

FEMALE GENITAL GRAFT-VERSUS-HOST DISEASE

Diagnosis, Treatment, Incidence, Long-term Prevalence,
and Impact on Androgen Hormones and Sexual Function

Eva Smith Knutsson MD

Department of Obstetrics and Gynecology

NU Hospital Group, Trollhättan

Department of Internal Medicine and Clinical Nutrition

Institute of Medicine

Sahlgrenska Academy, University of Gothenburg,



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Female genital Graft-versus-Host Disease

Diagnosis, treatment, incidence, long-term prevalence, and impact on androgen hormones and sexual function

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eva.sm@telia.com

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“Could this be genital Graft-versus-Host Disease?”
The first patient about her genital signs and symptoms.

For my grandchildren: It is never too late to learn!

ABSTRACT

BACKGROUND. Chronic graft-versus-host disease (cGvHD) is the major cause of morbidity after allogeneic hematopoietic stem cell transplantation (alloSCT), and contributes to non-relapse mortality. Caused by donor cells, cGvHD is a multi-organ syndrome involving tissue inflammation and fibrosis.

AIMS. To describe female genital cGvHD; its symptoms, signs, prevalence, incidence, severity, relationship to androgen levels, and long-term outcome.

STUDIES AND PARTICIPATING WOMEN. *Study I:* A cross-sectional, population-based study (n=42), median 80 (13-148) months after alloSCT. *Study II.* A cohort study (n=65), 55 (3-194) months after alloSCT, controls (n=140). *Study III,* n=41, ≤36 months post alloSCT. *Study IV,* n=38, 174 (120-232) months post alloSCT.

RESULTS. *Study I: Cross-sectional.* Prevalence of genital cGvHD was 52%.

Symptoms, signs: dryness, smarting pain, dyspareunia; vaginal stenosis (n=9).

Study II: Androgens and cGvHD. Corticosteroids and cGvHD were associated with low androgens.

Study III: Prospective study. Cumulative incidence of genital cGvHD 66%, extra-genital cGvHD 76%, at 3 years. Early diagnostic signs: lichen planus-like signs, and synechiae, with no symptoms in 30 %. Vaginal total stenoses (n=2). Genital cGvHD could vary over time.

Study IV: Follow-up study on women from Study I (n=38). Genital cGvHD prevalence 58%, no longer showing genital cGvHD (n=3), newly developed genital cGvHD (n=2). Prevalence and grade of cGvHD similar to Study I.

CONCLUSIONS. Female genital mucosa is a major target for cGvHD. The incidence of genital cGvHD is high, and the prevalence does not decrease over time. Fibrotic signs may not disappear. However, treatment may alleviate symptoms and signs. Independent of symptoms, early gynecologic surveillance is important. Close contact between gynecologist and hematologist, permitting early diagnosis and local and/or systemic treatment may diminish the risk of developing severe fibrosis. Chronic GvHD, especially in combination with glucocorticoid treatment, is associated with low androgens and may contribute to deteriorated quality of life and sexual health.

SAMMANFATTNING PÅ SVENSKA

INLEDNING: Allogen (från en annan människa) hematopoietisk (blodbildande) stamcellstransplantation (alloSCT) kan idag bota människor med maligna blodsjukdomar, tex leukemier. Det nya blodets T-lymfocyter angriper eventuella kvarvarande leukemiceller, Graft-versus-Leukemia-effekten, och bidrar till bot. Dessa T-lymfocyter kan även angripa patientens friska celler, Graft-versus-Host Disease (GvHD). Kronisk GvHD (cGvHD) är den vanligaste orsaken till sjuklighet och bidrar till död utan återfall efter alloSCT och inbegriper en inflammatorisk, fibrotiserande process som kan drabba alla organ men fr.a. slemhinnor i mun, ögon och genitalia. Kvinnors genitala cGvHD har länge varit underdiagnostiserad och otillräckligt behandlad.

FRÅGESTÄLLNINGAR: Vår övergripande avsikt var en kartläggning av kvinnors genital cGvHD. Prevalens, incidens och samband med annan cGvHD? När och hur debuterar genital cGvHD? Med vilka tecken och symptom, då och senare? Kan tidig behandling minska risken för irreversibla skador, t ex vaginalstenos? Är cGvHD relaterad till sänkta androgener? Kan cGvHD läka ut? Påverkas sexualfunktion och livskvalitet? Kan histopatologi bidra till diagnosen?

PATIENTER: Alla studier var populationsbaserade, Västra Götalandsregionen. *Studie I* var en cross-sectional studie av 42 kvinnor, 80 (13-148) mån efter alloSCT aug. 1996 - nov 2005. *Studie II* omfattade 65 kvinnor med alloSCT 1996 - 2012. Kontroller var kvinnor i) efter autolog SCT (får tillbaka egna stamceller) 1996 - 2010 (n=20), ii) med kortisonbehandling utan SCT (n=26), iii) friska åldersmatchade (n=94). *Studie III* var prospektiv, inkluderande kvinnor med alloSCT Sept 2005 - Febr 2010 (n=41), som följdes under 3 år. *Studie IV* är en långtidsuppföljning av alla överlevande kvinnor från Studie I (n=38).

METODER: Egenrapportering av genitala besvär i strukturerade formulär om gynekologisk historia, annan ohälsa, medicinering; Female Sexual Distress Scale och Beck Depression Inventory i Studie I, III o IV. Gynekologiska och allmänmedicinska undersökningar med strukturerad dokumentation i Studie I, III och IV, stansbiopsier i studie I, cytologprov och HPV-test i Studie IV; oftalmologisk och klinisk gradering av extra-genital cGvHD i Studie IV. Blodanalyser i Studie II avseende bl.a. androgena hormoner. Stansbiopsierna i Studie I bedömdes av två patologer. All cGvHD kategoriserades enligt internationella riktlinjer (National Institutes of Health (NIH) 2005 och 2014). Lokalbehandling i Studie III, inleddes med lokalt östrogen och därefter enligt strukturerat schema med clobetasol och tacrolimus. I Studie I ställdes diagnosen genital cGvHD först efter 6 veckors lokal östrogenbehandling.

RESULTAT: *Studie I:* Prevalensen av genital cGvHD var 52% (n=22); 12 kvinnor hade svår genital cGvHD (9 stenoser), och 5 isolerad genital cGvHD. Torrhet, sveda, smärta och dyspareuni vanligare vid genital cGvHD än utan (p<0.05). Tretton (31%) kvinnor hade både genital cGvHD och steroidkrävande extragenital cGvHD. Vi fann inget samband mellan histopatologi och klinisk diagnos. Alla kvinnor var i menopaus, naturlig eller prematur. *Studie II:* Låga serumandrogener var associerade med kortisonbehandling och cGvHD. *Studie III:* Första diagnostiska tecken var lichen planus-liknande förändringar och synekier - dock utan symptom i 30%. Kumulativ incidens 3 år efter alloSCT av extra-genital cGvHD 76% och genital 66%, flertalet med debut inom 1 år post alloSCT; senaste debut efter 30 mån. Fem kvinnor utvecklade partiell vaginalstenos varav 2 även total stenosis. Genital GvHD uppträdde intermittent. Lokal östrogenbehandling gavs till alla, systemisk hormonsubstitution till dem i för tidig menopaus, vilket drabbade alla som inte redan var i naturlig menopaus. Lokal immunosuppressiv behandling gavs till 13 kvinnor. *Studie IV:* prevalens av genital cGvHD 58%. Av 22 kvinnor med genital cGvHD i Studie I var 3 döda och 3 hade inte längre genital cGvHD. Två kvinnor hade utvecklat genital cGvHD 5-17 år efter alloSCT. Ingen skillnad sågs mellan Studie I och IV avseende prevalens eller svårighetsgrad av genital och extra-genital cGvHD på gruppnivå, men enskilda kvinnors svårighetsgrad av genital cGvHD kunde variera.

SLUTSATSER: Genital cGvHD är mycket vanlig efter alloSCT. Oberoende av symptom är tidig och systematisk gynekologisk kontroll viktig. Nära kontakt med hematolog, tidig diagnos och lokal behandling kan minska risken för utveckling av allvarlig fibros. Livslång kontroll är nödvändig på grund av ökad risk för epiteliala cancrar som t.ex. cervixcancer. Kronisk GvHD, särskilt vid samtidig kortisonbehandling, är associerad med låga androgener och kan bidra till försämrad livskvalitet och sexuell hälsa.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

- I. Smith Knutsson E, Björk Y, Broman AK, Helström L, Levin Jakobsen AM, Nilsson O, Sundfeldt K and Brune M. Genital chronic Graft-versus-Host Disease in females: a cross-sectional study. *Biol Blood Marrow Transplant*. 2014; 20:806-11.
- II. Björk Y, Smith Knutsson E, Ankarberg-Lindgren C, Broman A-K, Andersson I, Björkman L, Magnusson J, Bergmark K, Anderson H, Andersson P-O and Brune M. Androgens in women after allogeneic hematopoietic cell transplantation: impact of chronic GvHD and glucocorticoid therapy. *Bone Marrow Transplantation* (2017) 52, 431-437.
- III. Smith Knutsson E, Björk Y, Broman A-K, Helström L, Nicklasson M, Brune M and Sundfeldt K. A prospective study of female genital chronic graft-versus-host disease symptoms, signs, diagnosis, and treatment. *Acta Obstet Gynecol Scand* 2018; 97:1122-1129.
- IV. Smith Knutsson E, Nicklasson M, Björk Y, Helström L, Stenberg K, Sundfeldt K, and Brune M. Long-term follow-up of genital chronic Graft-versus-Host Disease in females after allogeneic stem cell transplantation. Manuscript.

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ABBREVIATIONS

ADL	activities of daily living
aGvHD	acute graft-versus-host-disease
alloHCT	allogeneic hematopoietic stem cell transplantation
alloSCT	allogeneic hematopoietic stem cell transplantation
AML	acute myeloid leukemia
ATG	anti-thymocyte globulin
autoSCT	autologous hematopoietic stem cell transplantation
BDI	Beck Depression Inventory
BMT	bone marrow transplant
cGvHD	chronic graft-versus-host disease
CI	cumulative incidence
CMV	cytomegalovirus
CR	complete remission
DHEAS	dehydroepiandrosterone sulfate
EBV	Epstein-Barr virus
FSDS	Female Sexual Distress Scale
GC	glucocorticosteroid /glucocorticoid
GI	gastrointestinal tract
GvHD	graft-versus-host disease
GvL	graft-versus-leukemia
HLA	human leucocyte antigen
HPV	human papilloma virus
HRT	hormone replacement therapy
KCS	keratoconjunctivitis sicca
MAC	myeloablative conditioning
MRD	measurable residual disease
MSD	matched sibling donor
MUD	matched unrelated donor
NIH	National Institutes of Health
NRM	non-relapse mortality
OCP	oral contraceptives
PBSC	peripheral blood stem cells
PR	partial remission
RICT	reduced intensity conditioning transplant
SCT	hematopoietic stem cell transplantation
SCC	squamous cell cancer
T	testosterone

PREFACE

My research on genital chronic Graft-versus-Host Disease (GvHD) started on the 25th of May 2000 – without my knowledge. That day at an antenatal clinic, the midwives had given an appointment to a woman, who recently had undergone an allogeneic stem cell transplantation. She had genital problems and was a fellow-worker. Her problems were caused by premature menopause, and easy to help with local and systemic hormone replacement therapy. A few months later new problems appeared. “Could this be genital Graft-versus-Host Disease?” the woman asked me. And that was the real beginning!

The hematologists started referring to me allografted women with genital complaints. By the summer of 2005 I had seen 10 women with genital chronic GvHD (cGvHD). I asked myself, what I should do with all this accumulating knowledge. I knew that other gynecologists knew very little about genital cGvHD. At that time, the hematologist Mats Brune called me. “There is a comprehensive new textbook on cGvHD, with only 20 lines on genital cGvHD. Shall we do a study of genital cGvHD?” There was only one answer to that!

1 BACKGROUND

1.1 Allogeneic Hematopoietic Stem Cell Transplantation

1.1.1 Introduction

Allogeneic hematopoietic stem cell transplantation (alloSCT) is an established treatment for malignant (e.g. acute and chronic leukemias) and also some non-malignant (e.g. aplastic anemia) diseases. The curative potential of a conventional alloSCT is due to a combination of the *myeloablative conditioning* (MAC) i.e. high-dose chemotherapy and/or irradiation, and an immunological graft-versus-leukemia (GvL-) effect, exerted by the donor's immunocompetent cells, mainly T lymphocytes. The GvL effect was first illustrated by Mary Horowitz 1990 in a registry study demonstrating an increased relapse rate if the donor was an identical twin rather than a matched sibling donor (1).

In Sweden, alloSCT is routinely performed at 6 university hospitals and, as in most other countries, the number of allografted patients has increased. Also, the indications for alloSCT have widened, from acute and chronic leukemias in young patients to an array of malignant and sometimes non-malignant diseases in patients up to the age of 70 years.

1.1.2 Toxicity

Unfortunately, the benefits of allografting either from a Human Leukocyte Antigen (HLA-) matched sibling donor (MSD) or from a voluntary HLA-matched unrelated donor (MUD), are partially offset by procedure-related toxicity, and *non-relapse mortality* (NRM), both of these problems increasing with patient's age. Actually, accumulated NRM in Acute Myeloid Leukemia (AML) patients is still 15-20%, also in younger patients (2). Indeed, one report from a contemporary study accounted for 34% NRM after MUD transplants in elderly patients (3).

1.1.3 Conditioning, myeloablative or reduced

There is a sustained rise in number of alloSCT which is mainly attributable to a rapid increase of *reduced intensity conditioning transplants* (RICT). The conceptual basis of RICT is to deliver a less toxic conditioning, and consequently benefit from the GvL effect. The intuitively attractive notion of reduced toxicity *and* an active GvL-effect has encouraged clinicians to perform RICTs in elderly and/or medically infirm patients. However, in a randomized trial, the reduced toxicity with RICT may come with an increased risk of relapse, outweighing the lower NRM(4).

1.2 Autologous SCT

Autologous SCT (autoSCT) relies on the effect of the heavy conditioning only. The stem cells have been collected from the patient, and kept frozen. After the

conditioning, the stem cells are infused and restore the patient's bone marrow function within 10-15 days. Obviously, there is no GvHD or any GvL effect following autoSCT.

1.3 Complications after alloSCT

1.3.1 Relapse

Although not formally a complication after alloSCT, relapse is by far the worst and most ominous event after alloSCT. The risk of relapse is dependent on the original disease – e.g. transplant of an AML patient with multiple chromosomal aberrations bears a high risk of early relapse, whereas an allografted patient with intermediate risk AML showing no *measurable residual disease* (MRD) has a much better prognosis (5).

1.3.2 Infections

After alloSCT, tissue damage and the eradication of neutrophils leave the patient highly vulnerable to infections. In particular, early Gram-negative bacterial infections are a great danger. Therefore, prophylactic antibiotics are used to prevent fatal infections. Following engraftment (normally occurring 12-15 days post-transplant) other agents including fungi and opportunistic viruses constitute a second wave of infectious threats. Fungal infections or activation of cytomegalovirus (CMV) are common problems. The allografted patient has an increased risk of infection for many years to come. Indeed, herpes zoster or human papilloma viruses (HPV) may flare-up years after transplant. In all, early or late infections account for one third of non-relapse mortality. Ongoing GvHD and ensuing immunosuppressive treatment markedly increase the risk of opportunistic infections (6).

A complete vaccination program is mandatory for all patients after alloSCT(7).

1.3.3 Other complications after alloSCT

Numerous late effects of alloSCT may strike the allografted patient. In addition to acute or chronic GvHD (see below) and the side effects of its treatment (systemic corticoid steroids), renal and pulmonary insufficiency, infections, anxiety, fatigue, and many more infirmities often reduce the patient's quality-of-life (8).

1.4 Graft-versus-Host Disease

1.4.1 Introduction

In 1966, R. Billingham defined a syndrome presented in an article called “The biology of graft-versus-host reactions”(9). This is a syndrome in which donor lymphocytes recognize and attack host tissues. Prophylaxis of acute GvHD consists of immunosuppressive drugs: ciclosporin or tacrolimus, and methotrexate or mycophenolate mofetil. Pre-transplant infusion of polyclonal

antibodies, anti-thymocyte globulin (ATG), decreases the risk of late onset (chronic) GvHD (see below)(10).

1.4.2 Acute GvHD

Acute GvHD (aGvHD) is a sometimes dramatic demonstration of donor cells' immunological capacity(11). Typically, donor lymphocytes attack skin, liver and gut within the first 4 months post-transplant. Primary treatment includes local immunosuppressive ointments and systemic high-dose corticosteroids. Response rate is 75%, but opportunistic infections (CMV, Epstein-Barr Virus, aspergillosis) are common. If there is no treatment response, the patient deteriorates, and mortality is high, usually due to intestinal acute GvHD and/or infection.

1.4.3.1 Chronic GvHD

Chronic GvHD “involves multiple, distinct interactions among alloreactive and dysregulated T and B cells and innate immune populations, including macrophages, dendritic cells, and neutrophils, that culminate in the initiation and propagation of profibrotic pathways” (12). Most common sites of cGvHD are mucous membranes in mouth, conjunctivae, and female genitals. However, almost any organ or tissue may be affected, e.g. skin (65-80%), mouth (48-72%), genitals (30-70%), eyes (18-47%), and liver (40-73%) (8). Fibrosis succeeding the inflammatory process may lead to scleroderma, bronchiolitis or vaginal stenosis. Thus, cGvHD may resemble any autoimmune disease including systemic sclerosis, Sjögren's syndrome, lichen planus and bronchiolitis obliterans. For a review of cGvHD, its symptoms, signs and categorization, see Lee S J (13).

Acute and chronic GvHD may appear in parallel (“overlap syndrome”) early after transplant. However, that is a rare scenario, and typically cGvHD comes with dry mouth and eyes, sometimes skin rash, 3-6 months after transplant. Risk factors are HLA mismatch, female donor to male patient, unrelated donor and transplants using peripheral blood stem cells (PBSC) (14). The incidence of cGvHD has increased over the last 20 years in spite of better HLA matching between unrelated donor and patient. The high amount of lymphocytes in the PBSC product is considered to be the factor behind the increased rate of cGvHD, which is the dominant problem in relapse-free patients after alloSCT.

Interestingly, mild cGvHD is associated with lower risk of relapse compared with no cGvHD, suggesting that the immunological mechanism(s) operative in cGvHD and GvL are identical. However, higher grades of cGvHD, and the systemic immunosuppression used, render risk of infections, impaired general health and wellbeing, and ensuing risk of mortality.

1.4.3.2 Treatment of cGvHD

Management of mild cGvHD includes topical corticosteroids and moisturizing ointments, whereas in moderate or severe cGvHD systemic corticosteroids are the mainstay of treatment. Chronic GvHD and its treatment increase the risk of opportunistic infections, in addition to cGvHD related fatigue, reduced quality-of-life, diabetes, alopecia and secondary cancers (15).

There are international guidelines for preventing and treating infectious complications after alloSCT (6, 8).

1.5 Genital chronic GvHD

1.5.1 Early reports

Genital cGvHD was first described by Stephen Corson et al. in *Obstetrics & Gynecology* in a report on five patients, four of whom developed vaginal stenosis (16). The treatment was local estrogen and surgery followed by use of vaginal dilators and systemic immunosuppression. Similar reports of genital cGvHD, describing vaginal stenosis with or without hematokolpos or hematometra, were published from 1999 onwards. Lönnqvist & Brune described two cases with dyspareunia and bloody discharge making coitus impossible. Signs and symptoms healed after 2 weeks of treatment with local hydrocortisone ointment (17).

1.5.2 Subsequent reports

In 2003, Spinelli et al. published a study of 213 women who had been referred because of gynecological symptoms after alloSCT. Retrospective analyses of the medical records indicated that 53 women had genital cGvHD (18). The authors proposed the first grading of genital cGvHD as minimal, moderate and severe. Vaginal adhesions or complete vaginal closure were considered diagnostic of severe genital cGvHD.

In the same year (2003), Spiryda et al. published “Graft-versus-Host Disease of the Vulva and/or Vagina: Diagnosis and Treatment” (19). Eleven cases of vaginal scarring were described. They reported good effect of topical ciclosporin only, or combined with surgery in severe cases. All women were on hormone replacement therapy (HRT) and six needed also topical estrogen due to vulvar and/or vaginal atrophy. Two women were treated for persistent dysplasia.

Zantomio et al. (2006) described a prospective surveillance program of female patients with genital cGvHD who survived at least 6 months post alloSCT (20). They concluded that genital cGvHD is common, and that early detection and topical immunosuppression, together with dilator use, appear to be effective at preventing progression. They stressed the importance of pre-transplant counsel, early hormone replacement, regular post-transplant gynecological examinations,

and the need for a close cooperation between hematologists and gynecologists.

In 2007, Stratton et al. (21) published data on 29 women and confirmed earlier findings presented by Zantomio . The authors showed that a combination of topical estrogen and a potent glucocorticoid, *and* vaginal dilator *and* estrogen ring (if vaginal scarring) was efficient treatment of genital cGvHD. An important observation was that women with ovarian function or HRT healed more rapidly than those who were hypoestrogenic. All women with genital cGvHD also had extra-genital cGvHD. Stratton modified the scoring of Spinelli and presented a list of “Severity scoring for vulvo-vaginal GvHD” with minimal, moderate and severe grades (or I-III). Twenty-eight women debuted with vulvar cGvHD and 13 of these patients either had or developed vaginal cGvHD. The authors concluded that vulvar cGvHD always precedes vaginal cGvHD. In accordance with previous findings, they noted that systemic immunosuppressive treatment of extra-genital cGvHD does not hinder development of genital cGvHD.

Hirsch et al. (22) suggested that local mucosal paleness actually is an early clinical manifestation, grade I, of genital cGvHD. They used the principles of the grading system proposed by Stratton, and concluded that “systematic, early and regular specialized gynecologic consultation should be performed in every alloSCT recipient”.

For a summery see Table 1.

Table 1. Previous papers on genital cGvHD.

Article	Recruit	n	Design	Incidence	Treatment	Comments
Spiryda 2003	Vaginal symptoms in spite of estrogen treatment	11	Retro-spective		Topical estrogen, cyclosporine vag cream. Prednison cyclosporine.	Description of vaginal cGvHD; local immunosuppressive therapy and surgery (7/11) and cervical dysplasia (2/11)
Spinelli 2003	AlloSCT 1980-99	213	Retro-spective	Cumulative incidence 24.9% at 8.9 years	Topical estrogen, triamcinolone, clobetasol	Own grading; no connection between parity or vaginal infection and GvHD; regards HRT as enough estrogen treatment of vulvo-vaginal mucosa
Zantomio 2006	AlloSCT 1999-2004	61	Retro-spective	Incidence 35% at 1 year, 49% at 2 years	HRT. Topical estrogen, hydrocortisone, & cyclosporine	Own severity grading similar to Spinelli's
Stratton 2007	Referred for genital symptoms or part of systemic evaluation	33	Observational		Topical estrogen, topical clobetasol	Modified Spinelli grading. Women with estrogen healed quicker than hypoestrogenic; vulvar cGvHD always preceded vaginal
Hirsch 2012	With genital GvHD AlloSCT 2000-10	32	Retro-spective		Local estrogen HRT, local corticosteroid, systemic cyclosporine	Modified Stratton grading; local paleness; emphasize the importance of long-term follow-up after alloSCT and long-term treatment

cGvHD, chronic Graft-versus-Host Disease; alloSCT, allogeneic stem cell transplantation; HRT, hormone replacement therapy

1.5.3 Clinical guidelines on the management of genital cGvHD

Typically, early guidelines described the female genitals as organs with endocrine dysfunction after alloSCT, and with an elevated risk for cervical malignancy(23, 24). Vaginal cGvHD is mentioned as causing strictures and synechiae.

From 2008, more attention is paid to the female genital cGvHD, and post-transplant examination of the genitals is recommended, in symptomatic women (25, 26) or all women (27-29).

1.5.4 Treatment of genital cGvHD

Corson recommended systemic prednisone and azathioprine as the treatment of genital cGvHD (16). In articles from 2003 and onwards, local immunosuppressive therapy is advised including topical use of clobetasol and/or ciclosporin or tacrolimus (18-21, 30). Most authors begin treatment with HRT and local estrogen to distinguish estrogen deficiency caused atrophy from genital cGvHD, and also because estrogen treated mucosa heals more rapidly on specific cGvHD therapy (21). If inadequate effect, systemic corticosteroids may be used (30). In vaginal cGvHD most authors propose the use of vaginal dilators, if coitus is not an option, both to keep the vaginal volume intact and to stretch the narrowing synechiae or circumferential vaginal bandings.

1.5.5 Genital cGvHD and cervical dysplasia

Savani et al. conducted a cross-sectional study of 35 patients at a minimum of 3 years posttransplant (31). HPV related cervical dysplasia was significantly associated with cGvHD requiring prolonged systemic immunosuppressive therapy. Most of these 35 women had normal cervical smears prior to alloSCT. In 2013, Sri et al. published a case report showing a relationship between the use of local immunosuppressive agents and HPV reactivation and spread (32). Wang et al. (33) performed a clinical study of cervical dysplasia in long-term survivors after alloSCT in Norway, and found that all alloSCT survivors were at high risk for cervical cytological abnormalities. Rizzo et al. studied a multi-institutional cohort of 28 874 allogeneic transplant recipients with 189 solid malignancies (34). The development of cGvHD was associated with a 5-fold increase in risk of squamous cell cancers (SCC).

1.6 Sexual function and depression after alloSCT

Humphreys et al. published a 3-years longitudinal study on sexual function in women after alloSCT (35). Half of the patients had had no discussion of sexuality with their health care providers during the 3 years. Baseline or pre-transplant level of depression was significantly related to sexual function at year 3 after alloSCT. Patients who were not sexually active reported fewer sexual difficulties. Those who had had a discussion of transplant effects on sexuality with their health care providers reported significantly fewer sexual functioning problems. In this paper, no data on the prevalence of cGvHD was presented, nor was the putative effect of genital cGvHD on sexual life discussed.

Li et al. in a review article stress the importance of screening for sexual dysfunction by asking about genital symptoms and performing genital examinations as part of the routine assessments after alloSCT (36). They noted the importance of physical necessary conditions for a functioning sexual life such as estrogen treatment of genital mucosa, treatment of genital cGvHD and

depression, regular medical review and elimination of polypharmacy to reduce sexual side effects.

Noerskov et al. made a prospective study for 1 year after alloSCT and found a significant decline in overall sexual function in both men and women. Of the women, 60% reported at least 1 physical sexual problem 1 year after alloSCT (37). Lack of libido and physical or sexual problems were rated equally as reasons for absence of sexual activity. Low arousal was the only sexual function correlated with depression. Women with cGvHD reported more sexual problems than those with no cGvHD. The authors ask for future studies on how the inevitable decline in endogenous sexual hormones influences sexual function in both men and women. Information is essential to improved understanding of the mechanisms behind the development of sexual dysfunction. In addition, the authors stress the importance of having a dialogue with the patients about sexual function as an integral part of cancer care.

Thygesen et al. published a review of the literature and summarized that some recovery of sexual activity and pleasure occurs during the first 2 years after transplantation (38). Yet, even 5-10 years after their cancer treatment survivors experienced more sexual dysfunction than controls. Typically, sexual dysfunctions were reported as lack of sexual interest, alterations in body image, vaginal dryness, vaginal tightness, fibrosis and painful intercourse.

1.7 Androgen hormones in women after alloSCT

Hovi et al. published an article where they had studied ovarian function and sex hormone production with special focus on androgens (39). Twenty-four young women (16-33 years of age) were followed up during 1.5–20 (mean 9) years after bone marrow transplantation (BMT). Subnormal testosterone levels were observed in 43% of BMT patients and subnormal dehydroepiandrosterone sulfate (DHEAS) levels in 34% of BMT patients, the latter being a constant finding during glucocorticoid therapy (GC) for cGvHD. Their question was whether the subnormal androgen levels are dependent on the result of dysfunctional ovaries or cGvHD with corticosteroid therapy, and secondary adrenal cortex insufficiency.

Davison et al. studied androgen levels in relation to age, menopause and oophorectomy in healthy adult females. They found that serum androgen levels decrease from the early reproductive years following a slow steady decline with age, unrelated to menopause (40).

1.8 Not yet studied?

Our project was planned in 2005, the same year as the National Institutes of Health (NIH) consensus document on “criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report” was published (41). There were no reports on population-based prevalence of genital cGvHD, no reports on prospective studies or long-term follow-up. No one had addressed the question raised by Hovi (39) regarding the putative effect of cGvHD on androgen levels in women.

2 AIMS

The overall aim of this thesis was to study genital cGvHD with respect to its prevalence, incidence, symptomatology, long-term prognosis and relationship to androgen hormone levels.

THE SPECIFIC AIMS WERE:

PAPER I

To determine the prevalence, signs, symptoms and histopathological features of genital cGvHD in a cross-sectional, population-based cohort of allografted women.

Paper II

To try and establish any impact of alloSCT and cGvHD, with or without systemic glucocorticoids, on androgen hormones. Controls were (i) women in remission after autologous transplantation, (ii) women with gluco-corticoid treatment for inflammatory diseases, (iii) healthy age-matched women.

Paper III

To follow a population-based cohort of allografted women to find out how, when and where genital cGvHD debuts, *and* to establish its cumulative incidence, *and* its relationship to extra-genital cGvHD *and* to depression and sex-related personal distress, *and* by early diagnosis and treatment try to hinder the development of more severe genital cGvHD.

Paper IV

To study the long-term outcome with respect to genital, ocular and other cGvHD in surviving women from the first study (Paper I).

3 PATIENTS AND METHODS

3.1 Patients

All patients were females living in the Region Västra Götaland (VGR), Sweden, an area with approximately 1.5 million inhabitants. Patients and controls signed informed consent. Ethical approvals for all studies were obtained from the Regional Ethical Review Board, Gothenburg.

Paper I. Eighty-six women were allografted from August 1996 to November 2005. Fifty surviving women in complete remission were identified and of these, 2 women declined participation, 3 were not invited due to mental disability, and 3 were excluded for other reasons. Thus, 42 women were enrolled (42).

Paper II. A total of 166 women from VGR underwent alloSCT at Sahlgrenska University Hospital from January 1996 to September 2012. Seventy-six surviving women in complete remission (CR) were identified living in Borås, Trollhättan and Gothenburg. Five were not invited to participate, owing to mental disability (n=1), insufficient Swedish language proficiency (n=3), and missing data (n=1). Six women declined participation. Thus, 65 women were enrolled. Forty-five of the 65 allografted women in Study II were also included in our other studies: 24 women took part in Study I and of these, 22 were also included in Study IV. Another 21 women were included in Study III (43).

Paper III. Fifty-two women scheduled for alloSCT from September 2005 to February 2010 were eligible. Four women declined to participate, 5 died or relapsed early, and 2 participated in study I. Thus 41 (79%) women were included at the pre-alloSCT gynecologic examination (n=22) or after transplant (n=19) (44).

Paper IV. Almost 7 years after Study I, 38 of the 42 participating women were still alive without relapse, and all of these accepted to participate in a long-term follow-up study. Four women had died: causes of death were cGvHD, pancreatic cancer, leukemia and amyotrophic lateral sclerosis (Manuscript).

For a summery see Table 2.

Table 2. Overview of the studies presented in this thesis.

Study	AlloSCT (n)	Incl. Period	In study (n)	Design	Aims	Follow-up months post alloSCT
I	84	1996 Aug 2005 Nov	42	Cross sectional	Prevalence	80 (13–148)
II	166	1996 Jan 2012 Sep	65	Cross sectional	Androgens & cGvHD	55 (3–194)
III	52	2005 Sep 2010 Feb	41	Prospective	Cumulative incidence	36
IV	84	1996 Aug 2005 Nov	38	Cross sectional	Late prevalence	174 (120–232)

alloSCT, allogeneic stem cell transplantation;

3.1.2 Controls

In Paper II, three groups of women served as controls:

1. Twenty women in complete, or partial remission (PR) with no ongoing chemotherapy after autologous SCT. Diagnoses were myeloma (n=7), multiple sclerosis, (n=1), lymphomas (n=12).
2. Twenty-six women without previous SCT but with ongoing glucocorticoid (GC) therapy (*GC group*). Indications for GC therapy were rheumatic disease without biological treatment (n=8), lung disease (n=6), or benign hematological disease: idiopathic thrombocytopenic purpura, autoimmune hemolysis (n=12).
3. Ninety-four healthy age-matched women with intact ovaries. Eighty-one of these were health care employees.

3.2 Methods

3.2.1 Clinical scoring according to NIH 2005

The traditional way of classifying cGvHD was to use the 3 terms *none*, *limited* or *extensive*. However, the basis for this division was imperfect and there was a lack of precise definition of the terms used.

In 2005, NIH published a clinical scoring system (0-3) for the evaluation of cGvHD involvement of individual organs and sites (NIH 2005) (41). Diagnosis of cGvHD should be made after assessment of signs and symptoms, laboratory tests, and (rarely) histopathological examination. The document defined criteria for scorings of organs including genitals, eyes, mouth, skin, nails, body hair, lungs, GI tract, liver, muscles, fascia, and joints.

As an example, scoring of ocular cGvHD was based on symptoms and signs.

Score 0: No symptoms.

Score 1: Mild dry eye symptoms i.e. eyedrops ≤ 3 times daily **or** asymptomatic signs of keratoconjunctivitis sicca (KCS).

Score 2: Moderate dry eye symptoms partially affecting activities of daily living (ADL) (eye drops > 3 times daily **or** punctal plugs, but without vision impairment

Score 3: Severe dry eye symptoms significantly affecting ADL, **or** unable to work **or** loss of vision.

For the clinical scoring of genital cGvHD, signs were categorized as mild, moderate or advanced; symptoms as minimal, mild, or severe. In practice, this symptombased scoring system implied that a woman with a complete vaginal stenosis, but with no symptoms, scored 0 for genital cGvHD (Table 3). Clinical scoring according to NIH 2005 was performed in Study I and III.

3.2.2 Clinical scoring of cGvHD according to NIH 2014

An update of the NIH consensus document was published 2015 (NIH 2014) with modifications of diagnostic criteria and scoring system *inter alia* for eyes and female genitals (45). The traditional Schirmer's test was withdrawn as a diagnostic tool, and KCS confirmed by an ophthalmologist in an asymptomatic patient (score 0) has been incorporated. Ocular score 2 was defined as new vision impairment due to KCS. Clinical score 3 was unchanged.

In contrast to NIH 2005, genital clinical scoring of cGvHD in the NIH 2014 classification focused on signs (see Table 3). NIH 2014 emphasizes that there are no symptoms specific for genital cGvHD. Clinical scoring according to NIH 2014 was performed in Study IV, and retrospectively in surviving women in Study I. The data in Study I were revised, on the basis of data on gynecological status, medical records and photographic evidence. Minor corrections were made, leading to the placement of 2 more women in the group with no extra-genital cGvHD.

Table 3. Genital clinical scoring according to NIH 2005 and 2014
Adapted from Filipovich (2004) & Jagasia (2015)

Genital clinical score	Score 0	Score 1	Score 2	Score 3
NIH 2005	No symptoms (independent of sign)	Symptomatic with mild signs on exam and no effect on coitus and minimal discomfort with gynecologic exam	Symptomatic with moderate signs on exam and with mild dyspareunia or discomfort with gynecologic exam	Symptomatic with advanced signs and severe pain with coitus or inability to insert vaginal speculum
NIH 2014	No signs	Mild signs and may have symptoms with discomfort on exam	Moderate signs and may have symptoms with discomfort on exam	Severe signs with or without symptoms

3.2.3 Global scoring of cGvHD

The global scoring system is based on the clinical scoring of affected organs. NIH 2005 proposed an assessment of severity (mild, moderate, or severe) of cGvHD by combining the number of organs involved, and clinical scoring of each affected organ. This global scoring system aimed to reflect the total clinical impact of cGvHD on the patient's functional status and remained by and large unchanged in the NIH 2014 document.

- *Mild* global score is the presence of 1-2 organs with clinical score 1.
- *Moderate* global score is the presence of
 - at least 1 organ or site with clinical score 2.
 - *or* 3 or more organs with clinical score 1 in all affected organs
 - *or* lung score 1
- *Severe* global score is the involvement of clinical score 3 in any organ *or* a lung score 2 or greater.

Global scoring was applied in Study I and Study IV. The global severity of each woman's cGvHD was categorized by combining genital and extra-genital clinical scores.

3.3. Other clinical cGvHD (not genital, not ocular)

All allografted women were followed as outpatients at the BMT¹ clinic. In Studies I and III clinical (not genital, not ocular) cGvHD data were retrieved

¹ Bone Marrow Transplantation Clinic. Section of Hematology. Sahlgrenska University Hospital, Gothenburg.

from medical records and registered by study hematologists (Y.B. and M.B.). For Study IV, each patient was examined with respect to genital, ocular and other cGvHD on the same day.

3.4 Ocular cGvHD

The ophthalmological examinations in Study I and Study III were sporadic and based on symptomatology. However, in Study IV all women were seen by an experienced ophthalmologist. Oral anamnesis included eye history, vision problems, cataract operation and use of lubricating eye drops and corticosteroid eye drops. Vision, Schirmer's test, ocular pressure, ophthalmoscopy and corneal microscopy of eyelids, conjunctiva and cornea were done. Break up time and chemosis were studied. All clinical signs were noted including those of inflammation, punctual erosions, papillary and follicular reaction. In NIH 2005 only distinctive signs were reported: new onset dry, gritty, or painful eyes, photophobia, cicatricial conjunctivitis, KCS and confluent areas of punctate keratopathy (41). In NIH 2014 signs as "New ocular sicca documented by low Schirmer's test with a mean value of 5 mm at 5 minutes" or "new onset of KCS by slit lamp exam with Schirmer's test 6 to 10 mm" are considered diagnostic for ocular cGvHD - if the signs are not due to other causes (45).

3.5 Genital cGvHD

Diagnosis of genital cGvHD according to both NIH 2005 and NIH 2014 requires at least 1 diagnostic sign or 1 distinctive cGvHD sign together with a diagnostic tissue biopsy *or* confirmed cGvHD in other organ(s) (41, 45).

The definitions of genital diagnostic signs in NIH 2005 were lichen planus-like features, and/or vaginal scarring. Erosions, fissures and ulcers were considered distinctive signs.

In NIH 2014 the distinctive signs remained the same.

- Diagnostic *mild* signs were erythema on vulvar mucosal surfaces, vulvar lichen-planus or vulvar lichen-sclerosus-like features.
- Diagnostic *moderate* signs included erosive inflammatory changes of the vulvar mucosa or fissures in vulvar folds.
- Diagnostic *severe* signs included labial fusion, labial agglutination, vulvar synechia, clitoral hood agglutination, fibrinous vaginal adhesions, circumferential fibrous vaginal banding (partial stenosis – author's comment), vaginal shortening or synechiae, dense sclerotic changes, and complete vaginal stenosis.

Gynecologic examinations included detailed structured documentation of vulvovaginal signs, and often photo-documentation of the vulva.

Examinations were done by one or both of the assigned gynecologists (A-KB and/or ESK). In Study I all women with atrophic mucous membranes were treated with local estrogen for a minimum of 6 weeks before the diagnosis of genital cGvHD was made. The reason was to avoid interpretation of genital

atrophia as genital cGvHD. All women were seen at least twice. For Study III, each woman was examined before alloSCT, and at +3, 6, 9, 12, 18, 24, 30 and 36 months after alloSCT. In Study IV the women were seen once by one gynecologist (ESK).

In Study I and Study III, the diagnosis of genital cGvHD was based solely on genital signs, according to the NIH 2005 consensus criteria. The NIH 2014 criteria were used in Study IV. A fibrotic sign i.e. a sore vaginal string was considered a diagnostic sign in Study III and Study IV (19, 21, 42). We also considered red and white spots occurring together, giving the mucous membrane a mottled appearance, a distinctive sign (22, 42).

At each study visit the woman completed or updated a comprehensive questionnaire on her general gynecological and medical history, gynecological symptoms, local and systemic medication. Symptoms were self-reported as 0, never; 1, seldom; 2, sometimes; 3, often; or 4, always. Symptoms asked for included itching, smarting pain, swelling, pain with and without touching, blisters, fissures/wounds, dryness, discharge, vaginal and/or vulvar constriction and dyspareunia. Cervical smear sampling was part of the protocol in Study IV only, but was performed according to the Swedish cervical cancer screening program and on clinical signs or symptoms in Studies I and III. HPV-test became more of a clinical routine during the project and was part of the protocol in Study IV.

3.5.1 Treatment of genital cGvHD

AlloSCT is known to affect the ovaries, causing premature menopause (28, 46). HRT was prescribed to all women in premature menopause. Local estrogen treatment was recommended for women with clinically atrophic mucous membranes. In Study I, the diagnosis of genital cGvHD was not made until after 6 weeks of local estrogen treatment. In Study III, first treatment of genital cGvHD was local estrogen therapy. Depending on signs, symptoms and histopathological findings, local immunosuppressive therapy was combined with continued local estrogen. Local ointments, clobetasol (0.05%) and/or tacrolimus (0.1-0.03%) were prescribed as first-line immunosuppressive therapy for genital cGvHD, and a dilator was recommended for vaginal cGvHD. We followed a structured treatment schedule (see Table 2, Paper III), based on the current Swedish recommendations for treatment of genital lichen sclerosis, published reports on tacrolimus use for atopic dermatitis and cutaneous cGvHD and in-house experience (47, 48). Systemic oral corticosteroid treatment was not included in the treatment schedule, but was prescribed by the hematologist in close cooperation with the gynecologist.

3.6 Sex-related personal distress and depression

The Female Sexual Distress Scale (FSDS), and the Beck Depression Inventory (BDI) were also completed at each study visit (49-51). A score of ≥ 15 in the

FSDS was used as the cut-off for sex-related personal distress. In the BDI, scores for depression ≥ 14 are ranked as mild, ≥ 20 as moderate, and ≥ 29 as severe. With the consent of the woman the gynecologist discussed the questions and answers in the FSDS and the BDI with the woman. Sexual advice and information were given as per the individual woman's request. The gynecologist ESK is also a clinical sexologist.

3.7 Genital biopsies for histopathological examination

In Study I, genital mucosal biopsies were obtained from 38 women from areas macroscopically suspicious for cGvHD (n=25), and from mucosa with no clinical cGvHD (n=31). Only the first biopsy taken from either vulva or vagina was used for the analysis of the relationship between clinical signs and histopathological features. Thus systemically skewed results due to multiple biopsies were avoided. Two pathologists examined each serial section. They used histopathological criteria to diagnose cGvHD (52). Assessments of findings of each biopsy were standardized into 1 of 4 categories: no cGvHD, possible cGvHD, consistent with cGvHD, or cGvHD. See Fig.1 in Paper I.

3.8 Laboratory methods

Comprehensive laboratory screening was performed in Study II. Serum levels of total and free testosterone (T) and DHEAS, were assessed at an accredited laboratory specialized on very low levels of androgens. HRT and oral contraceptives (OCP) were interrupted 24 h before blood sampling in all groups. The analyzing techniques are described in detail in Paper II.

3.9 Statistics

For dichotomous variables either Fisher's exact test or Pearson's chi-squared test were used in all studies and p-values < 0.05 were considered statistically significant. In Paper I and II for continuous variables Wilcoxon's rank-sum test was applied and nonparametric trend test was used to analyze ordered categorical variables (53). The simple linear regression model was used to analyze means in Paper II and the regression coefficients were compared by means of normal approximation. This was used to analyze the association between age and DEAS and free T respectively. Mann-Whitney-U-test was used in Paper III and IV to compare continuous variables.

4 RESULTS

4.1 Paper I. Genital chronic Graft-versus-Host Disease in females: a cross-sectional study.

The hypotheses of our first study was

- a. that genital cGvHD might be underdiagnosed in women after alloSCT
- b. that genital cGvHD implies suffering and discomfort
- c. that genital cGvHD has characteristic histopathological features.

4.1.1 Prevalence of genital chronic GvHD

Forty-two women were included in this cross-sectional study. The prevalence of genital cGvHD was 52% (n=22), to be compared with oral (43%) and ocular (40%) cGvHD. Seventeen of 42 (40%) women had fibrotic constrictions in the vagina (n=16), vulva (n=4) or in both locations (n=3). Six women had surgical treatment for vaginal stenoses before entering the study. Three of these women underwent surgery without cGvHD diagnosis and were prescribed only local estrogen as follow-up treatment. All 3 relapsed promptly.

Before inclusion, 10 women had been diagnosed with cGvHD by one of the gynecologists (ESK). In 12 cases genital cGvHD was unrecognized by the woman and found to be underdiagnosed. Clinical scoring was dominated by score 3 (n=12). The remaining women scored 2 (n=2), 1 (n=6) and 0 (n=2). The last two had no symptoms but showed diagnostic signs of genital cGvHD. The rate of severe global scoring was 15 of 42, mainly based on a genital clinical score of 3.

4.1.1.1 Symptoms

Suffering and discomfort associated with genital cGvHD was self-reported as described in 3.5. Compared to women without genital cGvHD, dryness, smarting pain, pain-when-touched, and dyspareunia was significantly more common in women with genital cGvHD ($p<0.05$).

4.1.2 Depression and sex-related personal distress

Data on depression and sex-related personal distress were not published in Paper I. There was no statistical difference between women with or without genital cGvHD as per the BDI and FSIDS instruments. To the BDI question No 21 about sexual interest, 17 of 37 women (46%) answered the alternatives “I am much less interested in sex now than earlier” or “I have completely lost my interest in sex.”

4.1.3 Histopathological examination

In women with clinical cGvHD at the location of the biopsy (n=14) the histopathological grades were evenly distributed from 0 to 3, i.e. “normal” to “confirmed” cGvHD. Biopsies were more often confirmative in mucosa without clinical cGvHD, with 17 of 24 biopsies graded 0-1 (normal - possible cGvHD). In 4 women with clinical vaginal cGvHD, biopsies from clinically normal vulvar mucosa showed grade 2-3, consistent with cGvHD. Three other biopsies were graded 2-3 (consistent with cGvHD) although they were obtained from clinically normal vulvar mucosa (the vagina was clinically normal). Taken together, we found no relationship between clinical findings and histopathology.

4.1.4 Effects of estrogen treatment

At their first visit, all women were in menopause, 11 natural and 31 premature as a consequence of the conditioning treatment. Twenty-nine women were on hormonal replacement therapy: 21 of these women had oral estrogen/gestagen, and 8 women were on local estrogen therapy. Thirteen of the 29 patients on HRT, and all the women not receiving hormonal therapy (n=13), needed complementary local estrogen because of mucosal atrophy.

At the second visit, genital symptoms and signs typically associated with estrogen deficiency – dryness, pain-when-touched, smarting pain – were alleviated in approximately one third of the women. The number of women reporting dyspareunia was reduced from 52% to 32% among those having coitus. Seven women with clinical signs of estrogen deficiency had no symptoms at their first visit, but nevertheless reported more well-being in their genitals when local estrogen treatment was used.

The main differences in signs at the second visit were the decrease of the thinness, and an increase of normal texture of the mucous membranes. However, estrogen treatment did not affect the diagnostic or distinctive signs of genital cGvHD, including reticular white lines and teleangiectatic areas, vaginal sore strings, synechiae or stenosis. See table 4.

Table 4. Genital signs before and after local estrogen therapy. In Study I, all participating women, n=42, underwent gynecologic examination, were prescribed complementary local estrogen therapy at their first study visit. Diagnosis of genital cGvHD was made at least 6 weeks later at a second visit.

Signs	Genital status	
	Before local estrogens	After local estrogens
Vulvar signs		
Normal	16	20
Red spots	18	14
White spots	9	8
Reticular white lines	6	7
Teleangiectatic areas	0	1
Vulvar mucous membrane		
Normal	6	14
Thin	35	27
Dry	7	6
Reddened	4	3
Edematous	1	2
Vulvar wounds		
Fissures	5	4
Vaginal signs		
Normal	23	26
Red spots	13	10
White spots	3	3
Teleangiectatic areas	2	4
Reticular white lines	2	2
Vaginal mucous membrane		
Normal	19	30
Thin	23	11
Dry	3	1
Edematous	1	1
Thick and non-elastic	3	1
Reddened	2	1
Synechia/ stenosis		
No stenosis/synechia	22	22
Synechia vulvae	6	6
Synechia vaginae	13 ¹	12
Sore vaginal string	5	5
Partial stenosis of the vagina	5	5
Total stenosis of the vagina	4	4

¹One small synechia was broken by the gynecological examination at the first visit and was completely disappeared after estrogen treatment.

One woman could have more than one sign.

4.1.5 Corticosteroids and genital cGvHD

The presence of genital cGvHD was associated with systemic corticosteroid treatment of extra-genital cGvHD ($p = 0.001$), older age ($p = 0.07$), and transplant from a sibling donor ($p = 0.002$).

Thirteen of the 22 women with genital cGvHD were on systemic corticosteroid therapy with extragenital cGvHD as the indication in all cases. Five women had only genital cGvHD, 3 of them with clinical score 3. The rate of genital cGvHD was similar among the women with follow-up on both sides of the median time after transplant.

4.1.6 Summary

The assessments in this cross-sectional study revealed that symptoms and signs of genital cGvHD were a common scenario, and often not correctly diagnosed or treated. The findings indicated a high prevalence of genital cGvHD similar to that of ocular and oral cGvHD. Genital cGvHD was associated with serious consequences for sexual life because of pain and may be present without any other cGvHD and, actually, even without genital symptoms if the women did not have intercourse. We found no relationship between clinical findings and histopathology.

4.2 Paper II. Androgens in women after allogeneic hematopoietic cell transplantation: impact of chronic GvHD and glucocorticoid therapy.

4.2.1 Background

Chronic GvHD and its effects on genital mucosa may cause sexual dysfunction, and premature ovarian failure affects all women after alloSCT. We did the clinical observation that sexual dysfunction prevailed also after substitution with local and systemic estrogens. Testosterone is positively associated with sexual function (54), and data on post-transplant levels are scarce. Dehydroepiandrosterone sulfate has after conversion multiple effects on physical well-being, including sexuality. However, data on androgen levels in female patients after alloSCT are limited (37, 55).

In this study we assessed serum T, free T and DHEAS in allografted women with/without cGvHD, and with/without treatment with GC.

4.2.2 Hypotheses

- Women after alloSCT have lower androgens than age-matched healthy women.
- Women after alloSCT have lower androgens compared to women after autoSCT

- Glucocorticoid treatment is associated with low androgens
- Chronic GvHD *per se* increases the risk of subnormal level of T and DHEAS.

4.2.3 Patients. Controls.

For this study 205 women (4 groups) were included.

1. *Allo-SCT*, n=65, age 51 (21-74) yrs; 55 (3–194) months post alloSCT
 - Ongoing cGvHD (n=33)
 - i. with prednisolone (n=23)
 - ii. without prednisolone (n=10)
 - No cGvHD (n=32), no corticoid steroids
2. *Auto SCT*, n=20; age 55 (34-65).
3. *Glucocorticoid group*, n=26; age 62 (23-82). Prednisolone for benign disease
4. *Controls*, healthy women, n=94; age 49 (26-74).

4.2.4 Methods

All samples for hormone determination were assessed at accredited laboratories.

Statistics:

- Androgen levels were compared between the 4 main groups.
- In a second step, the effect of cGvHD and ongoing GC therapy was studied by dividing the alloSCT group into subgroups, comparing them internally and with the other groups.

4.2.5 Results

- *AlloSCT group*. All androgens analyzed – T, free T and DHEAS – were lower compared to control and autoSCT groups ($P < 0.05$ for all).
 - AlloSCT/no-GvHD group: Free T similar, DHEAS lower than controls.
 - AlloSCT/GvHD group: Significantly lower androgen levels than alloSCT/no GvHD group; free T (3.2 vs 7.2 pmol/L, $p = 0.0001$), DHEAS (0.5 vs 1.7 $\mu\text{mol/L}$, $p < 0.0001$).
 - AlloSCT/cGvHD *with* GC group (n=23). Free T & DHEAS lower than in any other group.
 - AlloSCT/cGvHD/ *without* GC group (n=10): Both free T and DHEAS were lower than controls: 4.9 vs 8.6 pmol/L, $p = 0.004$, and 1.5 vs 2.5 $\mu\text{mol/L}$, $p = 0.0004$, respectively. But not significantly lower than the no-GvHD group: free T; $p = 0.068$, DHEAS $p = 0.18$.
- *AutoHCT group*: T and free T were higher, but DHEAS similar, to controls
- *Glucocorticoid group*: T, free T and DHEAS lower compared to control and autoSCT groups ($p < 0.05$ for all).

4.2.6 Summary

- AlloSCT but not autoSCT is associated with low androgens.
- Glucocorticoid treatment, is associated with low androgens.
- The lowest levels of androgens were seen in allografted women with cGvHD and GC treatment.
- Women after alloSCT with no cGvHD had similar free T, but lower DHEAS compared to controls.
- Compared to the no-cGvHD group, women with cGvHD but no GC treatment (n=10), had a tendency to lower T (p=0.068), but similar DHEAS.

4.3 Paper III. A prospective study of female genital chronic Graft-versus-Host Disease symptoms, signs, diagnosis, and treatment.

The hypothesis of this study of allografted women (n=41) was that regular gynecological surveillance, in close contact with the hematologist, might provide early diagnosis and intervention to reduce the problems associated with genital cGvHD and also decrease the risk of serious sequel.

Other questions that we sought to answer:

- What are the debut signs and symptoms of genital cGvHD?
- What is the relationship in time between genital and extra-genital cGvHD?

4.3.1 Rationale

In our first study (Paper I), we could see from medical reports that 8 of the 42 women had developed total vaginal stenosis in the period up to 36 months after alloSCT. Consequently, this study included regular gynecological examination with short intervals during the first year and longer intervals during the next 2 years and prompt local treatment at first sign of cGvHD. The aim was to try and reduce the incidence of severe genital cGvHD. The plan included close communication between gynecologist and hematologist.

4.3.2 Diagnosis and treatment

Genital cGvHD was diagnosed in 27 (66%) women with an incidence of 56% at 12 months and 66% at 36 months. Median time to first sign was 6 (1-30) months. Extra-genital cGvHD was observed in 21 women with genital cGvHD and 10 without genital cGvHD. Four women had their first sign of genital cGvHD later than 21 months. Thirteen women started local immunosuppressive treatment of genital cGvHD median 9 (1-31) months after alloSCT. Another 14 women did not get local immunosuppressive treatment because of pathological Pap smear, infection, relapse, fluctuation or disappearance of signs, or no symptoms.

4.3.3 Signs, symptoms, treatment

At first sign of genital cGvHD itching, dryness, smarting pain, dyspareunia and discharge were common symptoms. Notably, 30% of the women were symptom-free. The most common signs at first diagnosis were reticular white lines and synechiae, red-and-white spots and thin mucous membranes. More advanced fibrosis or partial stenosis were later findings. Despite treatment, we observed that signs and symptoms became chronic.

4.3.4 Five women with 7 circumferential fibrous vaginal bandings or partial vaginal stenoses and 2 total stenoses.

Five women had partial stenosis or circumferential fibrous vaginal banding. See Figure 1 concerning localization, thickness and duration of the stenoses. All 5 were prescribed local immunosuppressive treatment (see Treatment schedule, Table 2, Paper III).

Patient No. 1 no GC treatment. Signs of synechiae vaginae, and signs of cGvHD in mouth and eyes, occurred at 9 months during tapering of ciclosporin. Local immunosuppressive treatment started at 9 months. At 18 months she had sore vaginal strings and a circumferential fibrous vaginal banding, however still with few symptoms. The banding disappeared, but the sore vaginal strings continued and the synechiae remained at 36 months. The local immunosuppressive treatment continued with successive tapering and at 36 months she was on maintenance treatment.

No. 2, her genital cGvHD started with reticular white lines and fissures in the vulva and erythema in the vagina at 1 month after alloSCT, from 3 months she had active cGvHD in mouth and eyes. Full local genital immunosuppressive treatment started at 1 month, was reduced to maintenance treatment at 6 months. At 12 months full treatment was started again because of partial stenosis at 12-34 months, synechiae vaginae at 17-36 months and sore vaginal string at 19-27 months. (Like the others with active genital cGvHD she was seen also outside the protocol for clinical reasons). See figure 1. At 36 months she had red-and-white spots, was reddened in the vulva, had synechiae vaginae and was on maintenance local immunosuppressive treatment. Parallel with this the woman was treated with GC 25 mg when ciklosporin had been withdrawn at 16 months, successively tapered to 7.5 mg at 36 months.

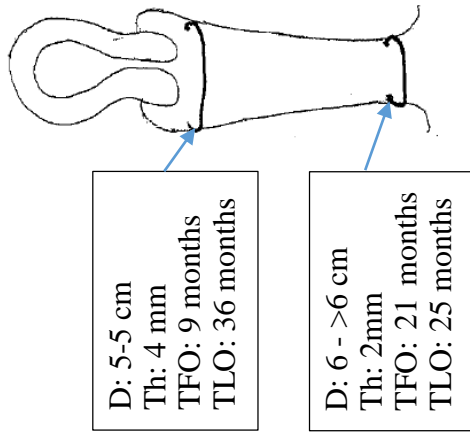
No. 3 never had any extra-genital cGvHD but got her first signs of genital cGvHD at 9 months, sore vaginal string and synechiae vaginae. Daily local immunosuppressive treatment was prescribed. Two weeks later there was a partial stenosis. The woman had only used the treatment once a week. On adequate local treatment she got better and then worse again. At 21 months she had circumferential fibrous vaginal banding on two different levels in the vagina, as shown in figure 1. The cooperative hematologist prescribed GC

20 mg/day which 2 months later was tapered to 7.5 mg every other day. At 36 months the dosage was 2.5 mg every other day. The local immunosuppressive treatment had continued. The outer circumferential fibrous vaginal banding disappeared. The woman was seen at the clinic after the study. The inner partial stenosis became successively wider and when coitus started at 41 months post alloSCT there was no dyspareunia.

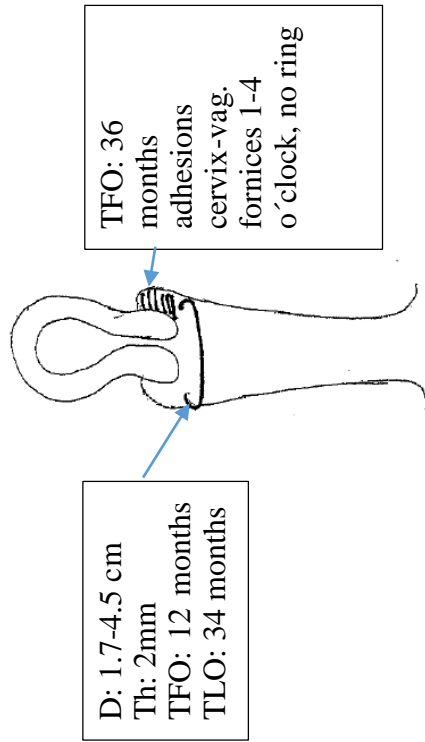
The two total stenoses both had the form of a membrane covering the cervix. Woman No. 4 in Figure 1 started local immunosuppressive treatment of the vulva because of distinctive signs and extra-genital cGvHD at 4 months, tapered at 6. Because of extra-genital cGvHD with global score 3 systemic GC were prescribed 50 mg/day at 6 months, at 9 months 15 mg and one month later 10 mg/day. At 9 months post alloSCT the woman re-started scheduled local treatment of the vagina because of sore vaginal string and synechiae vaginae et vulvae. At 12 months the systemic GC were 12,5 mg/day. The genital cGvHD signs included now also partial and complete vaginal stenosis, and fissures and reticular white lines in the vulva. She had extra-genital cGvHD in the mouth, eyes and skin. At 13 months post alloSCT she relapsed and died one month later.

The genital cGvHD of woman No. 5 started slowly with reticular white lines in the vulva at 12 months with no symptoms. Local estrogen treatment was used. GC treatment was 5 mg/day. At 18 months the genital signs were vaginal sore string, synechiae and partial stenosis, vulvar red-and-white spots and reticular white lines. Local immunosuppressive treatment of the vulva and vagina was started according to schedule. The synechia vaginae disappeared, but sore vaginal strings continued and another circumferential fibrous vaginal banding developed. At 36 months she had vaginal strings and fibrous vaginal banding on two different levels as seen in Figure 1 together with the membrane covering the cervix. She had extra-genital cGvHD in the eyes with clinical score 1 and systemic GC 5 mg/day.

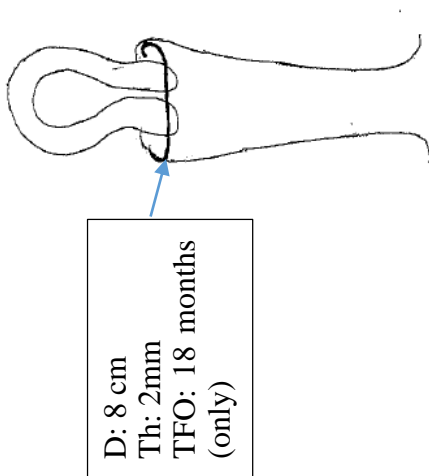
Pat nr 3



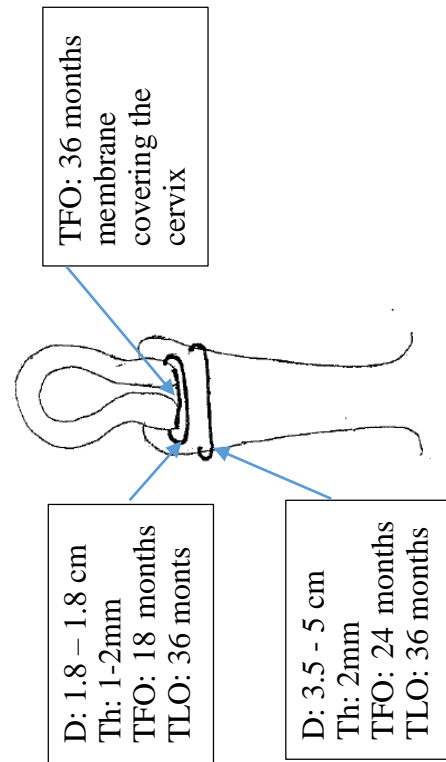
Pat nr 2



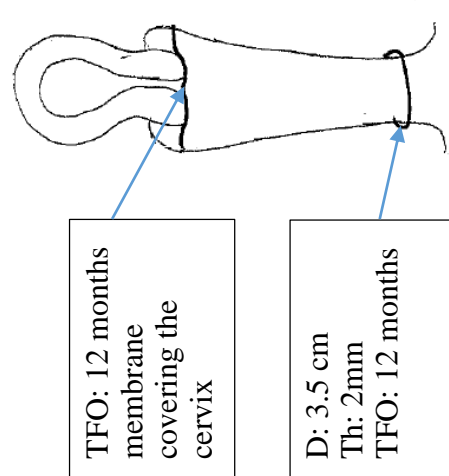
Pat nr 1



Pat nr 5



Pat nr 4



Relapse at 13 months,

Dead at 14 months

Figure 1. The vaginal stenoses in study III, a prospective study up to 36 months after alloSCT, partial stenoses or circumferential fibrous vaginal banding (n=5) and total stenoses (n=2) (pt No 4 and 5).
D, diameter of fibrous ring around the vaginal lumen (first – last observation); cm, centimeter; Th, thickness of the banding; mm, millimeter; TFO, time for first observation of the ring; TLO, time for last observation of the ring. All observations are months after alloSCT. The total stenoses had the form of complete covering of the cervix.

4.3.5. Depression and sex-related personal distress

The presence of genital cGvHD was associated with higher BDI at 6 and 24 months after alloSCT ($p=0.011$ and $p=0.024$ respectively). FSDS did not differ significantly between the groups with or without genital cGvHD. In the total study cohort, maximum FSDS (≥ 15) was reported in 29% of the responders at 24 months after alloSCT, compared with 18% before and 36 months after alloSCT.

4.3.6 Summary

The incidence of genital cGvHD is high and typically develops during the first year after alloSCT. Signs and symptoms fluctuate with or without local treatment, and the disease is likely to last for more than 36 months. Treatment intervention may halt progression to severe genital cGvHD.

4.4 Paper IV. Long-term follow-up of genital chronic Graft-versus-Host Disease in females after allogeneic stem cell transplantation.

We hypothesized that signs and symptoms of genital and other cGvHD may alleviate over time.

4.4.1. Study design

This was a long-term, follow-up study on surviving women ($n=38$) from Study I. Median time since transplant was 14.5 (10-19.3) years, and 8.4 (5.8-12) years after inclusion in Study I. Each woman was evaluated by an ophthalmologist (KS), a gynecologist (ESK) and 1 of 2 hematologists (MB or YB), on the same day. Diagnosis and stage of cGvHD was based on the NIH 2014 guidelines. For the comparisons between Study I and IV, data from Study I were examined and recategorized as per NIH 2014.

4.4.2 Results

Twenty-five women (66%) had stopped using adequate local estrogen therapy prescribed as part of Study 1, and subsequently had symptoms from atrophic genital mucosa at the late follow-up.

Ocular cGvHD was seen in 24 women, genital cGvHD in 22, and extra-genital (including eyes) in 25 (missing data, $n=1$). Concurrent genital and extra-genital cGvHD were identified in 16 women. See Table 5. Six women had only genital cGvHD, and 9 only extra-genital cGvHD. Six women (16%) had no cGvHD.

Table 5. Genital and non-genital cGvHD in all surviving women from Study I (original study) and in Study IV.

	Original study (n=38)	Follow-up study (n=38)	P value
Genital cGvHD (Yes/No)	19/19	22/16	0.646
Non-genital cGvHD (Yes/No)	22/16	¹ 25/12	0.476
Chronic GvHD (Yes/No)	28/10	31/6	0.399
Oral glucocorticoids (Yes/No)	13/25	11/27	0.805
GvHD global score (NIH 2014)			
None	10	6	
Mild	7	6	
Moderate	5	4	
Severe	16	21	
None/Mild vs Moderate/Severe	17/21	¹ 12/25	0.345

¹ One patient, data missing on extra-genital cGvHD in follow-up study.

cGvHD, chronic Graft-versus-Host Disease, NIH, the National Institutes of Health

Three women with genital cGvHD in Study I had no genital cGvHD at follow-up; but two of them had extragenital cGvHD in Study IV (missing data, n=1).

Six women with no genital cGvHD in Study I, now had signs of genital cGvHD. Two of these 6 women had labial fusions and one of these women had also a stenosis of the cervix. Apparently, their genital cGvHD had developed between the two studies i.e. between 5-12 years and 9-17 years after alloSCT, respectively. Compared with findings in Study I, both these women had more extra-genital cGvHD in the follow-up study: in 1 woman mouth, eyes, and joints were affected; the second woman showed involvement of eyes, gastro-intestinal tract, skin, mouth, and joints.

All cervical smears were normal. However, HPV types associated with development of cervical cancer were found in 3 women.

4.4.3 Treatment

Local treatment was not part of the study protocol. Local immunosuppressive ointments, clobetasol (0.05%) and tacrolimus (0.1-0.03%) were prescribed following a structured treatment schedule, modified according to signs and symptoms. See Table 2 in Paper III.

4.4.4 Depression and Sex-related personal distress

Using the BDI and FSIDS instruments, there were no statistical differences between women with or without genital cGvHD, although a comparison of the original and follow-up studies indicated a tendency to improvement with respect to both BDI and FSIDS. However, dyspareunia score 2-4 (*sometimes to always*) was self-reported by 15 of 27 (56%) of women with genital cGvHD having or tried coitus.

4.4.5 Summary

Women's genital mucosa is a major target for cGvHD. Structural signs such as vaginal stenosis rarely disappear over time. Genital cGvHD may debut many years after transplant. The use of local estrogen therapy continues to be important. Obviously, cGvHD rarely heals even after a very long time. However, depressive symptoms seem to decline over time.

Looking at each woman's global cGvHD in the original and follow-up study (Table 5, Paper IV), we observed movements between global severity scores in many patients. Movements were noted in both directions, and reasonably reflect the clinical experience that signs – and also symptoms – of cGvHD in affected tissues or organs may differ in severity over time.

5 DISCUSSION

5.1 Introduction

Study I describes the prevalence of female genital cGvHD, 52%, median 80 (13-148) months after alloSCT.

Study II accounts for androgen levels in women after alloSCT with/without cGvHD, and on glucocorticoids. There are 3 control groups: i) women after autoSCT; ii) women on glucocorticoid treatment for non-malignant disease; iii) age-matched healthy women.

Study III is a prospective population-based study of genital cGvHD with cumulative incidence 56% at 12 months and 66% at 36 months.

Study IV is a long-term follow-up study of surviving women from Study I. Median follow-up was 174 (120-232) months after alloSCT with prevalence cGvHD the same as in Study I.

See Table 2, page 13, for summary of the studies.

5.2 Diagnosing cGvHD

The diagnoses of genital and extra-genital cGvHD were made according to NIH 2005 in Study I, II and III, and to NIH 2014 in Study IV. In Study III most (85%) genital cGvHD debuted during the 1st year after alloSCT; later debuts occurred up to 30 months. Even later debuts were seen in Study IV, 5-17 years after transplant. Earlier investigators have reported lower figures; Zantomio found 35% at 1 year, 49% at 2 years (20). Our higher figures may be due to a strict application of the NIH criteria. NIH 2014 stressed all fibrotic signs both in the vagina, as NIH 2005 did, and the vulva with labial fusion, and clitoral hood agglutination as examples. Vulvar erythema and lichen sclerosus were also considered as diagnostic signs.

The diagnosis could be difficult, even if the two gynecologists always tried to examine the woman together. Reticular white lines could be vague, both in the genitals and in the mouth. Erythema might mimic local infection. The assessments of extra-genital cGvHD in Study I and III were obtained from medical records, with some risk of non-uniform cGvHD grading. Sometimes time solved the problem with appearance of more distinct diagnostic signs, or disappearance of the vague suspected signs. We assumed that red and white spots, described in Study III and IV are identical to the “local mucosal paleness” described by Hirsch (22). They give the mucous membrane a very characteristic mottled appearance. Actually, in Study IV all women with red and white spots also had other distinctive and diagnostic signs of genital cGvHD.

In Study IV, two women without genital cGvHD in Study I had developed labial fusions, a fibrotic sign of genital cGvHD, and more extra-genital cGvHD. Their genital cGvHD had developed between the two studies i.e. between 5-12 years and 9-17 years after alloSCT. Thus, very late debuts of genital cGvHD.

As is pointed out by Jagasia, there is a want of distinction between active disease and a fixed deficit like a fibrotic stenosis from prior tissue damage (45). Four women in Study IV illustrate this. Photos from Study I of 4 women without the diagnosis genital cGvHD were compared with the findings of their labial fusions in Study IV. We found that they also had labial fusion in the first study without any symptoms. For 2 women the photos showed the same labial fusions in both Studies I and IV. In the follow-up study one woman had no symptoms and the other had smarting pain, itching and dryness, and often fissures. The labial fusion in the first woman might be an end stage of passed genital cGvHD, and not an active disease (45). The genital cGvHD in the second woman seems to be an active disease according to her fissures and symptoms.

The photos of the other 2 women with labial fusion in the original study revealed that a development to more severe labial fusions had occurred until the follow-up study. Both had developed bilateral labial fusion along the whole lips and one had also clitoral hood agglutination. Neither of them had extra-genital cGvHD. Both needed local estrogen. Their genital cGvHD might be a more progressive type of genital cGvHD.

Stratton concluded that genital cGvHD always starts in the vulva (21). However, in our Study III the first sign of genital cGvHD was in the vulva in 18 women, in the vagina in 4, and in both the vulva and the vagina in 5. Throughout the study 15 of 27 women had cGvHD in both the vagina and the vulva. One woman had only vaginal cGvHD during the study, 11 only vulvar cGvHD. Preemptive treatment of the vagina, simultaneously with treatment of vulvar genital cGvHD, did not hinder the development of vaginal cGvHD.

5.3 Effects of estrogen

Estrogen did not affect the signs of genital cGvHD in the mucous membranes in Study I (see Table 4). In Study III, treatment of genital cGvHD with clinical score 0-1 started with local estrogen in 9 women. During estrogen treatment, 6 women with diagnostic or distinctive signs of cGvHD in the vulva and/or the vagina cleared all signs of cGvHD. Reversible signs included teleangiectatic areas, reticular white lines, synechiae, reddened mucosa, fissures, red-and-white spots, and sore vaginal string. There was no corresponding effect of estrogen in Study I. This difference in response to estrogen treatment was at least partially due to the differences in follow-up time since transplant between the two groups. In the prospective Study III, the transplant was recent and the signs of genital cGvHD even more recent, whereas in Study I the women had long duration since transplant, and the signs were probably older than in Study III. The fresher signs in Study III may have been easier to affect. However, immunosuppressive therapy is more effective with concomitant estrogen treatment (21). Time is another factor influencing what is happening. The genital cGvHD in itself is a pendulating disease as illustrated by the fact that of the 6 women mentioned above one woman had had vulvar cGvHD 21 and 24

months before that “only-estrogen-treated” period, and in another woman the diagnosis of vulvar cGvHD reappeared 9 months after the described period. See table 5 in Paper IV.

In Study IV the women were seen only once. Sixty-six percent had stopped local estrogen therapy and had developed atrophic mucous membranes. Their self-reported genital symptoms were a mix of atrophic symptoms and symptoms from genital cGvHD. Therefore genital symptoms could not be ascribed any of the causes atrophia or genital cGvHD, e.g. the dyspareunia described in 4.4.4.

5.4 Local immunosuppressive treatment

We have chosen a treatment with both the ointments clobetasol (glucocorticosteroid group IV) and tacrolimus (calcineurin inhibitor) with the intention of achieving a maintenance treatment with tacrolimus together with local estrogen. The intention was to avoid clobetasol once the mucous membranes had improved and perhaps become more sensitive to the attenuating effect of the corticosteroid. Temporary burning sensations were reported at start of treatment by 9 women in Study III. However, none of them had to stop using ointment because of that. We did not prescribe immunosuppressive ointment more than once a day. Other authors suggest start twice a day on clobetasol (29). A treatment with both ointments, i.e. clobetasol and tacrolimus, once every day would also be an alternative aggressive local treatment, hopefully reducing the need for systemic corticosteroid treatment or surgery. When using local tacrolimus, analyses of whole blood levels should be performed to avoid nephrotoxic levels. Only once we had to reduce the genital use of tacrolimus because of nephrotoxic risk. We have seen the fibrotic process respond to treatment as long as the woman continued to treat. As soon as the treatment is tapered, the fibrotic process starts again, see e.g. patient No 3 in 4.3.4. If the local treatment fails, systemic corticoids are indicated after agreement with the hematologist.

5.5 Other treatments for genital cGvHD

Fibrotic vaginal cGvHD was treated with a dilator, if coitus without dyspareunia was not possible, or wished for. To avoid the development of vaginismus the woman was advised to use the dilator with an adequate amount of lubricant, inserting the dilator very slowly to avoid pain, and waiting for muscular contractions to relax before continuing insertion.

Surgery was not performed in any of the studies, only before Study I and between Studies I and IV. Surgically opening of vaginal stenoses with only estrogen treatment afterwards, lead to prompt relapses. Obviously, surgery is a temporary solution; it does not cure genital cGvHD. In our studies 5 women have been surgically treated for their stenoses 8 times in spite of adequate treatment after. As soon as the woman has stopped her local treatment, the fibrosis relapses again.

There is a necessity of immuno-suppressive treatment after surgery.

5.6 Treatment risks

Local immunosuppressive treatment brings risks. HPV may reactivate and stimulate development of squamous cell atypias into malignant cells(32). That is one reason for starting the treatment of genital cGvHD with local estrogen only, or waiting and seeing. We have seen the signs of genital cGvHD disappear without further treatment, as described above, or remain at the same level for a long time without deteriorating (Study III). But we have also seen fast impairment as described among the women with partial stenosis in Study III. All our women were attending the Swedish cervical cancer screening program before and during the studies, and were investigated, when atypical cells were found, and treated when the histopathological assessment showed pathology. During the Studies I, III and IV no genital malignancy was found. Fourteen women in Study IV, and 15 in Study III had had atypical cells; of those 22 women, 11 from each group, were also diagnosed with genital cGvHD. Between Study I and IV, 5 women had been treated for 6 different malignancies, 3 in the oral cavity, 2 basaliom and 1 colon cancer; a reminder of the increased risk for malignancies after alloSCT (34).

5.7 Depression and sex-related personal distress

We used the completed BDI and FSIDS questionnaires at each study visit to discuss the answers given by the woman in relation to depression and sexual function in the Studies I, III and IV. This facilitated the giving of sexual advice to the women, when wanted, and the diagnosing of depression for referral and treatment.

Our approach thus included a frequent psychosexual intervention that might have influenced the results, at least in the prospective Study III. The women in our studies showed considerably less sexual distress (14-29%) than was found in other studies, where 66-80% of female survivors of alloSCT were affected by sexual distress in addition to dyspareunia, problems with sexual dysfunction, libido, sexual activity and satisfaction (35, 56, 57). According to Humphreys assessing and addressing depression at pre-transplant stage may reduce sexual dysfunction in long-term survivors. The depression frequency decreased from 28% before transplant to 14% at 36 months. Mosher et al. found that 15% of the sample of alloSCT men and women reported moderate to severe depressive symptoms, and these symptoms were higher among allogeneic transplant recipients and those with lower functional status (58). Between the original Study I and the follow-up Study IV moderate to severe depression was lowered from 18% to 5%. The personal sexual distress changed from 55% to 37%.

5.8 Clinical and global scoring

The clinical scoring according to NIH 2014, applied in Study IV, is more adequate and easier to implement than NIH 2005. However, the scoring of 3 for all levels of fibrosis implies that many women by definition will have a severe global score even although they have minor signs of fibrosis. Symptom-free labial fusion of the vulva without any extra-genital cGvHD will reasonably not affect survival.

5.9 Fertility

Fertility and pregnancies were not part of the protocol in any of our studies. Salooja et al. 2001 reported 312 pregnancies in 232 patients or partners of patients among 19412 alloSCT and 17950 autoSCT patients, i.e. 0.6% (59). After our studies 3 women have become pregnant, 2 spontaneously and 1 after assisted fertilization. The possibilities to help couples with assisted fertilization is increasing to-day. However, the infertility that follows alloSCT is a problem and a life-long sorrow for younger allografted women.

5.10 Fibrotic genital cGvHD

The finding that 2 women with vaginal stenosis, 1 total and 1 partial, in the follow-up Study IV did not have other cGvHD, implies that the genital fibrotic process may not be reversible. In Study III however, synechiae were signs disappearing on only estrogen treatment, and circumferential fibrous vaginal bandings disappeared on local immunosuppressive treatment (see Figure 1). It seems that there are different kinds of fibrotic genital cGvHD. A chronic variant that is active as soon as treatment tapers, and relapses without tendencies to reverse spontaneously. Another variant of fibrotic genital cGvHD is partial stenosis, disappearing on treatment without relapse during observational time. And then there is the very slow developing fibrosis of the vulva in the form of labial fusions. We have not studied if this process occurs in parallel with inflammatory periods of sore mucous membranes with lichen planus-like signs. Many women report that those periods often are connected with viral infections e.g. in the form of upper respiratory infections.

5.11 Androgens and cGvHD

Hovi found that low DHEAS were a consistent finding during GC therapy for cGvHD, and ask if that might be due to adrenal insufficiency and/or dysfunctional ovaries (39). In our Study II, androgen-producing ovarian cells seemed to be resistant to high-dose chemotherapy, but sensitive to GC, and possibly also to cGvHD. In non-transplanted women with GC therapy, androgens were significantly lower than in controls. This suggests that the GC-effect on adrenals and a secondary adrenal insufficiency is the cause of the lowered androgens seen in these non-transplanted women. However, we do not

know if low androgens in women with cGvHD and on GC treatment, also is dependent on secondary adrenal insufficiency, or the cGvHD effect on the adrenals, or both. AlloSCT, irrespective of cGvHD and GC therapy, was associated with low DHEAS levels. This might indicate an effect from the alloSCT in itself on both ovaries and adrenals.

5.12 Summary

To our knowledge **Study I** is the first to try to establish a population-based prevalence of female genital cGvHD, while **Study III** is the first population-based prospective study of genital cGvHD. In **Study IV** a long-term follow-up of surviving women from Study I, median 8.4 years after the original study, is described. **Study II** is a contribution to a field not yet much described, androgen levels in women after alloSCT.

6 CONCLUSIONS

Chronic cGvHD is the major cause of morbidity after alloSCT, and contributes to NRM. Female genital mucosa is a major target for cGvHD.

Study I. We observed that the prevalence of genital cGvHD in our cross-sectional, population-based study was similar or higher than in previous reports.

Study IV. This was a long-term follow-up of surviving women from Study I. The time lag between the two examinations was median 8.4 (5.8-12) years. The main finding was that prevalence and severity of chronic GvHD was almost identical to findings in Study I. One third of the women was still on systemic corticosteroids. Two women had a very late debut of genital cGvHD.

Study III. In this prospective study, allografted women were regularly examined and treated for 3 years. Cumulative incidence of genital cGvHD was 66%. However, in spite of local treatment, 5 women developed partial vaginal stenoses. But the incidence of total stenoses was seemingly lower compared with the findings in Study I (2 *versus* 8). The activity of the genital cGvHD was fluctuating with periods of more or less signs and symptoms. HRT should be prescribed to women with premature menopause. Local estrogen treatment do relieve symptoms and facilitate local immunosuppressive treatment of genital cGvHD.

Study II. Androgen levels were assessed in a cohort of allografted women, and in 3 control groups. The main finding was that women on GC treatment had low androgens, with an additive effect of cGvHD.

We conclude that genital cGvHD after alloSCT principally debuts the first year after transplant, and is a common and unresolved constant clinical problem associated with impaired sexual life, and inconvenience in terms of necessary local treatment increasing the risk for genital malignancy. The conclusion is the necessity of continuous contact with a dedicated gynecologist.

7 FUTURE PERSPECTIVES

Reducing frequency and severity of genital cGvHD is a major challenge for the future. Ways to pursue that aim may include:

- 1) A more frequent use of bone marrow instead of peripheral blood stem cells.
- 2) Improved pre-transplant prophylaxis against cGvHD by e.g. anti-thymocyte globulin in doses adapted to a risk-benefit analysis.
- 3) Close contacts between transplant physicians and experienced gynecologists.
- 4) Systemic corticosteroids if aggressive genital cGvHD signs appear.
- 5) Guidelines for the prevention and treatment of genital cGvHD, HPV vaccination and a schedule for more intensive local immunosuppressive therapy.

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REFERENCES

1. Horowitz MM, Gale RP, Sondel PM, Goldman JM, Kersey J, Kolb HJ, et al. Graft-versus-leukemia reactions after bone marrow transplantation. *Blood*. 1990;75(3):555-62.
2. Koreth J, Schlenk R, Kopecky KJ, Honda S, Sierra J, Djulbegovic BJ, et al. Allogeneic stem cell transplantation for acute myeloid leukemia in first complete remission: systematic review and meta-analysis of prospective clinical trials. *Jama*. 2009;301(22):2349-61.
3. Russell NH, Kjeldsen L, Craddock C, Pagliuca A, Yin JA, Clark RE, et al. A comparative assessment of the curative potential of reduced intensity allografts in acute myeloid leukaemia. *Leukemia*. 2015;29(7):1478-84.
4. Scott BL, Pasquini MC, Logan BR, Wu J, Devine SM, Porter DL, et al. Myeloablative Versus Reduced-Intensity Hematopoietic Cell Transplantation for Acute Myeloid Leukemia and Myelodysplastic Syndromes. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2017;35(11):1154-61.
5. Buccisano F, Hourigan CS, Walter RB. The Prognostic Significance of Measurable ("Minimal") Residual Disease in Acute Myeloid Leukemia. *Current hematologic malignancy reports*. 2017;12(6):547-56.
6. Tomblyn M, Chiller T, Einsele H, Gress R, Sepkowitz K, Storek J, et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation*. 2009;15(10):1143-238.
7. Cordonnier C, Einarsdottir S, Cesaro S, Di Blasi R, Mikulska M, Rieger C, et al. Vaccination of haemopoietic stem cell transplant recipients: guidelines of the 2017 European Conference on Infections in Leukaemia (ECIL 7). *The Lancet Infectious diseases*. 2019.
8. Mohty M, Apperley JF. Long-Term Physiological Side Effects After Allogeneic Bone Marrow Transplantation. *Hematology*. 2011;2010(1):229-36.
9. Billingham RE. The biology of graft-versus-host reactions. *Harvey lectures*. 1966;62:21-78.
10. Kroger N, Solano C, Wolschke C, Bandini G, Patriarca F, Pini M, et al. Antilymphocyte Globulin for Prevention of Chronic Graft-versus-Host Disease. *The New England journal of medicine*. 2016;374(1):43-53.
11. Sung AD, Chao NJ. Concise review: acute graft-versus-host disease: immunobiology, prevention, and treatment. *Stem cells translational medicine*. 2013;2(1):25-32.
12. Cooke KR, Luznik L, Sarantopoulos S, Hakim FT, Jagasia M, Fowler DH, et al. The Biology of Chronic Graft-versus-Host Disease: A Task Force Report from the National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation*. 2017;23(2):211-34.
13. Lee SJ. Classification systems for chronic graft-versus-host disease. *Blood*. 2017;129(1):30-7.
14. Gratwohl A, Sureda A, Cornelissen J, Apperley J, Dreger P, Duarte R, et al. Alloreactivity: the Janus-face of hematopoietic stem cell transplantation. *Leukemia*. 2017;31(8):1752-9.
15. Garnett C, Apperley JF, Pavlu J. Treatment and management of graft-versus-host disease: improving response and survival. *Therapeutic advances in hematology*. 2013;4(6):366-78.
16. Corson SL, Sullivan K, Batzer F, August C, Storb R, Thomas ED. Gynecologic manifestations of chronic graft-versus-host disease. *Obstetrics and gynecology*. 1982;60(4):488.
17. Lonnqvist B, Brune M. Hydrocortisone ointment intravaginally for dysparunia in chronic GVHD. *Bone marrow transplantation*. 1999;24(5):573.
18. Spinelli S, Chiodi S, Costantini S, Van Lint MT, Raiola AM, Ravera GB, et al. Female genital tract graft-versus-host disease following allogeneic bone marrow transplantation. *Haematologica*. 2003;88(10):1163-8.

19. Spirya LB, Laufer MR, Soiffer RJ, Antin JA. Graft-versus-host disease of the vulva and/or vagina: diagnosis and treatment. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation*. 2003;9(12):760-5.
20. Zantomio D, Grigg AP, MacGregor L, Panek-Hudson Y, Szer J, Ayton R. Female genital tract graft-versus-host disease: incidence, risk factors and recommendations for management. *Bone marrow transplantation*. 2006;38(8):567-72.
21. Stratton P, Turner ML, Childs R, Barrett J, Bishop M, Wayne AS, et al. Vulvovaginal chronic graft-versus-host disease with allogeneic hematopoietic stem cell transplantation. *Obstetrics and gynecology*. 2007;110(5):1041-9.
22. Hirsch P, Leclerc M, Rybojad M, Petropoulou AD, Robin M, Ribaud P, et al. Female genital chronic graft-versus-host disease: importance of early diagnosis to avoid severe complications. *Transplantation*. 2012;93(12):1265-9.
23. Wingard JR, Vogelsang GB, Deeg HJ. Stem cell transplantation: supportive care and long-term complications. *Hematology American Society of Hematology Education Program*. 2002:422-44.
24. Rizzo JD, Wingard JR, Tichelli A, Lee SJ, Van Lint MT, Burns LJ, et al. Recommended screening and preventive practices for long-term survivors after hematopoietic cell transplantation: joint recommendations of the European Group for Blood and Marrow Transplantation, the Center for International Blood and Marrow Transplant Research, and the American Society of Blood and Marrow Transplantation. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation*. 2006;12(2):138-51.
25. Couriel D, Carpenter PA, Cutler C, Bolanos-Meade J, Treister NS, Gea-Banacloche J, et al. Ancillary therapy and supportive care of chronic graft-versus-host disease: national institutes of health consensus development project on criteria for clinical trials in chronic Graft-versus-host disease: V. Ancillary Therapy and Supportive Care Working Group Report. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation*. 2006;12(4):375-96.
26. Dignan FL, Scarisbrick JJ, Cornish J, Clark A, Amrolia P, Jackson G, et al. Organ-specific management and supportive care in chronic graft-versus-host disease. *British journal of haematology*. 2012;158(1):62-78.
27. Majhail NS, Rizzo JD, Lee SJ, Aljurf M, Atsuta Y, Bonfim C, et al. Recommended screening and preventive practices for long-term survivors after hematopoietic cell transplantation. *Hematology/oncology and stem cell therapy*. 2012;5(1):1-30.
28. Shanis D, Merideth M, Pulanic TK, Savani BN, Battiwalla M, Stratton P. Female long-term survivors after allogeneic hematopoietic stem cell transplantation: evaluation and management. *Seminars in hematology*. 2012;49(1):83-93.
29. Carpenter PA, Kitko CL, Elad S, Flowers ME, Gea-Banacloche JC, Halter JP, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: V. The 2014 Ancillary Therapy and Supportive Care Working Group Report. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation*. 2015;21(7):1167-87.
30. Wolff D, Gerbitz A, Ayuk F, Kiani A, Hildebrandt GC, Vogelsang GB, et al. Consensus conference on clinical practice in chronic graft-versus-host disease (GVHD): first-line and topical treatment of chronic GVHD. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation*. 2010;16(12):1611-28.
31. Savani BN, Stratton P, Shenoy A, Kozanas E, Goodman S, Barrett AJ. Increased risk of cervical dysplasia in long-term survivors of allogeneic stem cell transplantation--implications for screening and HPV vaccination. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation*. 2008;14(9):1072-5.
32. Sri T, Merideth MA, Pulanic TK, Childs R, Stratton P. Human papillomavirus reactivation following treatment of genital graft-versus-host disease. *Transplant infectious disease : an official journal of the Transplantation Society*. 2013;15(4):E148-51.

33. Wang Y, Brinch L, Jebsen P, Tanbo T, Kirschner R. A clinical study of cervical dysplasia in long-term survivors of allogeneic stem cell transplantation. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation*. 2012;18(5):747-53.
34. Rizzo JD, Curtis RE, Socie G, Sobocinski KA, Gilbert E, Landgren O, et al. Solid cancers after allogeneic hematopoietic cell transplantation. *Blood*. 2009;113(5):1175-83.
35. Humphreys CT, Tallman B, Altmaier EM, Barnette V. Sexual functioning in patients undergoing bone marrow transplantation: a longitudinal study. *Bone marrow transplantation*. 2007;39(8):491-6.
36. Li Z, Mewawalla P, Stratton P, Yong AS, Shaw BE, Hashmi S, et al. Sexual health in hematopoietic stem cell transplant recipients. *Cancer*. 2015;121(23):4124-31.
37. Noerskov KH, Schjodt I, Syrjala KL, Jarden M. Sexual function 1-year after allogeneic hematopoietic stem cell transplantation. *Bone marrow transplantation*. 2016;51(6):833-40.
38. Thygesen KH, Schjodt I, Jarden M. The impact of hematopoietic stem cell transplantation on sexuality: a systematic review of the literature. *Bone marrow transplantation*. 2012;47(5):716-24.
39. Hovi L, Saarinen-Pihkala UM, Taskinen M, Wikstrom AM, Dunkel L. Subnormal androgen levels in young female bone marrow transplant recipients with ovarian dysfunction, chronic GVHD and receiving glucocorticoid therapy. *Bone marrow transplantation*. 2004;33(5):503-8.
40. Davison SL, Bell R, Donath S, Montalto JG, Davis SR. Androgen levels in adult females: changes with age, menopause, and oophorectomy. *The Journal of clinical endocrinology and metabolism*. 2005;90(7):3847-53.
41. Filipovich AH, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ, 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 : journal of the American Society for Blood and Marrow Transplantation*. 2005;11(12):945-56.
42. Smith Knutsson E, Bjork Y, Broman AK, Helstrom L, Levin Jakobsen AM, Nilsson O, et al. Genital chronic graft-versus-host disease in females: a cross-sectional study. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation*. 2014;20(6):806-11.
43. Bjork Y, Smith Knutsson E, Ankarberg-Lindgren C, Broman AK, Andersson I, Bjorkman L, et al. Androgens in women after allogeneic hematopoietic cell transplantation: impact of chronic GvHD and glucocorticoid therapy. *Bone marrow transplantation*. 2017;52(3):431-7.
44. Smith Knutsson E, Bjork Y, Broman AK, Helstrom L, Nicklasson M, Brune M, et al. A prospective study of female genital chronic graft-versus-host disease symptoms, signs, diagnosis and treatment. *Acta obstetrica et gynecologica Scandinavica*. 2018;97(9):1122-9.
45. Jagasia MH, Greinix HT, Arora M, Williams KM, Wolff D, Cowen EW, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation*. 2015;21(3):389-401.e1.
46. Sanders JE, Buckner CD, Amos D, Levy W, Appelbaum FR, Doney K, et al. Ovarian function following marrow transplantation for aplastic anemia or leukemia. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 1988;6(5):813-8.
47. Reitamo S, Rissanen J, Remitz A, Granlund H, Erkko P, Elg P, et al. Tacrolimus ointment does not affect collagen synthesis: results of a single-center randomized trial. *The Journal of investigative dermatology*. 1998;111(3):396-8.
48. Elad S, Or R, Resnick I, Shapira MY. Topical tacrolimus--a novel treatment alternative for cutaneous chronic graft-versus-host disease. *Transplant international : official journal of the European Society for Organ Transplantation*. 2003;16(9):665-70.

49. Derogatis LR, Rosen R, Leiblum S, Burnett A, Heiman J. The Female Sexual Distress Scale (FSDS): initial validation of a standardized scale for assessment of sexually related personal distress in women. *Journal of sex & marital therapy*. 2002;28(4):317-30.
50. Beck AT, Steer, Robert A & Brown, Gregory K. Beck Depression Inventory - Second Edition: Harcourt; 1996.
51. Beck AT, Steer, Robert A & Brown, Gregory K. BDI-II, Beck Depression Inventory - second edition. Manual Svensk version: Harcourt Assessment, Inc, USA; Psykologiförlaget AB; 2005.
52. Shulman HM, Kleiner D, Lee SJ, Morton T, Pavletic SZ, Farmer E, et al. Histopathologic diagnosis of chronic graft-versus-host disease: National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: II. Pathology Working Group Report. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation*. 2006;12(1):31-47.
53. Cuzick J. A Wilcoxon-type test for trend. *Statistics in medicine*. 1985;4(1):87-90.
54. Basaria S, Dobs AS. Clinical review: Controversies regarding transdermal androgen therapy in postmenopausal women. *The Journal of clinical endocrinology and metabolism*. 2006;91(12):4743-52.
55. Tauchmanova L, Selleri C, De Rosa G, Esposito M, Orio F, Jr., Palomba S, et al. Gonadal status in reproductive age women after haematopoietic stem cell transplantation for haematological malignancies. *Human reproduction (Oxford, England)*. 2003;18(7):1410-6.
56. Syrjala KL, Kurland BF, Abrams JR, Sanders JE, Heiman JR. Sexual function changes during the 5 years after high-dose treatment and hematopoietic cell transplantation for malignancy, with case-matched controls at 5 years. *Blood*. 2008;111(3):989-96.
57. Dyer G, Gilroy N, Bradford J, Brice L, Kabir M, Greenwood M, et al. A survey of fertility and sexual health following allogeneic haematopoietic stem cell transplantation in New South Wales, Australia. *British journal of haematology*. 2016;172(4):592-601.
58. Mosher CE, DuHamel KN, Rini C, Corner G, Lam J, Redd WH. Quality of life concerns and depression among hematopoietic stem cell transplant survivors. *Supportive care in cancer : official journal of the Multinational Association of Supportive Care in Cancer*. 2011;19(9):1357-65.
59. Salooja N, Szydlo RM, Socie G, Rio B, Chatterjee R, Ljungman P, et al. Pregnancy outcomes after peripheral blood or bone marrow transplantation: a retrospective survey. *Lancet (London, England)*. 2001;358(9278):271-6.