fenylephrine) were performed at the first visit and subsequently whenever relevant. On each visit the patient filled in the Visual Analog Scale score (severity rating 0-10), Ocular Surface Disease Index (OSDI) and Visual Functioning Questionnaire 25 (VFQ-25). The questionnaires were evaluated using the appropriate guidelines[10-12].

The endpoint of study participation was development of ocular GvHD as described by Filipovich *et al*. According to these criteria, development of low Schirmer test values with a mean value of both eyes≤ 5 mm at five minutes or a new onset of KCS diagnosed by slit-lamp examination with mean values of 6 to 10 mm on the Schirmer's test are sufficient for the diagnosis of ocular GVHD if accompanied by distinctive manifestations in at least one other organ. Conjunctival changes matching ocular GvHD were as well evaluated but not used for the diagnosis of ocular GvHD[9].

Schirmer's test

Three minutes prior to the Schirmer's test topical anaesthetic eye drops (oxybuprocaine 0,4%) 4 mg/ml; 0,4 ml, Thea Pharma) were administered. The Schirmer's test was performed using sterilised filters strips (Dina strip, Schirmer-Plus, Gecis, France) that were placed to the lower temporal eyelid margin. The strips were left in place for five minutes with closed eyes.

TBUT measurement

Fluorescein 2.5 mg/ml, 0.5ml eye drops were administered before TBUT measurement by slit lamp. A TBUT of less than five seconds was considered decreased.

Fluorescein staining

Fluorescein staining was performed using fluorescein 2.5 mg/ml, 0.5ml eye drops and was graded using the Oxford grading Scheme for dry eyes (Figure 1)[13].

Patients

Twenty three patients were included that underwent allo-SCT at the haematology department of our centre

	No. of patients (%)
Acute myeloid leukemia	3 (13%)
Chronic lymphoblastic leukemia	3 (13%)
Multiple myeloma	6 (26%)
Mantle cell lymphoma	3 (13%)
B-cell Non Hodgkin Lymphoma	2 (9%)
Myeloproliferative neoplasms	6 (26%)

Table 1.
Indications for allogeneic stem cell transplantation

	No. of patients (%)
Myeloablative allo-SCT	11/23 (48%)
Fludarabine, Melphalan, (ATG)*	3 (27%)
Fludarabine, Busilvex, Alemtuzumab	1 (9%)
Fludarabine, Busilvex, T-cell and CD-19 depletion	2 (18%)
Fludarabine, Melphalan, Alemtuzumab	5 (45%)
Non-Myeloablative allo SCT	12/23 (52%)
Fludarabine, TBI, (ATG)**	11 (67%)
Fludarabine, Cyclofosfamide, TBI	1 (8%)

ATG: anti-thymocyte globulin; TBI: total body irradiation
*N=1 received ATG in addition to Fludarabine and Melphalan
**N=3 received ATG in addition to Fludarabine and TBI

Table 2.
Conditioning regimens

from May 2012 till August 2013 and completed six months follow-up. Allo-SCT indications are listed in Table 1. The patients received the patient information folder on this study prior to their allo-SCT. Exclusion criteria were a history of Sjögren's syndrome, documented dry eye prior to allo-SCT and a history of or risk factors for non-compliance. After obtaining written informed consent, the patients were scheduled for three ocular examinations, before allo-SCT, three months and six months after allo-SCT. All examinations were performed by masked investigators. Between separate visits, phone consultations were made to check on use of medication and possible side effects. Baseline characteristics included age, gender, haematological diagnosis, type of allo-SCT, conditioning regimen, immunosuppressive therapy and development of acute

and/or chronic GvHD. In addition, ocular medical history and possible co-morbidity were recorded.
Myeloablative and non-myeloablative conditioning regimes and T-cell depleted and repleted grafts were given (Table 2). In matched unrelated donors anti-thymocyte globulin was added to the conditioning regimen.

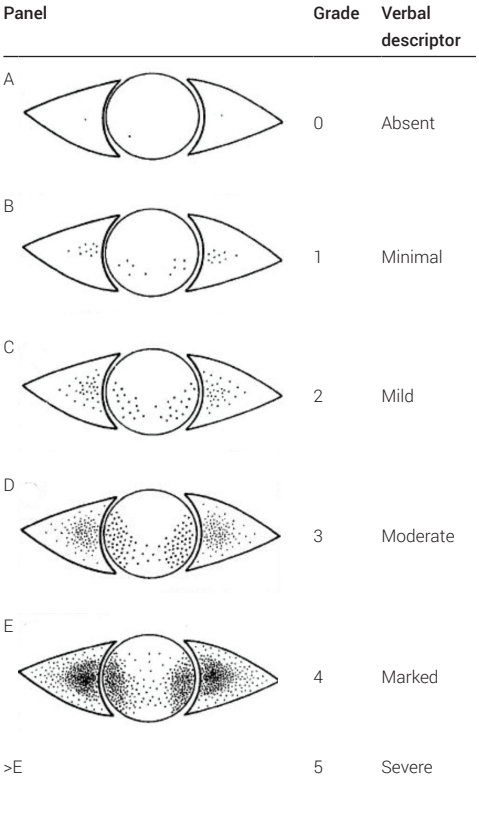


Figure 1.
Oxford grading scheme for fluorescein staining in dry eyes

Treatment

All patients started therapy with eye drops they were randomised to: either Cyclosporin A 0.05% (Restasis, Allergan) or Polyvidone 50 mg/ml (Oculotect unidose, Novartis Pharma B.V.) two times a day in each eye starting one month after transplantation till six months after transplantation (overall medication duration of five months).

Statistical analysis

The statistical calculations were done with SPSS statistics. The Pearson chi-square test was used for data on incidence and prevalence of ocular GVHD between different treatment groups. Differences in the results of diverse functional tests between the two study groups and the results of the questionnaires were statistically analysed using the Independent sample t-test, Fisher's exact test, Pearson chi-square test and the Mann-Whitney U test. A p-value of ≤0.05 was considered significant.

Results

General characteristics

The patient demographic characteristics are summarized in Table 3. Twenty-three patients were included in the study of which eleven patients were randomized in the Polyvidone group and 12 patients in the Cyclosporin A treatment group. The mean age was 57 years (SD 11.3, range 23-73) and not significantly different between both treatment groups (p=0.6). More male than female patients were included (70% vs. 30%) with no significant difference between both groups (p=1.0).
Systemic manifestations of acute GvHD developed in 8/23 (35%) of patients and affected organs included

	All patients N=23	Polyvidone group N=11	Cyclosporin A group N=12	P-value
Mean age, SD, range	57, 11.3, 23-73	55, 14.7, 23-73	58, 7.5, 44-69	P=0.6*
Male gender, N (%)	16 (70%)	8 (73%)	8 (67%)	P=1.0**
Diagnosis, N (%)				
Multiple Myeloma	6 (26%)	2 (18%)	4 (33%)	P=0.7**
AML	3 (13%)	2 (18%)	1 (8%)	
CLL	3 (13%)	2 (18%)	1 (8%)	
Mantle Cell Lymphoma	3 (13%)	2 (18%)	1 (8%)	
B-cell non-Hodgkin lymphoma	2 (9%)	2 (18%)	-	
Myeloproliferative neoplasms	6 (26%)	1 (9%)	5 (42%)	
Type of transplantation, N (%)				
Unrelated donor	13 (57%)	7 (64%)	6 (50%)	P=0.8**
Related donor	8 (35%)	3 (27%)	5 (42%)	
Cord Blood	2 (9%)	1 (9%)	1 (8%)	
Conditioning regimen, N (%)				
TBI based	11 (48%)	8 (73%)	3 (25%)	P=0.02***
Chemotherapy based	12 (52%)	3 (27%)	9 (75%)	
GvHD, N (%)				
Acute	6 (26%)	2 (18%)	4 (33%)	P=0.8**
Chronic	12 (52%)	7 (64%)	5 (42%)	
Both	2 (9%)	1 (9%)	1 (8%)	
No GvHD	3 (13%)	1 (9%)	2 (17%)	
Death, N (%)	4 (17%)	2 (29%)	2 (22%)	P=1.0**

SD: standard deviation; AML: Acute Myeloid Leukemia; CLL: Chronic Lymphoid Leukemia; ALL: Acute Lymphoid Leukemia; TBI: Total Body Irradiation; GvHD: Graft-versus-Host Disease.
*Independent Sample t-Test; **Fisher's Exact Test; ***Pearson Chi-Square.

Table 3.
Baseline characteristics

the gastrointestinal tract, the hands and skin. Chronic GvHD developed in 14/23 (61%) of patients and included the gastrointestinal tract, hands, skin, mouth and liver. Two of these patients developed systemic manifestations of both acute and chronic GvHD. Three/23 (13%) patients did not develop any systemic signs of GvHD. Acute GvHD was severe in two patients, of which one patient died because of acute GvHD and the second patient died because of subsequent severe chronic GvHD. Systemic manifestations of chronic GvHD were severe in four patients of which one patient died because of GvHD. In the remaining

	Polyvidone group N= 22 eyes	Cyclosporin A group N = 26 eyes	P-value
Snellen visual acuity; median (IQR)			
before allo-SCT	1.0 (IQR 0.3)	1.0 (IQR 0.1)	P=0.06
3 months after allo-SCT	1.2 (IQR 0.4)	1.0 (IQR 0.1)	P=0.2
6 months after allo-SCT	0.8 (IQR 0.7)	1.0 (IQR 0.4)	P=0.5
Schirmer's test; mean (SD, range)			
before allo-SCT	15.5 mm (10.2, 3- 30)	8.1 mm (5.1, 3-20)	P=0.3
3 months after allo-SCT	8.7 mm (8.3,0-30)	11.5 mm (9.1, 0-30)	P=0.7
6 months after allo-SCT	14.8 mm (13.5, 0-30 mm)	16.9 mm (11.7, 2-30)	P=0.02
Presence of corneal fluorescein staining*, N (%)			
before allo-SCT	1 (5%)	5 (19%)	P=0.2
3 months after allo-SCT	0 (0%)	1 (4%)	P=1.0
6 months after allo-SCT	0 (0%)	6 (23%)	P=0.13
Presence of conjunctival chemosis, N (%)			
before allo-SCT	8 (44%)	6 (60%)	P=0.8
3 months after allo-SCT	8 (36%)	10 (42%)	P=0.7
6 months after allo-SCT	9 (75%)	12 (67%)	P=0.7

Allo-SCT; allogeneic stem cell transplantation, IQR; interquartile range, SD; standard deviation
* corneal fluorescein staining was Grade I or II according to the Oxford scheme in all patients with presence of corneal staining.

Table 4.
Ocular findings before and after allogeneic stem cell transplantation

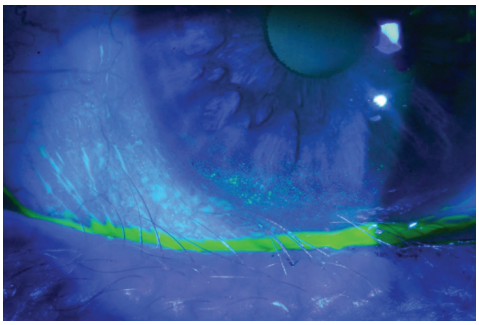


Figure 2.
Corneal fluorescein staining and trichiasis in patient with ocular Graft-versus-Host Disease

ten patients systemic manifestations of chronic GvHD were mild. Manifestations of acute and/or chronic systemic GvHD did not significantly differ between the two treatment groups (p=0.8).

The conditioning regimen was TBI based in 11/23 (48%) of patients, 8/23 in the Polyvidone group and 3/23 in the Cyclosporin A treatment group which was a significant difference (P=0.02). Of all 23 patients four (17%) died during follow-up, two in each treatment group.

Seven out of 23 patients did not finish the six months follow-up. Of these seven patients two died during follow-up, two had relapse of their disease and were too ill for ophthalmic examination at six months, two patients decided to stop study participation because of psychological stress and one additional patient had to withdraw because of difficulties with administering the eye drops because of tremor of the hands. One of the patients that withdrew had graft failure and was diagnosed with KCS at three months of follow-up which was treated with tear substitutes. Of these seven patients, two were randomised in the Cyclosporin A treatment and five in the Polyvidone treatment group.

	Polyvidone before allo-SCT	Cyclosporin A before allo-SCT	P-value	Polyvidone 3 months after allo-SCT	Cyclospo-rin A 3 months after allo-SCT	P-value	Polyvidone 6 months after allo-SCT	Cyclospo-rin A 6 months after allo-SCT	P-value
VFQ-25 Median (IQR)	96.5 (IQR 2.7)	96.0 (IQR 3.4)	P=1.0	96.7 (IQR 8.8)	94.8 (IQR 5.5)	P=0.4	96.1 (IQR 4.1)	97.7 (IQR 5.8)	P=0.6
OSDI Median (IQR)	6.2 (IQR 4.0)	2.1 (IQR 6.8)	P=0.31	2.5 (IQR 0)	3.2 (IQR 20.4)	P=0.8	13.6 (IQR 15)	2.3 (IQR 13.7)	P=0.4
VAS Ocular Pain Median (IQR)	0	0	P=1.0	0	0	P=0.38	0 (IQR 1)	0	P=0.54
VAS Limitations Median (IQR)	0 (IQR 1)	0	P=0.12	0	0	P=0.97	0.5 (IQR 2)	0	P=0.02

Allo-SCT; allogeneic stem cell transplantation, IQR; interquartile range, VFQ-24; visual functioning questionnaire-25, OSDI; ocular surface disease index, VAS; visual analogue scale, Na; not applicable.
P-values were calculated using the Mann-Whitney U test

Table 5.
Results of questionnaires before and after allo-sct

Ocular GvHD

One out of 23 patients developed ocular GvHD 84 days after allo-SCT together with systemic manifestations of overall grade III GvHD of the gastrointestinal tract. He was treated with prednisone 2mg/kg for his systemic manifestations. This patient was in the Cyclosporin A eye drops group. His Schirmer test values were five and seven mm ODS respectively, he had, fluorescein staining Grade II of his cornea in both eyes according the Oxford grading scheme for dry eyes (Figure 1), a low TBUT together with conjunctival fluorescein staining and trichiasis of his right eye due to conjunctival cicatrisation (Figure 2).

The comparison of ocular variables between patient of both treatment groups who finished the study is shown in Table 4.

Visual acuity did not significantly differ at all three measuring points, before allo-SCT (p=0.06), three months after allo SCT (p=0.2) and six months after allo-SCT (p=0.5).

Schirmer test values did not significantly differ between treatment groups before allo-SCT (p=0.3) and three months after allo-SCT (p=0.7). However six months after allo-SCT, Schirmer test values were higher in the Cyclosporin A group compared to Polyvidone group (p=0.02) with a mean value of 14.8 mm (sd 13.5, range 0-30 mm) in the Polyvidone group and a mean value of 16.9 mm (sd 11.7, range 2-30 mm) in the Cyclosporin A group. Using a generalized linear model correcting for the use of both eyes and baseline Schirmer's test value measurement this difference remained significant (p=0.04). Both fluorescein staining and prevalence of conjunctival chemosis did not significantly differ between the treatment groups at all three measurement points (Table 4).

Questionnaires

The results of the questionnaires are displayed in Table 5. In short, the OSDI scores and overall VFQ-25 scores were not significantly different between the groups at all measuring points. The VFQ-25 subgroups were not analysed in this one-year evaluation due to a limited number of patients. The Visual Analog Scale limitations score 6 months after SCT was significantly (p=0.02) higher in the Polyvidone group.

Discussion

The results of this one year evaluation disclose that patients treated with Cyclosporin A eye drops had significantly better Schirmer's test values six months after allo-SCT than patients treated with Polyvidone eye drops. Visual acuity was not significantly different between the treatment groups before allo-SCT and during follow up. The OSDI and VFQ-25 did not show any significant differences between the treatment groups at all three measuring points (before all-SCT, three and six months after allo-SCT), but the Visual Analog Scale showed a trend toward more visual limitations six months after allo-SCT in the Polyvidone treatment group. Ocular GvHD developed in one patient in the Cyclosporin A treatment group and in none

of the patients in the Polyvidone treatment group. So far, definitive conclusions cannot be drawn from this one year evaluation since the number of included patients was low and there was a significant loss of patients during the follow-up period.

Cyclosporin A is a cyclic polypeptide produced by the fungus Tolypocladium inflatum Gams and topical use inhibits the proliferation of T-lymphocytes and the production and the release of lymphokines from activated T-cells in the conjunctiva[14]. T-cells play an important role in GvHD and in both acute and chronic GvHD of the eyes T-cell infiltration has been detected in the conjunctival tissue[15,16]. The T-cells damage the basal cells that have the capacity to induce regeneration of damaged epithelial tissue[15]. In acute ocular GvHD, T-lymphocyte infiltration of the lacrimal gland has been shown in murine models[17].

Wang *et al*. showed an improvement in the mucin layer, decrease of inflammatory cell numbers, an increased level of the goblet cell density and MUC5AC mRNA expression in patients with ocular GvHD that were treated with topical Cyclosporin A. The authors concluded that Cyclosporin A eye drops decreased ocular surface inflammation[18].

Topical Cyclosporin A has shown to be safe and effective in the treatment of moderate to severe dry eyes of various causes[19-21]. Phase I, II and III studies have been performed concerning the use of Cyclosporin A ointment in dry eye disease. A phase II study with 162 patients (129 patients: Cyclosporin A group, 33 patients: vehicle group) by Stevenson *et al*. concluded that Cyclosporin A ophthalmic emulsions (0.05%, 0.1%, 0.2% and 0.4%) are safe and well tolerated and significantly improve the ocular signs and symptoms[21]. A phase III study by Sall *et al*. with 877 patients (292 patients Cyclosporin A 0.05%, 292 patients Cyclosporin A 0.1%, 293 vehicle) showed that the Cyclosporin A ointments were safe and effective in the treatment of moderate to severe dry eye disease with improvement in both objective and subjective measures[20].

A beneficial effect of topical Cyclosporin A specifically on ocular GVHD has been shown in several small studies, but a large prospective randomized study is lacking[18,22-25]. Wang *et al*. investigated the effect of 0.05% topical Cyclosporin A (made by the pharmacy at the Keio University Hospital; Cyclosporin A 0.05 g, a-Cyclodextrin 8.00 g, Sodium Chloride 0.695 g, sterilized water 100 ml) on ocular surface and tear functions in severe dry eye patients with ocular GVHD who were refractory to the maximal conventional treatments (artificial tears, autologous serum and punctal plug occlusion) in a prospective comparative study with 14 eyes in the topical Cyclosporin A treatment group and 12 eyes in the control group[18]. They found a significant improvement of TBUT, vital staining scores, impression and brush cytology parameters with 0.05% topical Cyclosporin A treatment four times per eye per day during one month[18]. Rao *et al* performed a prospective study in eight patients with dry eye associated with ocular GVHD unresponsive to artificial tears. After three months of 0.05% topical Cyclosporin A (Restasis, Allergan) there was an improvement in dry eye signs and symptoms in seven out of eight patients[23]. Kiang *et al*. describe five cases with ocular GvHD and conclude that the apparent benefit of topical Cyclosporin A (1% solution) in these patients may result from the pharmacological action of Cyclosporin A[24]. More recently a study has

been performed in which 81 patients received topical Cyclosporin A 0.05% (Restasis Allergan) starting one month before allo-SCT and a control group in which patients (n=24) did not receive topical Cyclosporin A until at least six months after allo-SCT. In this study the dry eye symptoms were significantly ($P<0.05$) more severe in the control group (at three months, one and two years after BMT). After one year the mean Schirmer's test value was significantly higher in the treatment group (9.5 mm versus 5.2 mm)[25]. The data on better Schirmer's test results in topical Cyclosporin A group are in concordance with our results.

Several problems were encountered during the conduction of the present study. Primarily, the follow-up of severely ill patients was difficult and caused premature termination of study participation and/or missing data in five/23 patients. In addition, a loss to follow-up occurred in two/23 patients because of their death. The changes in transplantation techniques during the preparation of our study as well as a prolonged immune suppression in some patients might cause patients to develop less (severe) GvHD or develop GVHD later in time than within six months after allo-SCT. In this study two patients were conditioned with CD19 and T-cell depletion in which less development of GvHD is expected. These changes in transplantation techniques might also explain the low prevalence of ocular GVHD within six months after allo-SCT in the total group of followed patients. Therefore, in the meantime we prolonged the originally planned follow-up of six months to at least one year. So far, ocular GvHD developed only in one patient who was randomized in the Cyclosporin A treatment group. At this point of time, however, we cannot draw any conclusions and need to include more patients.

The Polyvidone group was significantly more often conditioned with TBI which might in part be responsible for the lower tear production. However, the tear production was significantly lower in the Polyvidone group only at six months of follow-up and not at three months after allo-SCT. This makes it unlikely that the lower tear production in Polyvidone group was caused by TBI. There are other factors than the type of conditioning regimen which may have influenced the results such as the severity of systemic GvHD, however in our one year follow-up evaluation we could not include these variables in our statistical analysis due to a limited number of patients.

In conclusion, our study shows higher Schirmer's test values six months after the allo-SCT in patients treated with preventively Cyclosporin A drops. The only case of ocular GVHD however developed also in the Cyclosporin A-treated group. The number of included patients in this one year evaluation is too limited to draw any definitive conclusions on the efficacy of Cyclosporin A eye drops in preventing ocular GvHD. Two additional university hospitals will be included in this on-going study to accelerate patient inclusion and to include more patients with various forms of conditioning regimens. The future results of this study aiming on prevention of ocular GvHD are of great importance for all patients undergoing allo-SCT.

References

1. Westeneng, A.C., Hettinga, Y., Lokhorst, H., Verdonck, L., van Dorp, S., & Rothova, A. (2010). Ocular graft-versus-host disease after allogeneic stem cell transplantation. Cornea, 29 (7), 758–63.

2. Balaram M, Rashid S, Dana R. (2005) Chronic ocular surface disease after allogeneic bone marrow transplantation. Ocul Surf, Oct; 3 (4): 203-11.

3. Tabbara KF, Al-Ghamdi A, Al-Mohareb F, *et al*. (2009) Ocular findings after allogeneic hematopoietic stem cell transplantation. Ophthalmology, 116 (9): 1624–9.

4. Westeneng AC, Hettinga Y, Lokhorst H, Verdonck L, van Dorp S, Rothova A. (2010)Ocular graft-versus-host disease after allogeneic stem cell transplantation. Cornea, 29 (7): 758–63.

5. Ogawa Y, Okamoto S, Wakui M, *et al*. (1999)Dry eye after haematopoietic stem cell transplantation. British Journal of Ophthalmology, 83 (10): 1125–1130.

6. Hirst, L., Jabs, D., & Tutschka, P. (1983). The Eye in Bone Marrow Transplantation: I. Clinical Study. Archives of ophthalmology 101, 580–584.

7. Jack, M. K., Jack, G. M., Sale, G. E., Shulman, H. M., & Sullivan, K. M. (1983). Ocular manifestations of graft-v-host disease. Archives of ophthalmology, 101 (7), 1080–4.

8. Riemens, A., Te Boome, L. C. J., Kalinina Ayuso, V., Kuiper, J. J. W., Imhof, S. M., Lokhorst, H. M., & Aniki, R. (2013). Impact of ocular graft-versus-host disease on visual quality of life in patients after allogeneic stem cell transplantation: questionnaire study. Acta ophthalmologica, april 22 (2013) Epub ahead of print.

9. Filipovich, A. H., Weisdorf, D., Pavletic, S., Socie, G., Wingard, J. R., Lee, S. J., Flowers, M. E. D. (2005). National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation, 11 (12), 945–56.

10. Mangione CM, Lee PP, Gutierrez PR, Sprit- zer K, Berry S & Hays RD (2001): Devel- opment of the 25-item National Eye Institute Visual Function Questionnaire. Arch Ophthalmol 119: 1050–1058.

11. Miller KL, Walt JG, Mink DR, Satram-Ho- ang S, Wilson SE, Perry HD, Asbell PA & Pflugfelder SC (2010): Minimal clinically important difference for the ocular surface disease index. Arch Ophthalmol 128: 94– 101.

12. Walt J (2004): Ocular surface disease index (OSDI) administration and scoring manual. Irvine, CA: Allergan, Inc. Warrian

13. Bron, A.J., Evans, V.E., & Smith, J.A. (2003). Grading of corneal and conjunctival staining in the context of other dry eye tests. Cornea, 22 (7), 640–50.

14. Nussenblat R.B., Palestine AG (1986). Cyclosporine: immunology, pharmacology and therapeutic uses. Surv Ophthalmol, 31: 159–169.

15. Tatematsu Y, Ogawa Y, Shimmura S, *et al*. (2012): Mucosal microvilli in dry eye patients with chronic GVHD. Bone marrow transplantation, 47: 416–25.

16. Saito T, Shinagawa K, Takenaka K, *et al*. (2002) Ocular manifestation of acute graft- versus-host disease after allogeneic peripheral blood stem cell transplanta- tion. Int J Hematol, 75: 332–334.

17. Hassan AS, Clouthier SG, Ferrara JLM, Stepan A, Mian SI, Ahmad AZ &Elner VM (2005): Lacrimal gland involvement in graft-versus-host disease: a murine model. Investigative ophthalmology & visual science, 46: 2692–7.

18. Wang, Y., Ogawa, Y., Dogru, M., Kawai, M., Tatematsu, Y., Uchino, M., … Tsubota, K. (2008). Ocular surface and tear functions after topical cyclosporine treatment in dry eye patients with chronic graft-versus-host disease. Bone marrow transplantation, 41 (3), 293–302.

19. Barber LD, Pflugfelder SC, Tauber J, Foulks GN. (2005) Phase III safety evaluation of cyclosporine 0.1% ophthalmic emulsion administered twice daily to dry eye disease patients for up to 3 years. Ophthalmology, 112: 1790–1794.

20. Sall K, Stevenson OD, Mundorf TK, Reis BL. (2000) Two multicenter, randomized studies of the efficacy and safety of cyclosporine ophthalmic emulsion in moderate to severe dry eye disease. CsA Phase 3S tudy Group. Ophthalmology, 107: 631–639.

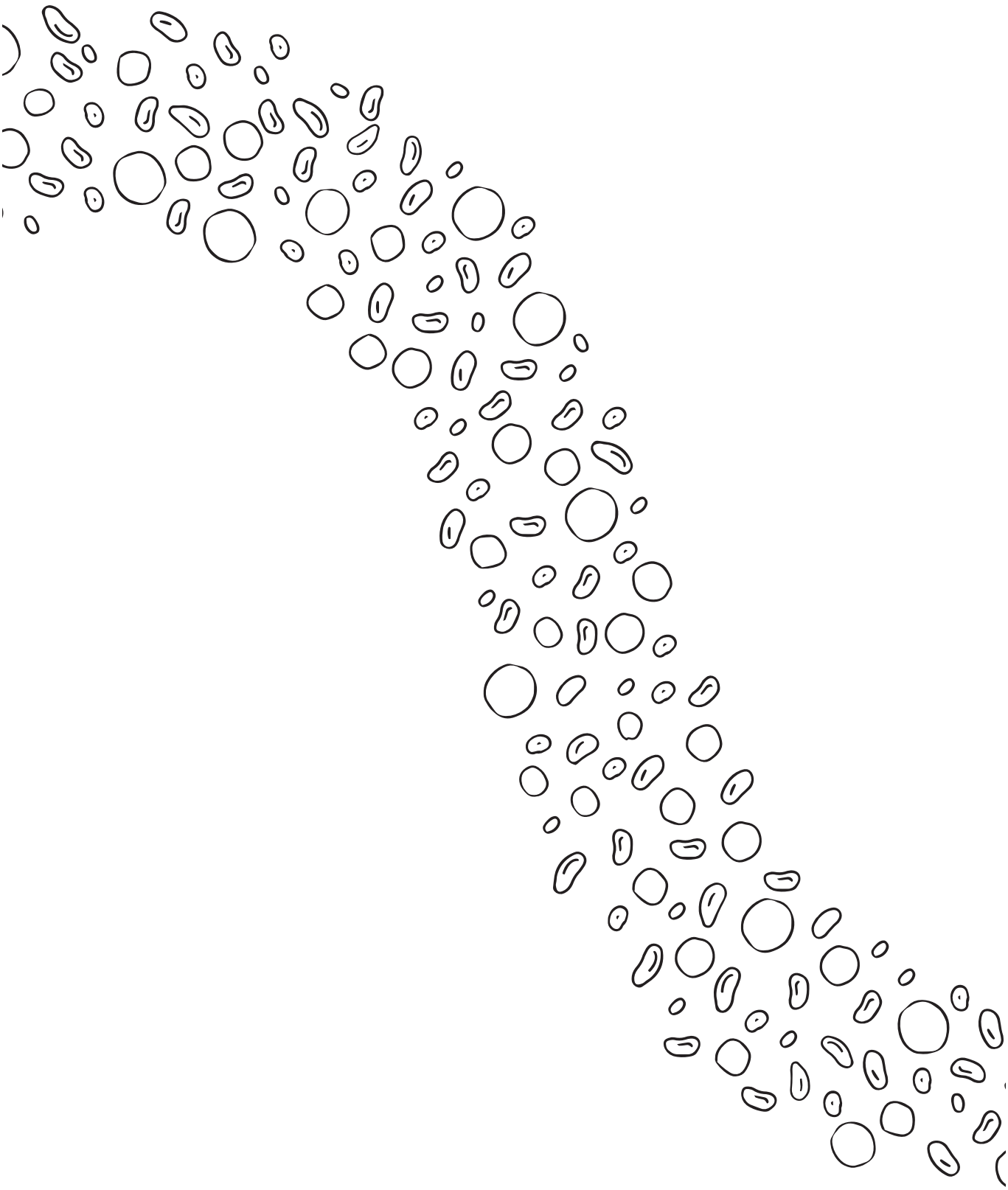
21. Stevenson D, Tauber J, Reis BL. (2000) Efficacy and safety of Cyclosporin A ophthalmic emulsion in the treatment of moderate-to-severe dry eye disease: a dose-ranging, randomized trial. The Cyclosporin A Phase 2 Study Group. Ophthalmology, 107: 967–974.

22. Lelli GJ Jr, Musch DC, Gupta A, *et al*. (2006) Ophthalmic cyclosporine use in ocular GVHD. Cornea, 25: 635–638.

23. Rao SN, Rao RD. (2006) Efficacy of topical cyclosporine 0.05%in the treatment of dry eye associated with graft versus host disease. Cornea, 25: 674–678.

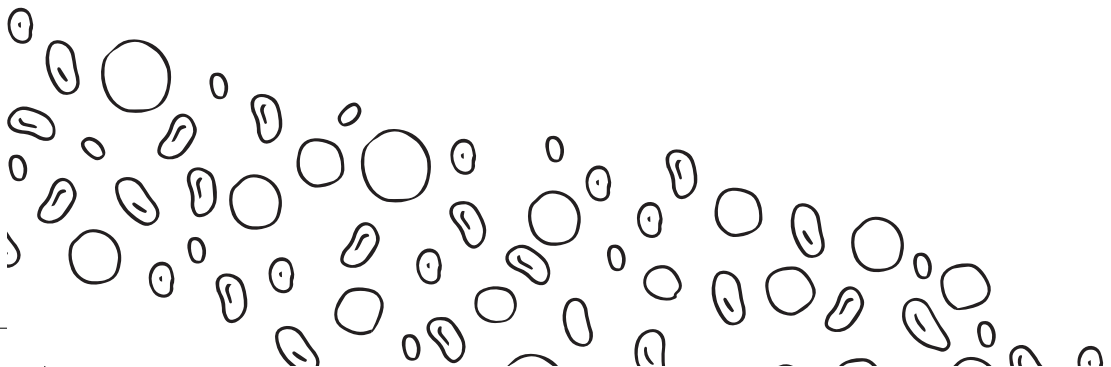
24. Kiang E, Tesavibul N, Yee R, *et al*. (1998)The use of topical Cyclosporin A in ocular graft-versus-host-disease. Bone Marrow Transplant, 22: 147–151.

25. Malta JB, Soong HK, Shtein RM *et al*. (2010) Treatment of ocular graft-versus-host disease with topical cyclosporine 0.05%. Cornea, 29: 1392-1396.



Chapter 11

Summary and conclusions Closing remarks and future perspectives



Summary and conclusions

This thesis describes the involvement of the eye in haematological malignancies and focuses on two topics; primary vitreoretinal lymphoma (PVRL) and ocular Graft-versus-Host Disease (GvHD). The aim of this thesis is first: to compare the efficacy of diverse treatment options of PVRL with regard to prevention of subsequent central nervous system lymphoma (CNSL) and second: to provide an insight into clinical manifestations, pathogenesis and treatment strategies of ocular GvHD.

Chapter 1 gives a general introduction and summarizes the state of the art of PVRL and GvHD. The clinical impact of both disorders is pointed out. In this chapter we indicate that PVRL represents a rare ocular malignancy with a high morbidity and mortality rate because of subsequent CNSL development in approximately 80% of the patients. PVRL mimics the signs and symptoms of uveitis (and is therefore called a "masquerade syndrome"), which often causes a substantial delay in diagnosis. The ophthalmologist can play a life-saving role by making an early diagnosis of this malignant disorder. Confirmation of the clinical suspicion of PVRL requires ocular fluid or tissue sampling for cytologic and immunophenotypic analysis. PVRL is mostly of B-cell origin and can be classified as diffuse large B-cell lymphoma (DLBCL). Unfortunately vitreous specimens frequently do not contain neoplastic cells and often multiple biopsies are needed to confirm the suspected diagnosis, which causes a delay in diagnosis and start of treatment. Moreover, the best treatment for isolated PVRL is not yet determined since the diverse treatment regimens were not systematically compared. In addition, it is not known whether the lymphoma is of multifocal origin and arises independently in the eye and/or central nervous system (CNS), or whether this lymphoma has one site of origin and spreads subsequently from one site to the other.

The best therapeutic approach for patients with isolated PVRL without documented CNS manifestations has not achieved consensus. In **Chapter 2** we retrospectively studied the currently employed treatment options for PVRL in 17 participating centers in Europe. In this study 95 patients were included with a median follow-up of 57 months. The primary outcome was subsequent CNSL and was evaluated in the following treatment groups: 1. local ocular treatment (radiotherapy and/or intravitreal chemotherapy (n=31; 33%)), 2. extensive treatment (intravenous and/or intrathecal chemotherapy and/or cerebral irradiation (n=21; 22%)) and 3. the combination of both treatments (n=23; 24%). Additionally, 20 patients were not treated initially for PVRL. CNSL developed in 45/95 (47%) of all patients and the incidence of CNSL development was similar in the three treatment groups (32-43%, p=0.76) whereas untreated patients developed CNSL significantly more often (85%). The Kaplan-Meier ten-year survival curves (with CNS manifestations as outcome) as well as the five-year cumulative survival rate showed no significant differences in the three treated groups (p=0.91 and p=0.10 respectively). Treatment complications developed in all treatment groups, however these were less serious and only local after ocular treatment whereas complications in patients receiving systemic chemotherapy included acute renal failure in five/40 patients (13%) receiving

this treatment modality.
We illustrate the lack of a consistent treatment approach for patients with PVRL and we show a similar incidence of CNSL manifestations among patients with PVRL treated with various ocular and extensive treatment regimens.

In **Chapter 3** we report on nine patients who were diagnosed with a combination of testicular and vitreoretinal DLBCL of whom seven also developed CNS manifestations.
The CNS, eyes and testes are all immune privileged (IP) sites. Previously, only occasional cases of concurrent vitreoretinal and testicular lymphoma have been reported and it was hypothesized that, in IP sites, the malignant cells can escape the immunosurveillance and thereby escape from the destruction initiated by the immune system as well as from chemotherapy.
Our study favours the hypothetical existence of IP-site lymphoma. Our finding of the extensive intervals (up to 10 years) between the development of lymphoma at the different IP sites, suggest that the lymphoma might develop at each site separately though we can certainly not exclude the spread from one site to another. The awareness of the existence of this type of lymphoma might improve its recognition and provides a new platform for future research on the pathogenesis of this type of lymphoma.

In **Chapter 4** we review the literature regarding ocular GvHD following allogeneic stem cell transplantation (allo-SCT) and its treatment options. Ocular GvHD develops in 40–60% of all patients after allo-SCT and can cause severe ocular surface disease. Data on long-term ocular complications is lacking. New allo-SCT techniques, such as HLA matching, reduction in conditioning regimen intensity and T-cell depletion, might help to diminish the incidence and severity of (ocular) GvHD. There are no widely accepted guidelines for the treatment of ocular GvHD and usually solely symptomatic treatment is being employed. In addition to treatment with artificial tears, topical steroids, punctal occlusion and contact lenses, recent treatment options include topical anti-inflammatory medications including Cyclosporin A and tacrolimus. Unfortunately, none of the treatment regimens is completely satisfactory and systematic data on the efficacy of these agents are lacking. Treatment is usually initiated during the symptomatic stage of ocular GvHD and by that time the damage to the lacrimal gland and conjunctival tissue might already be permanent. So far, prophylactic treatment strategies for ocular GvHD have not been investigated. We conclude that data on the long-term consequences of ocular GvHD is needed and targeted therapeutic and pre-emptive strategies need to be explored in prospective studies.

In **Chapter 5** we describe new criteria for diagnosing chronic ocular GvHD which were developed by the International Chronic Ocular GvHD Consensus Group.
So far, the National Institute of Health (NIH) consensus criteria from 2005 are being used for diagnosis of ocular GvHD but these criteria do not suit the current clinical situation. These criteria require the presence of chronic GvHD in at least one other organ in addition to ocular manifestations and in fact preclude the

early diagnosis of GVHD for example in patients that present with, in the first instance, only ocular manifestations of GvHD. In this chapter, we refine, together with other members of the international Consensus group, the definition, classification and grading of chronic ocular GvHD.
We elected four variables to measure subjective and objective findings in patients after allo-SCT, specifically: the ocular surface disease index (OSDI), Schirmer test score without anaesthesia, fluorescein staining of the cornea and conjunctival vascular injection. These variables were implemented in a scoring system in which patients are categorized as having no ocular GVHD, probable ocular GVHD and definitive ocular GVHD. In addition consideration was given to the presence or absence of systemic GVHD.
In this chapter we provide a novel up-to-date classification of the presence and severity of ocular GVHD, which can be used for clinical diagnosis and will allow international comparison in future (prospective) studies.

In **Chapter 6** we determine the impact of ocular GVHD on visual quality of life (QoL) three years after allo-SCT. Two widely accepted and validated questionnaires were used for this assessment: the visual function questionnaire-25 (VFQ-25) and the OSDI. Ocular GVHD was present in 26% (14/54) out of all 3 years survivors after allo-SCT and 71% of these patients (10/14) received treatment for ocular GVHD. OSDI and VFQ-25 scores were significantly impaired in patients with ocular GVHD three years after allo-SCT compared to patients with no ocular GVHD ($p = 0.02$). The scores of the VFQ-25 were significantly lower in the domains of general health, ocular pain, social functioning and role difficulties, which are, for example, similar to findings in Sjögren's disease. This study points out the severe consequences of ocular GvHD and clearly shows impaired vision-related QoL, even on the long-term follow-up in patients surviving haematological malignancies after allo-SCT. These findings underline the need for more insight into appropriate treatment of and preventive options for ocular GVHD.

In **Chapter 7** we study the risk factors for the development of ocular GvHD in a large series of adults and children after allogeneic hematopoietic SCT (HSCT) in a retrospective study design. We reviewed clinical data of one hundred seventy consecutive patients who underwent systematic ophthalmologic evaluations after HSCT and we evaluated these patients for possible risk factors. Ocular GvHD developed in 29/126 (23%) of the adult patients within one year after HSCT and in 9% of the children (4/44), which shows a significantly lower incidence in children than adults ($p=0.04$). In children, ocular GvHD required treatment in all affected patients, however treatment with tear drops only was sufficient in all. In contrast, ocular GvHD in adults often required multiple types of treatment including various local immunosuppressive medications as well as other treatment modalities such as scleral lenses. Children who developed ocular GvHD were significantly older than children without ocular GVHD (median age 13 vs. 5 years). Adults and children had a similar incidence of systemic manifestations of acute and chronic GvHD ($p=0.4$ and $p=0.16$). Development of ocular GvHD was associated with older age ($p=0.002$) in the entire study population. No association was found between the development of ocular GvHD and gender ($p=0.7$), malignancy as HSCT

indication (p=0.2), HLA-disparity (p=0.4), source of stem cells (p=0.3), conditioning regimen (p=0.8), acute GvHD (p= 0.2) and death (p=0.5) for all patients included. Using a multivariate Cox survival analysis, age was, in adults and children together, the only significant factor associated with development of ocular GVHD (p=0.007). We established that rising age is a predictor for the development of ocular GvHD.

In **Chapter 8** we determine the incidence of intraocular infections after allo-SCT in 135 patients who underwent allo-SCT in 2006 and 2007. Data of the period of systemic immunosuppression and antibacterial and antiviral prophylaxis (up to 6 months after allo-SCT) was evaluated and in the subsequent period of time without immunosuppression (median follow up of 15 months). In the past, severe sight threatening ocular infections were reported after allo-SCT, however less aggressive conditioning regimens, subsequent regular laboratory monitoring and use of antibacterial antiviral prophylaxis might have improved the incidence of ocular infections in the post transplant period. In our study, 59/135 patients (44%) developed systemic infection(s) during follow-up, but intraocular infection occurred solely in one of 135 consecutive adult patients (0.7%). This patient developed infectious chorioretinitis together with osteomyelitis, endocarditis and brain abscess with fungus Scedosporium. The patient was successfully treated with a combination of voriconazole, amphotericin B and surgical interventions. Viral and/or bacterial intraocular infections were not observed in this study population. Mild non-infectious uveitis anterior developed in two patients and was associated with an exacerbation of systemic GvHD in one patient and with ocular ischemia after radiotherapy in the second patient. The results of this study point out that intraocular infections after allo-SCT are currently uncommonly due to current systematic use of pre-emptive treatment regimens, frequent check-ups and early treatment of systemic infections. Although the modern prophylactic protocol is successful in reducing the probability of intraocular bacterial and viral infections, the awareness of a possible intraocular fungal infection and its timely recognition are of significant importance for visual prognosis of post allo-SCT patients.

In **chapter 9** we investigated cytokines in tear fluid of patients after allo-SCT and specifically studied the profile of two elevated cytokines, interferon (IFN)-γ and interleukin (IL)-6, and determined their relation to the presence and manifestations of ocular GvHD. IFN-γ is a T-cell associated cytokine which is considered to play a central role in the pathogenesis of GvHD and IL-6 is a pivotal pro-inflammatory cytokine with numerous downstream targets that affects multiple tissues and cell types.
The levels of IFN-γ and IL-6 in tear fluid in ocular GvHD patients were significantly elevated in comparison to patients without ocular GvHD and in healthy controls ($p<0.005$ for each). The levels of IFN-γ correlated with low tear production ($p<0.0001$) and low tear break up time (p=0.03). High IL-6 levels in tear fluid correlated with complaints of dry eyes (p=0.02), low tear production ($p<0.0001$), fluorescent staining of the cornea (p=0.01) and with the OSDI score (p=0.005). Our results suggest that IFN-γ is elevated during the early stages, while IL-6 is involved in later stages of ocular GVHD. The independent role of these cytokines is furthermore delineated by the observation that each of them correlated with separate symptoms of

dry eye disease. Previous experimental and clinical evidence has revealed IFN-γ plays a major role in the pathogenesis of GvHD and as well showed unequivocal evidence for a prominent role of IL-6 in promoting the onset of systemic GvHD and severity of GvHD. Although the number of available samples in this study was limited, the results support the current concepts of pathogenesis of ocular GvHD.

In **Chapter 10** we explore the possibility of prevention of ocular GVHD in a prospective randomized, single masked study in patients that underwent allo-SCT. All patients started therapy with eye drops and were randomised to either Cyclosporin A 0.05% (Restasis) or Polyvidone 50 mg/ml (Oculotect unidose) two times a day in each eye, starting one month after transplantation till six months after transplantation (overall duration of medication being five months). Ocular examination was performed before allo-SCT and three and six months after allo-SCT. Here we present an interim analysis of 23 patients (12 patients randomized to Cyclosporin A treatment and 11 patients randomized in the Polyvidone group). The results demonstrate that six months after allo-SCT, Schirmer's test values were higher in the Cyclosporin A group compared to the Polyvidone group (p=0.02). Both fluorescein staining grades and prevalence of conjunctival chemosis did not significantly differ between the treatment groups at all measurement points. One out of 23 patients developed 84 days after allo-SCT ocular GvHD together with systemic manifestations of GvHD of the gastrointestinal tract. This patient was randomized in the Cyclosporin A treatment group.
The number of included patients in this one year evaluation is too limited to draw definitive conclusions about our hypothesis that the administration of Cyclosporin A eye drops might prevent or modify ocular GvHD. Topical administration of Cyclosporin A reaches higher levels in the lacrimal gland and conjunctival tissue and has less side effects than systemically administered immune suppressive agents. The future results of this ongoing study aiming for prevention of ocular GvHD are of great importance for all patients undergoing allo-SCT.

Closing remarks and future perspectives

This thesis explores the underexposed involvement of the eye in haematological malignancies focusing on primary vitreoretinal lymphoma (PVRL) and ocular Graft-versus-Host Disease (GvHD). We delve into various clinical aspects of both entities and focus on (future) treatment options. Giving the accurate treatment modality in PVRL might be of life-saving value whereas preventive treatment of ocular GvHD might greatly ameliorate the quality of life in patients surviving haematological malignancies after allo-SCT.

We studied the treatment outcomes of patients with isolated PVRL without evidence of CNSL on MRI or in cerebrospinal fluid (CSF). Our study shows that all three treatment options employed in our 95 patients; ocular treatment, extensive treatment (e.g. systemic chemotherapy) and a combination of both all had similar outcomes with regard to subsequent CNSL development. The patients who received extensive treatment suffered from more severe side effects than patients treated with ocular treatment only. Our findings might determine the future approach to patients with PVRL and might imply that in the future patients with isolated PVRL would only receive local treatment and a more aggressive extensive treatment options could be given in case CNSL manifests later in time. For this approach however, the development of future screening regimen might be necessary. Our study has a retrospective design and a large prospective trial with international collaboration might be extremely valuable and of crucial importance for patients affected by this malignancy.

In this thesis we report on nine patients with concurrent intraocular, testicular and central nervous system lymphoma. The mechanism behind this combination of development of lymphoma at diverse immunoprivileged sites remains unknown. The existence of this so called "immunoprivileged site lymphoma" is of particular interest because it might reveal further insight into the pathogenesis of ocular and CNS lymphoma and into lymphoma in general. Future research should be focusing on mutual (immunologic and genetic) factors in lymphoma cells at the different sites as well as on unmasking the mechanism behind this concurrent localization. One intriguing question is whether lymphoma of immune privileged sites develops independently at each site or spreads from one site to another. Lymphoma tissue from different sites should be (prospectively) analysed for histological, immunological and genetic comparisons and special emphasis should be paid to the clonal identity of the lymphoma cells from the different sites. The outcome would provide not only a better insight into pathogenesis, but more importantly could reveal key information on what treatment strategy should be used with regard to CNS prophylaxis in patients with testes and/or intraocular lymphoma.

Ocular Graft-versus-Host Disease (GvHD) can develop after allogeneic SCT (allo-SCT) and cause debilitating ocular surface disease. The International Chronic Ocular GvHD consensus group, in which we participate, developed new diagnostic criteria which are described in this thesis. A prospective study is

needed, evaluating ophthalmic examinations in the pre- and post-transplantation period using these new guidelines to diagnose new onset dry eye or other ocular GVHD-related complications. Hopefully, such studies will provide more insight into incidence, prevalence and severity of ocular GvHD and confirm that the eye can be the primary localisation of GvHD, which was precluded in previous guidelines. This could lead to earlier diagnosis and treatment of ocular GvHD which might reduce tissue damage caused by GvHD and reduce the severity of the disease. The collaboration and communication between ophthalmologist and haematologist are of essential importance concerning the diagnosis, dose of systemic and local immunosuppressant and the time of commencement and cessation of treatment.

Ocular GvHD develops in a considerable number of patients after allo-SCT and we show that ocular GvHD has a significant impact on the visual quality of life in patients surviving haematological malignancies, even during long-term follow-up (three years) after allo-SCT. These findings stress the urge for the development of appropriate therapeutic and moreover preventive measures specifically aimed at decreasing (the severity of) ocular GvHD.

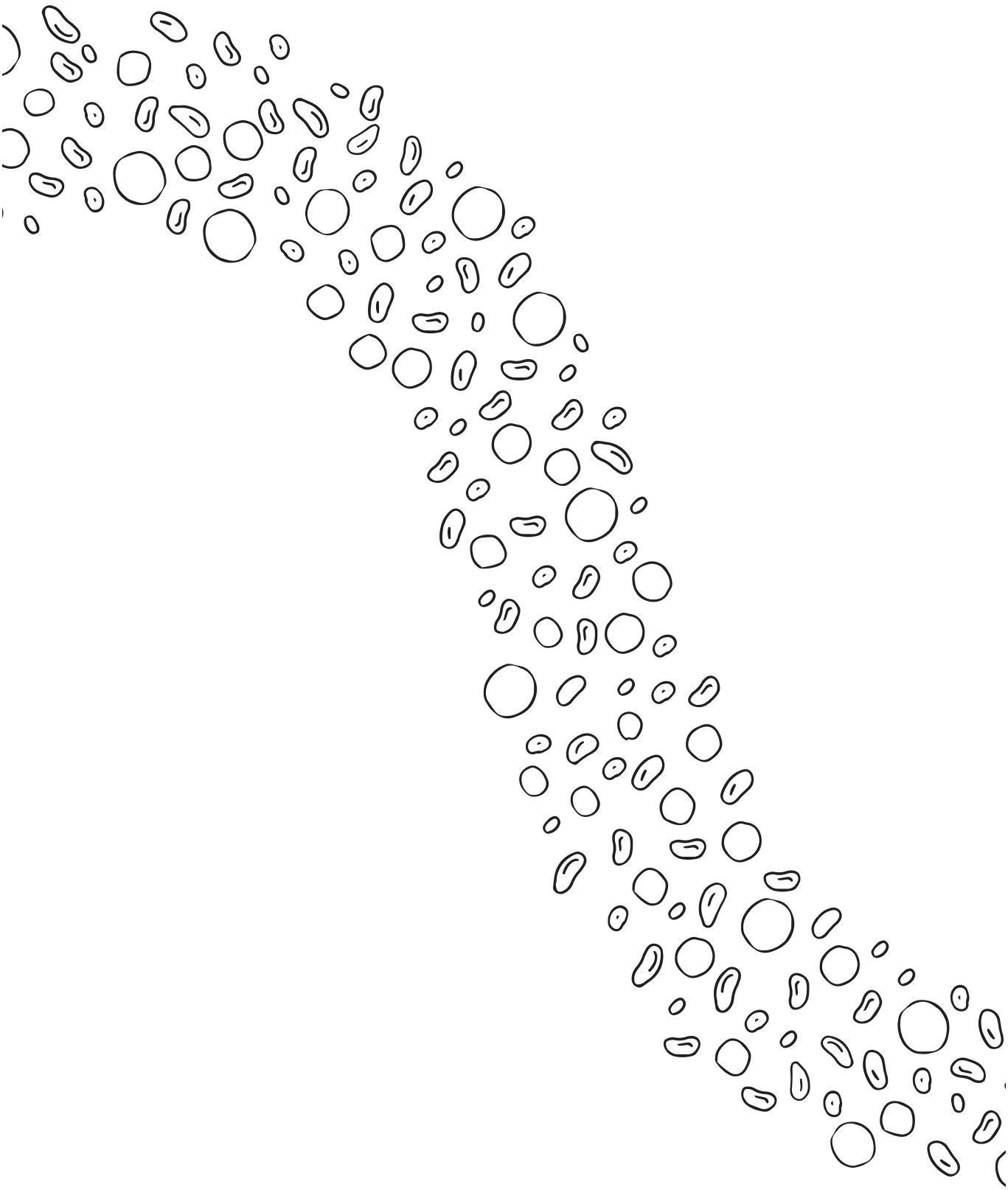
We established that rising age is a major predictor for the development of ocular GvHD in both adults and children. These findings indicate that future preventive treatment protocols should be focusing on older children and adults. In addition, these results can form a fundament for further research on why and how age is associated with GvHD.

It has been repeatedly reported that after allo-SCT, mainly during post-transplant treatment with immunosuppressive medications, severe sight threatening ocular infections can occur. We show that intraocular bacterial and viral infections are currently rare after allo-SCT and that contemporary preventive and therapeutic regimens are efficient for viral and bacterial infections. However a focus on intraocular fungal infections is needed since these infections might still occur.

There is a need for better insight into the chronology of immunologic processes and the resulting damage of the lacrimal gland and conjunctival tissue. We demonstrate that interleukin (IL)-6 and interferon (IFN)-y both play a distinct role in the tissue damaging process in ocular GvHD. Our results warrant further more detailed studies, especially focusing on the initial stages of the lacrimal gland and conjunctival involvement, to provide more understanding and potential new targets for early treatment modalities of ocular GvHD.

Until now, satisfying treatment and/or preventive options for severe dry eye disease encountered in ocular GvHD are lacking. The treatment is confined to symptomatic relief of symptoms with artificial tears and immunomodulating eye drops, however these types of treatment are frequently insufficient and associated with adverse effects. Since allo-SCT is currently more widely used and life expectancy after this procedure is increasing, there is a need for both better symptomatic and preventive treatment options for ocular GvHd. We investigated the preventive potential of administration of topical Cyclosporin A teardrops. The results of the one-year evaluation of this study presented in this thesis seem promising and show better

Schirmer's test values six months after allo-SCT in patients who obtained this preventive treatment. So far, definitive conclusions on its value cannot be drawn due to a limited amount of patients. Our study will continue as a multicentre trial and we expect to obtain final results within one year after publication of this thesis. If our hypothesis, that topical Cyclosporin A might lessen inflammation and prevent fibrosis, proves correct, application of eye drops two times a day for five months after allo-SCT could prevent the debilitating ocular surface disease caused by ocular GvHD and improve visual quality of life in patients after allo-SCT.



Nederlandse samenvatting

Summary in Dutch

Dankwoord

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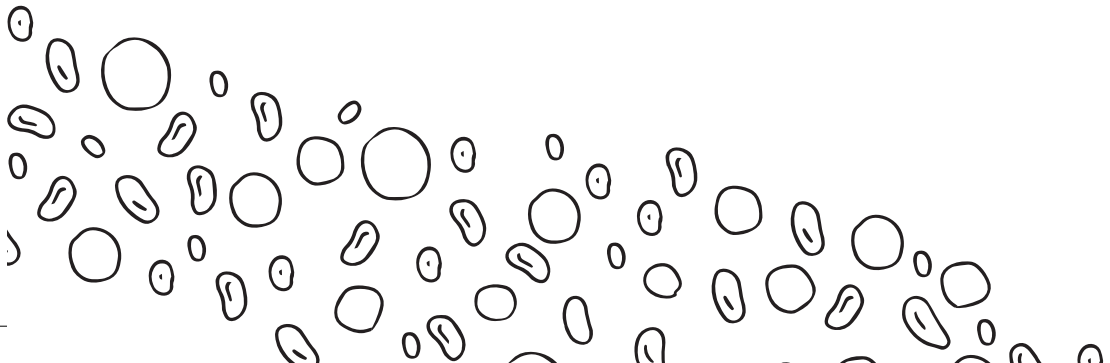
Contributors

Curriculum Vitae

Publications



Nederlandse samenvatting

In dit proefschrift wordt de betrokkenheid van het oog bij hematologische maligniteiten (kwaadaardige aandoeningen van het bloed) besproken. We richten ons op twee specifieke onderwerpen: oogheelkundige Graft-versus-Host Ziekte (GvHD) en het primaire vitreoretinale lymfoom (PVRL).

In **Hoofdstuk 1** wordt een algemene introductie gegeven over deze twee onderwerpen.
Oogheelkundige GvHD is een aandoening die zich kan ontwikkelen na een donor beenmerg of stamcel-transplantatie. Deze transplantaties worden gebruikt om kwaadaardige hematologische aandoeningen zoals leukemie en lymfoom te behandelen en kunnen deze aandoeningen zelfs genezen. Helaas hebben deze transplantaties (ernstige) bijwerkingen, waarvan de meest bekende GvHD is. De basis van GvHD berust op het feit dat de cellen van het transplantaat (van de donor) de cellen van de patiënt als vreemd beschouwen en deze vervolgens proberen af te breken. Dat is enerzijds gunstig omdat deze reactie zich ook richt tegen de kankercellen, maar aan de andere kant ook schadelijk, als deze reactie ook gezonde weefsels van de patiënt aantast. Daarom krijgen alle getransplanteerde patiënten afweerremmende medicatie om de afstoting en de omgekeerde afstoting (de Graft-versus-Host reactie) te onderdrukken. Specifiek op oogheelkundig gebied kunnen de traanklier en de slijmvliezen rond het oog door deze Graft-versus-Host reactie beschadigd raken, waardoor de ogen zeer droog kunnen worden en diverse complicaties zich kunnen ontwikkelen. Aangedane ogen kunnen veel pijn en ongemak veroorzaken en oogheelkundige GvHD kan tot blijvende gezichtsscherptedaling leiden.

PVRL is een zeer zeldzame vorm van de ziekte van Non-Hodgkin die zich in het oog openbaart. De tumorcellen bestaan uit witte bloedcellen (lymfocyten) en daarom wordt deze kwaadaardigheid lymfoom genoemd. Bij PVRL gaan deze kwaadaardige cellen in het oog woekeren in het glasvocht en het netvlies, vaak in beide ogen tegelijk. Patiënten gaan hierdoor minder goed zien en in het oog worden door de oogarts vaak aspecifieke troebelingen gezien, waarvan de oorsprong onduidelijk is. Dit maakt de vroege diagnose lastig en vaak worden de maligne cellen voor een onschuldige ontsteking aangezien. De diagnose kan gesteld worden door een zogenaamde vitrectomie, waarbij het glasvocht uit het oog tijdens een operatie verwijderd wordt en de cellen nader onderzocht worden. Helaas wordt over de hele wereld de diagnose van PVRL vaak pas laat gesteld, meestal na meer dan 10 maanden vanaf het begin van de oogklachten. Een groot percentage van de patiënten met PVRL ontwikkelt in een later stadium een hersenlymfoom (CNSL), een aandoening waar veel patiënten aan overlijden. Het mechanisme achter deze relatie tussen PVRL en CNSL is vooralsnog niet bekend. Dit maakt de keuze voor behandeling van PVRL erg lastig. Er zijn meerdere behandelingsmogelijkheden. Er kan bijvoorbeeld chemotherapie of bestraling van het oog gegeven worden, dan wordt alleen het oog behandeld. Een andere benadering, chemotherapie via de bloedbaan of bestraling van de hersenen, is veel intensiever, maar gaat ook met vele bijwerkingen gepaard. Deze laatste therapie heeft potentieel levensbedreigende bijwerkingen zoals ernstige dementie, en wordt daarom bij voorkeur

alleen gegeven als de aanwezigheid van het lymfoom in het centraal zenuwstelsel ook echt bewezen is.
In dit proefschrift worden diverse aspecten van oogheelkundige GvHD en PVRL besproken welke hier zullen worden samengevat.

In **hoofdstuk 2** wordt een studie besproken waarin onderzocht is hoe PVRL op dit moment in diverse Europese landen behandeld wordt. Er is met name keken naar hoe vaak patiënten CNSL ontwikkelen binnen de diverse behandelingsstrategieën en welke complicaties er opgetreden zijn. Dit onderzoek is een samenwerking tussen 17 Europese universiteits- en oogziekenhuizen en 95 patiënten werden geïncludeerd. De verschillende behandelingen zijn onderverdeeld in 3 groepen: 1. alleen behandeling van het oog (31 patiënten, 33%) 2. intensieve behandeling (21 patiënten, 22%) en 3. combinatie van behandeling van het oog en intensieve systemische behandeling (23 patiënten, 24%). Tevens waren er 20 patiënten die niet behandeld werden voor PVRL.
Van alle 95 patiënten ontwikkelden er 45 (47%) CNSL en de percentages tussen de 3 behandelingsgroepen verschilden weinig en waren niet significant (32-43%, p=0.76).
In alle behandelingsgroepen traden diverse complicaties op: in de systemische groep ontwikkelden 5/40 patiënten acuut nierfalen (13%), in de lokale behandelingsgroep waren de complicaties minder ernstig en bleven beperkt tot het oog. In onze studie laten we zien dat PVRL niet consistent behandeld wordt en dat er veel verschillende therapieën worden toegepast. Verder laten we zien dat de ontwikkeling van CNSL vergelijkbaar is tussen de verschillende behandelingsgroepen (dus er is weinig verschil tussen diverse lokale oogbehandelingen, al dan niet gecombineerd met intensieve systemische therapie). Het lijkt erop dat het effect van intensieve systemische therapie niet beter is dan van de behandeling van het oog alleen. Omdat de intensieve behandelingsbenaderingen zo divers waren, kunnen wij weinig zeggen over de eventuele effecten van een specifieke benadering, zoals bijvoorbeeld stamceltransplantatie of een intraveneuze hoge dosis methotrexaat. Onze studie laat duidelijk zien dat prospectief internationaal onderzoek noodzakelijk is om de waarde van de specifieke benaderingswijzen te onderzoeken om zo de therapie van PVRL te verbeteren.

In **hoofdstuk 3** bespreken we 9 patiënten die een lymfoom ontwikkelden van zowel het oog als de testes. Van deze 9 patiënten ontwikkelden 7 patiënten ook een lymfoom van de hersenen. Zowel het oog als de testes en de hersenen hebben een barrière tussen het orgaan en de bloedbaan waardoor afweercellen uit de bloedbaan niet zomaar in deze organen kunnen komen. Hierdoor is de samenstelling van het immuunsysteem in deze organen anders dan in de rest van het lichaam (immune privilege).
Eerder werden er al enkele patiënten beschreven die een lymfoom ontwikkelden in zowel het oog, de testes als de hersenen. Een mogelijke hypothese is dat kwaadaardige cellen in deze organen met “immune privilege” kunnen ontsnappen aan de afweercellen van het immuunsysteem en bij voorkeur op deze plaatsen woekeren. Het is niet bekend of het lymfoom zich uitzaait van de ene naar de andere plek of dat het los van elkaar op verschillende locaties ontstaat. Bij de 9 patiënten die wij bespreken was een lange interval te zien (tot 20 jaar) tussen het ontwikkelen van het lymfoom op de verschillende plaatsen, wat

zou kunnen pleiten voor het individueel ontstaan van het lymfoom op de verschillende lokalisaties. Echter, deze bevinding sluit het andere mechanisme, het uitzaaien van de lymfoomcellen, niet uit. Het bewustzijn van het bestaan van deze vorm van lymfoom is van belang zodat artsen het beeld vroegtijdig kunnen herkennen en hierop kunnen anticiperen. Onze bevindingen kunnen een basis vormen van waaruit verder onderzoek gedaan kan worden naar het ontstaan van dit type lymfoom.

In **hoofdstuk 4** geven we een overzicht van wat er in de literatuur beschreven is over oogheelkundige GvHD en gaan we in op de behandeling van deze aandoening. Zoals we eerder al beschreven, kan oogheelkundige GvHD zich ontwikkelen na een donor stamceltransplantatie, ook wel allogene stamceltransplantatie (allo-SCT) genoemd. Oogheelkundige GvHD ontwikkelt zich in 40-60% van alle patiënten die een allo-SCT ondergaan en kan een ernstig beeld van droge ogen en verlittekening van de slijmvliezen rond de ogen tot gevolg hebben. Er is weinig bekend over de gevolgen op lange termijn van deze oogaandoening. Tevens worden steeds meer nieuwe allo-SCT technieken ontwikkeld die erop gericht zijn om GvHD van zowel de ogen als van andere organen te verminderen.
De behandeling van oogheelkundige GvHD is momenteel nog niet vastgelegd in een (internationale) richtlijn en de behandeling is vooral gericht op het onderdrukken van de symptomen. De therapeutische opties bestaan uit traandruppels, prednisondruppels, het afsluiten van de traanpuntjes en speciale contactlenzen. Recent worden er ook druppels toegepast die het immuunsysteem onderdrukken (net zo als prednison) zoals ciclosporine A en tacrolimus. Helaas is geen van de behandelingsopties optimaal en zijn er geen gegevens beschikbaar uit (grote) studies. De behandeling wordt meestal gestart als patiënten ernstige oogklachten hebben en in dit stadium is er vaak al zodanige schade opgetreden aan traanklier en slijmvliezen, dat de situatie onomkeerbaar is. De mogelijkheid tot preventieve behandeling van oculair GvHD, die al wordt gestart voordat de schade is opgetreden, is tot dusver niet systematisch onderzocht. In dit hoofdstuk concluderen wij dat er onderzoek verricht moet worden naar de gevolgen van oogheelkundige GvHD op lange termijn, en vooral dat het onderzoek naar de therapie en de profylactische behandeling van oculair GvHD in goed opgezette, prospectieve studies zeer nuttig zal zijn.

In **hoofdstuk 5** bespreken we de nieuwe criteria voor de diagnose oogheelkundige GvHD, die zijn ontwikkeld door de "International Chronic Ocular GvHD Consensus Group". Tot nu toe werden andere criteria gebruikt, ontwikkeld in 2005 door the National Institute of Health (NIH), maar deze richtlijnen zijn niet meer geschikt voor de huidige situatie. De NIH criteria vereisen de aanwezigheid van GvHD in een ander orgaan behalve het oog en sluiten dus de diagnose GvHD uit indien alleen het oog betrokken is. In dit hoofdstuk bespreken we de nieuwe criteria, de classificatie en het scoringssysteem welke samen met de andere leden van de "International Chronic Ocular GvHD Consensus Group" zijn ontwikkeld. We hebben 4 variabelen gekozen om zowel objectieve als subjectieve symptomen te meten: een vragenlijst over droge ogen, (de ocular surface disease index (OSDI)), een test om de traanproductie te meten (Schirmer test), een test om de schade van het hoornvlies ten gevolge van de droogheid te meten (fluoresceïne aankleuring), en de

mate van roodheid (vaatinjectie) van de slijmvliezen rond de ogen. Deze variabelen zijn gebruikt in een scoringssysteem waarin patiënten in de volgende categorieën onderverdeeld kunnen worden: 1. geen oogheelkundige GvHD, 2. waarschijnlijk oogheelkundige GvHD en 3. zeker oogheelkundige GvHD. Verder wordt er ook aandacht geschonken aan de aanwezigheid van GvHD in andere organen, echter dit is geen criterium meer om de diagnose oculair GvHD te kunnen stellen.
Onze nieuwe criteria en het scoringssysteem kunnen worden gebruikt om de diagnose oogheelkundige GvHD te stellen en door deze criteria te gebruiken kunnen studies naar deze aandoening internationaal beter met elkaar vergeleken worden.

In **hoofdstuk 6** bepalen we in invloed van oogheelkundige GvHD op de kwaliteit van leven van patiënten op de lange termijn (3jaar) na een allo-SCT. Hiervoor hebben we 2 vragenlijsten gebruikt die wereldwijd bekend zijn en veel gebruikt worden bij oogklachten: de visual function questionnaire-25 (VFQ-25) en de ocular surface disease index (OSDI). Deze vragenlijsten hebben we naar alle patiënten gestuurd die een allo-SCT ondergingen in 2006 of 2007 en in leven waren. Van deze patiënten had 26% (14/54) oogheelkundige GvHD en hiervan werd 71% (10/14) behandeld. Uit de resultaten van beide vragenlijsten blijkt dat de patiënten met oogheelkundige GvHD minder goed scoorden en dat hun oogheelkundige kwaliteit van leven dus minder goed was dan van patiënten zonder oogheelkundige GvHD (p=0.02).
De VFQ-25 liet zien dat de patiënten met oogheelkundige GvHD slechter scoorden op bepaalde domeinen: algehele gezondheid, pijn aan de ogen, sociaal functioneren en het uitvoeren van dagelijkse taken.
Deze studie toont de ernstige consequenties aan van oogheelkundige GvHD op lange termijn na allo-SCT. Het geeft aan dat de oogheelkundige kwaliteit van leven verlaagd is bij patiënten die een ernstige, kwaadaardige aandoening hebben overleefd en daarna oogheelkundige GvHD hebben ontwikkeld. De resultaten onderstrepen het belang van meer inzicht in de juiste behandeling en preventie van oogheelkundige GvHD.

In **hoofdstuk 7** onderzoeken we in een retrospectieve studie de risicofactoren voor het ontwikkelen van oogheelkundige GvHD. We hebben de klinische gegevens van 170 patiënten verzameld die een allo-SCT hebben ondergaan en bij wie een oogheelkundig onderzoek is uitgevoerd. Oogheelkundige GvHD ontwikkelde zich binnen 1 jaar na allo-SCT in 29/126 (26%) van de volwassenen en in 4/44 (9%) van de kinderen, wat laat zien dat oogheelkundige GvHD significant minder vaak ontwikkelde bij kinderen dan bij volwassenen (p=0.04). Kinderen met oogheelkundige GvHD werden allemaal behandeld, echter behandeling met traandruppels alleen was voldoende. Daarentegen hadden volwassenen met oogheelkundige GvHD vaak meerdere typen behandeling nodig zoals lokale immunosuppressiva (o.a. prednison oogdruppels) en andere vormen van behandeling zoals speciale contactlenzen. Kinderen die oogheelkundige GvHD ontwikkelden waren significant ouder dan kinderen die dit niet ontwikkelden (mediane leeftijd 13 versus 5 jaar). Volwassenen en kinderen hadden een vergelijkbare incidentie van systemische manifestaties van zowel acute als chronische GvHD (p=0.4 en p=0.16). De ontwikkeling van oogheel-

kundige GvHD hield verband met een hogere leeftijd (p=0.002) in de gehele studiepopulatie (volwassenen en kinderen bij elkaar). In alle patiënten die deelnamen aan deze studie werd er geen associatieverband gevonden tussen de ontwikkeling van oogheelkundige GvHD en geslacht (p=0.7), maligniteit als indicatie voor allo-SCT (p=0.2), mate van HLA-mismatch (p=0.4), herkomst van de stamcellen (p=0.3), type conditionering (p=0.8), voorafgaande acute GvHD (p=0.2) en overlijden (p=0.5). Ook na statistisch corrigeren voor alle mogelijke risicofactoren die werden meegenomen in deze studie was leeftijd de enige significante risicofactor (p=0.007). In deze studie laten we zien dat een hogere leeftijd een voorspellende factor is voor het ontwikkelen van oogheelkundige GvHD.

In **hoofdstuk 8** bepalen we de incidentie van infecties in het oog, zogenoemde intra-oculaire infecties, bij 135 volwassen patiënten die een allo-SCT ondergingen in 2006 en 2007. De gegevens werden geëvalueerd gedurende de periode dat systemische immunosuppressieve medicatie en antibacteriële en antivirale profylaxe werd gegeven (tot 6 maanden na allo-SCT) en gedurende de periode hierna waarin geen immunosuppressiva en profylaxe werd gegeven (mediane follow-up van 15 maanden). In het verleden werden ernstige, potentieel blind makende, infecties van het oog gerapporteerd na allo-SCT. Echter, verbeterde technieken bestaande uit minder agressieve conditionering, beter toezicht op eventuele infecties door middel van frequente controle van infectiewaarden in het bloed, en het gebruik van antibacteriële en antivirale middelen zouden de incidentie van intra-oculaire infecties na allo-SCT hebben kunnen verlagen. In onze studie ontwikkelden 44% (59/135) van de patiënten een systemische infectie gedurende de follow-up, echter slechts 1 patiënt ontwikkelde een intra-oculaire infectie (0.7%). Deze patiënt ontwikkelde een ontsteking van het netvlies en ook een ontsteking van het bot, het hartzakje en een hersenabces, welke alle werden veroorzaakt door een schimmel met de naam Scedosporium. De patiënt werd met succes behandeld met diverse medicatie en chirurgische interventies. In de gehele studiepopulatie werden geen intra-oculaire infecties geobserveerd die veroorzaakt waren door bacteriën en/of virussen. Milde, niet infectieuze ontstekingen voorin het oog, ook wel uveitis anterior genoemd, ontwikkelden zich in twee patiënten, bij 1 patiënt bij verergering van de systemische GvHD en bij de tweede patiënt na bestraling. De resultaten van deze studie laten zien dat infecties in het oog na een allo-SCT weinig voorkomen. De verbetering ten opzichte van het verleden is een gevolg van de huidige profylactische behandelingsstrategieën, frequente controles en vroege behandeling van eventuele systemische infecties. Het huidige profylactische behandelingsprotocol is effectief in het verminderen van het risico op bacteriële en virale infecties in het oog, echter het alert zijn op een mogelijke door een schimmel veroorzaakte intra-oculaire infectie is van belang.

In **hoofdstuk 9** bestuderen we moleculen in het immuunsysteem die een rol spelen bij de afweer. Deze stoffen heten cytokines en degenen welke we bestuderen zijn interferon-γ en interleukine 6. We hebben deze cytokines gemeten in het traanvocht van patiënten die een allo-SCT hebben ondergaan en we hebben gekeken naar hun relatie tot de manifestaties van oogheelkundige GvHD. Het gehalte van interferon-γ en

interleukine 6 was in onze studie significant hoger bij patiënten met oogheelkundige GvHD in vergelijking met patiënten zonder oogheelkundige GvHD en gezonde controles (P<0.005 voor beiden). Het gehalte van interferon-y stond in verband met een lage traanproductie (p<0.0001) en een instabiele traanfilm (p=0.03). Hoge waardes van interleukine 6 correspondeerden met klachten van droge ogen (p=0.02), een lage traanproductie (p<0.0001), aankleuring met fluoresceïne van het hoornvlies (deze meting geeft celschade ten gevolge van droogheid van het hoornvlies aan, p=0.01) en met de score van een vragenlijst over klachten van droge ogen (OSDI, p=0.005). Onze resultaten suggereren dat interferon-y verhoogd is in een vroeg stadium van oogheelkundige GvHD en interleukine 6 in een later stadium van deze aandoening. Dat beide cytokines ieder een eigen rol spelen wordt onderstreept doordat ze ieder correleren met specifieke symptomen van oogheelkundige GvHD. Eerdere studies lieten zien dat interferon-y betrokken is bij het ontstaan van systemische GvHD en dat interleukine 6 een belangrijke rol speelt bij zowel de ernst van systemische GvHD als bij het ontstaan van deze aandoening. Onze studie ondersteunt de huidige concepten over het ontstaan van oogheelkundige GvHD.

In **hoofdstuk 10** onderzoeken we de mogelijkheid van preventie van oculaire GvHD bij patiënten die een allo-SCT ondergaan. Wij hebben een prospectieve gerandomiseerde klinische trial opgezet waarin wij trachten de ontwikkeling van oogheelkundige GvHD te voorkomen. In deze studie beginnen patiënten in een vroeg stadium na de allo-SCT, met ciclosporine A oogdruppels of, als ze in de controlegroep zijn ingedeeld, met traandruppels. De patiënten gebruiken deze druppels gedurende vijf maanden twee keer per dag, vanaf een maand na de transplantatie tot zes maanden na de transplantatie. Er wordt een oogheelkundig onderzoek uitgevoerd voorafgaand aan de allo-SCT en drie en zes maanden na de allo-SCT. In dit hoofdstuk laten we de interim resultaten zien van het eerste jaar van deze studie waarin we 23 patiënten hebben geïncludeerd (12 patiënten in de ciclosporine A groep en 11 in de kunsttraangroep). De resultaten laten zien dat zes maanden na de allo-SCT de traanproductie in de groep met patiënten die ciclosporine A oogdruppels gebruikten beter was dan in de groep met patiënten die kunsttraandruppels gebruikten (p=0.02). Andere tekenen van droge ogen, zoals celschade van het hoornvlies ten gevolge van droge ogen en zwelling van de slijmvliezen rond het oog, verschilden niet significant tussen de twee behandelingsgroepen op alle drie momenten van oogonderzoek. Slechts een van de 23 patiënten in deze studie ontwikkelde oogheelkundige GvHD, 84 dagen na de allo-SCT, tegelijkertijd met systemische manifestaties van GvHD van het maagdarm-kanaal. Deze patiënt was ingedeeld in de groep patiënten die ciclosporine A oogdruppels kreeg.
Het aantal patiënten in deze interim analyse is te beperkt om conclusies te kunnen trekken over de hypothese dat toediening van ciclosporine A oogdruppels oogheelkundige GVHD zou kunnen voorkomen of de ernst ervan zou kunnen verminderen.
Toediening van ciclosporine A in de vorm van oogdruppels zorgt voor hogere concentratie van het middel in de traanklier en de slijmvliezen rond de ogen en heeft minder bijwerkingen dan wanneer het oraal toegediend wordt. De toekomstige resultaten van deze nu nog lopende studie, die gericht is op het voorkomen van oogheelkundige GvHD, zijn van groot belang voor alle patiënten die een allo-SCT ondergaan.

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Anjo

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Curriculum Vitae

Anjo Riemens werd geboren op 5 november 1983 in het St. Antonius ziekenhuis te Nieuwegein. Ze groeide op in 't Goy, een klein dorp in de provincie Utrecht. Na het behalen van haar VWO-diploma aan het college de Heemlanden te Houten begon ze haar studie geneeskunde aan de Universiteit Utrecht. Tijdens haar studie maakte ze meerdere lange reizen naar zowel Azië als Latijns Amerika welke ze combineerde met vrijwilligerswerk en (talen)cursussen. Haar interesse en fascinatie voor de oogheelkunde werden gewekt tijdens een coschap oogheelkunde in Nepal in 2006. Hierna volgde ze een tweetal (keuze) coschappen oogheelkunde in het UMC Utrecht en voerde ze een wetenschappelijke studie uit naar intraoculaire HIV in Chiang Mai in Thailand onder begeleiding van Prof. Dr. Aniki Rothova. Vanaf maart 2014 zal zij voor een jaar naar Rwanda en Suriname gaan om onderzoek te doen op het gebied van retinoblastoom en uveitis waarna ze in het voorjaar van 2015 zal starten met haar opleiding tot oogarts.

List of Publications

1. Pathanapitoon, K., Riemens, A., Kongyai, N., Sirirungsi, W., Leechanachai, P., Ausayakhun, S., Kalinina Ayuso, V., Kunavisarut, P., de Groot-Mijnes, J., Rothova, A. (2011). Intraocular and plasma HIV-1 RNA loads and HIV uveitis. AIDS,25 (1): 81–86.

2. Riemens, A., te Boome, L., Imhof, S., Kuball, J., Rothova, A. (2010). Current insights into ocular graft-versus-host disease. Current opinion in ophthalmology, 21 (6): 485–94.

3. Riemens, A., Stoyanova, E., Rothova, A., Kuiper, J. (2012). Cytokines in tear fluid of patients with ocular graft-versus-host disease after allogeneic stem cell transplantation. Molecular vision, 18: 797–802.

4. Riemens, A., Te Boome, L. C. J., Kalinina Ayuso, V., Kuiper, J. J. W., Imhof, S. M., Lokhorst, H. M., Rothova, A. (2013). Impact of ocular graft-versus-host disease on visual quality of life in patients after allogeneic stem cell transplantation: questionnaire study. Acta ophthalmologica, 22 April: 1–6, Epub ahead of print.

5. Riemens, A., Missotten, T., Baarsma, S., Vidovic-Valentincic, N., Novac-Andrejcic, K., Erckens, R., Kuijpers, R., Lokhorst, H., Rothova, A. (2013). Diffuse large B-cell lymphoma in immunoprivileged sites: association of vitreoretinal, testicular and central nervous system lymphoma. Acta ophthalmologica, 2 July: 1–2, Epub ahead of print.

6. Stoyanova, E. I., Riemens, A., Lokhorst, H. M., Te Boome, L., Rothova, A. (2013). Absence of Intraocular Infections after Hematopoietic Stem Cell Transplantation at a Single Center: The Experience with Current Preventive Regimens. Ocular immunology and inflammation, 9 October: 1–5, Epub ahead of print.