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Chronic Graft-Versus Host Disease - Single Center Experience

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Abstract

Background. With the increasing number of patients surviving peritransplant complications of stem-cell transplantation (SCT), the incidence of chronic Graft Versus Host Disease (cGVHD) has increased too. cGVHD now develops in approximately 30-50% of patients undergoing SCT. Risk factors associated with the development of cGVHD are: previous acuteGVHD (aGVHD), prior cytomegalovirus infection, use of a matched unrelated donor and combination of a male recipient with a female donor. Not all patients who develop aGVHD progress to cGVHD. The latter may occur de novo.

Aim. To describe clinical and patohistological findings and influence of cGVHD on survival in allogeneic stem cell recipients.

Material and methods. During 7 years period we have treated 40 patients with allogeneic SCT from HLA identical sibling. aGVHD prophylaxis comprised Seattle protocol.

Results. 12 patients (6 males, 6 females; mean age: 34,5) developed cGVHD (7 de novo). Distribution according to diagnosis: Acute Myeloid Leukemia (n=8), Chronic Myeloid Leukemia (n=3), Primary myelofibrosis (n=1). Six of them had limited disease, and 6 had extensive. Treatment comprised: corticosteroids + CsA (6), Mophetyl mycophenolate (4), Tacrolimus (2), Photochemiotherapy (3). 10 (83%) patients are alive; 2 (17%) died.

Conclusion. Despite advances in histocompatibility matching and immunosuppressive drugs, GVHD has continued to be a common and often lethal complication of SCT.

Introduction

Over the last three decades, the allogeneic bone marrow transplantation (BMT) has become a widely accepted treatment for hematological malignancies and other diseases. However, despite of major advances in histocompatibility matching and discovery of more efficient immunosuppressive drugs, GVHD is stil common and often lethal complication of marrow transplantation. The interaction of donor immune cells and host target tissues in GVHD is proven to be a complex conundrum, involving multiple organs, different cytokines and effector cells (1). Acute GVHD (aGVHD) results in an immediate multiorgan inflammatory syndrome affecting the skin, liver, and digestive tract but also produces a long-term immune deficiency and is assotiated with increased frequency of chronic GVHD (cGVHD). For many years the central paradigm of aGVHD has been that it is a cell-mediated immunological attack of donor T cells against the host. Initial attempts to prevent GVHD by T depletion have resulted in increased failure of engraftment and increased leukemic relapse with little

overall improvement in long-term survival.

Whereas the T-cell allo-response remains the core of aGVHD, recent research supports a complex interweaving of other cell populations and cytokines in the pathophysiology of aGVHD (2). Cell damage from radiation and other components of preparative regimens cause transient release of inflammatory cytokines, such as IL-1, and TNF-alpha, and increase the immunogenecity of host antigen presenting cells. Donor T cells responding to host alloantigens release IL-2 and, most important, IFN-gamma which activates macrophages and NK cells. Activated macrophages and NK cells can then be triggered by gut bacteria or by latent infections to release large quantities of inflammatory cytokines and active nitrogen intermediates, resulting with a "cytokine storm" (3,4), manifested by loal tissue damage in gut, skin and liver. The critical aspects of aGVHD are therefore, first, that the donor Tcell population produces T-helper 1 (Th-1) cytokines-IL-2, and IFN-gamma in response to host antigens. Second, the main effectors in aGVHD are not cytotoxic T-cells, but rather NK and proinflammatory macrophages. Finally, inflammatory cytokines released by these cells mediate tissue injury, weight loss, and lethal complications.

Donor T-cells are indisputably necessary to induce GVHD. T-cell depletion from the donor inoculum has effectively prevented GVHD. Recent studies have addressed effects of both marrow depletion or in vivo depletion of CD3, CD5, CD6, CD52, CD90, TCR-alfa/beta cells (4-6). Chronic GVHD is a Th-2-Mediated B-cell Stimulatory Disorder. Although the immune deficits, observed initially in aGVHD merge gradually into the long-lasting deficiencies the cGVHD is a disorder of immune dysregulation, not merely deficiency. It is characterized by autoantibody production and increased collagen deposition, resulting in clinical symptoms similar to those seen in patients with autoimmune disease, particularly scleroderma and Sjogren's disease. Whereas aGVHD is associated with hypogammaglobulinaemia, serum IgM and IgG levels are elevated in cGVHD, associated with monoclonal gammopathies or homogenous Ig components indicative of clonal dysregulation (7). Even when the same organs are affected, the pathology may be distinct in aGVHD and cGVHD, with necrosis dominating in aGVHD and fibrosis in cGVHD. The skin lesions of aGVHD are characterized by necrosis of epithelial cells of the basal layer, with minimal lymphoid infiltrates. In cGVHD, in contrast, there is a significant lymphocytic or lymphoplasmocytic infiltration and extensive dermal fibrosis.

The pathology of cutaneous GVHD is subtle yet clinically devastating, experimentally reproducible yet etiologically mysterious, and target-cell-specific yet promiscuous regarding epidermal or dermal injury. The enigma of cutaneous GVHD is emphasized by its typical clinical features. Acute lesions occur after allogeneic bone marrow transplantation, and degree of histocompatibility disparity between donor and host correlates with severity. However, not all recipients of partially unmatched transplants develop clinical disease, and the characteristic exanthema may occur in syngenic (identical twin) transplants or independent of transplantation altogether.

Acute GVHD generally is observed as a maculopapular rash that may be generalized. The onset may be as early as several weeks after marrow transplantation, and clinical confusion with viral and drug related exanthemas is common. Often there is palm and sole involvement, a valuable clue to GVHD as a cause. In addition, the rash may be punctuate, corresponding to early involvement of hair follicles. In rare individuals, cutaneous involvement may be as severe as epidermal sloughing in a manner similar to toxic epidermal neurolysis.

Chronic GVHD occurs at least several months after transplantation and may be either localized or generalized in distribution. The initial alterations are limited to the more superficial skin layers, with the formation of lichen-planus-like papules or scaling erythematosus plaques often first involving facial skin, palms, and soles. Gradual spread to other body sites generally occurs within 4 weeks and may be associated with diffuse erythema and induration, mottled hypo and hyperpigmentation, and alopecia. Mucous membrane involvement results in excessive dryeness of the mouth, conjuctiva, trachea, and vagina to produce sicca-like syndrome. Ultimately, persistent alterations include brawny induration indistinguishable from skin lesions of progressive systemic sclerosis, epidermal thinning with prominent teleangiectasiae, and anomalous pigment distribution.

Not all patients who develop acute GVHD progress to chronic GVHD, and the latter may occur de novo, without antecedent acute disease. Therefore, it remains unclear whether acute and chronic GVHD are continua of the same disorder or independent but temporally related conditions. Perhaps either form of GVHD may sometimes be clinically covert, accounting for the over predominance of one or the other in certain individuals. Chronic GVHD, on the other hand, may represent a distinctive autoimmune phenomenon set into motion by immunological imbalances resulting

from acute cytotoxicity to the immune system, one cause of which might be acute GVHD. The clues to these and other mysteries remain locked in GVHD target tissues, which include skin, liver, gut, and lymphoid organs. The epidermal manifestation of chronic GVHD is far less subtle than those observed in acute disease. Common to both is the finding of satellitosis, with epidermotropic lymphocytes surrounding degenerating and necrotic keratinocytes. The inflammatory infiltrate in chronic GVHD is not sparse and angiocentric, however., it may rather fill the papillary dermis in a bandlike manner. It is associated with transformation of degenerating basal cells into cells with flattened, polyhedral contours (squamitization of the basal-cell layer). A major differential diagnostic consideration in hyperplastic epidermal forms of cGVHD in lichen-planus-like drug eruptions.

The aim of this study was to describe clinical and patohistological findings and influence of chronic GVHD on survival in allogeneic stem cell recipients, treated at the University Hematology Clinic in Skopje, Republic of Macedonia.

Material and methods

During a 7 year period, from September 2000 to September 2007, we have treated 130 patients with different hematological malignancies with high-dose chemotherapy and subsequent stem cells transplantation. Distribution according to diagnose was: Acute myeloblastic leukemia 59 (49.2%), Acute lymphoblastic leukemia 6 (5%), Chronic myeloid leukemia 6 (5%), Chronic lymphocytic leukemia 1 (0.8%), Non Hodgkin Lymphoma 15 (12.5%), Hodgkin disease 13 (10.8%), Multiple myeloma 18 (15%), Severe aplastic anemia 1 (0.8%), Primary myelofibrosis 1 (0.8%). Peripheral stem cells were used as a source of hematopoietic stem-cells in 100, while bone marrow in 30 procedures. Median number of infused CD34+ cells were: 3,24x10^6/kg. Allogeneic: 40 from HLA identical sibleeng and Autologous: 90 HSCT. Gender: Male: 67 Female:63. Median age: 34 years (12-64 years). Patients were treated in sterile room, conditioned with HEPA filters, and low microbes diet. HLA-DNA histocompatibility test was performed at the Institute of Immunobiology and Human Genetics, Medical Faculty, Skopje, Republic of Macedonia.

In this study we have retrospectively analyzed the incidence and characteristics of chronic GVHD in our group of patients (Table 1). Diagnosis was made according to EBMT diagnostic criteria (Shulmann) (8). All patients were treated with myeloablative chemo-

Table 1: Characteristics of patients with chronic GVHD.

Patients	Dg	Date	Disease Status	Donor/ Recipient	Grading of cGVHD	Months post SCT
1. P. E.	AML	11/2001	AD	F/F	extensive	A +80
2. D. Z.	AML	11/2001	AD	F/M	extensive	D+48
3. B. J.	AML	08/2002	CR	M/F	limited	A+70
4. D. L.	CML	09/2003	AD	F/M	extensive	D+12
5. P. V	CML	08/2004	Chr.Ph	F/M	extensive	A+45
6. M. O.	AML	11/2004	CR	M/M	limited	A+43
7. G. N.	CML	01/2005	Chr.Ph	F/M	extensive	A+40
8. S. Z.	MP	02/2006	Chr.Ph	M/F	limited	A+28
9. K. S.	AML	03/2006	CR	M/M	extensive	A+27
10. S. A.	AML	03/2007	CR	M/F	limited	A+12
11. D. S.	AML	08/2007	CR	M/F	limited	A+10
12. P. T.	AML	09/2007	CR	M/F	limited	A+9

Abbreviations: cGVHD=chronic graft-versus-host disese; SCT=stem cell transplantation; AML=acute myeloblastic leukemia; CML=chronic myeloblastic leukemia; MP=multiple myeloma; AD=scute disese; CR=complete remison; ChrPh=chronic phase.

therapy regimen Busulphan-Cyclophosphamide 2. During the early posttransplantat period every patient had received 0.2 mg/kg b.w intravenous immunoglobulins every week until day +90. Acute GVHD prophylaxis regimen consisted of Cyclosporine A and Methotrexate (on day +1, 3, 6, 11) according to Seattle protocol. In the case of cutaneous chronic GVHD, skin biopsies were used for diagnosis. Patohistological examination was made at the Institute of Pathology or Clinic of Dermatology using standard procedures for skin biopsy. As a first line therapy in cGVHD corticosteroids 1 mg/kg b.w. and Cyclosporine A 3 mg/kg b.w.were used, while in a case of nonresponders Mofetyl mycophenolate 1 gr/

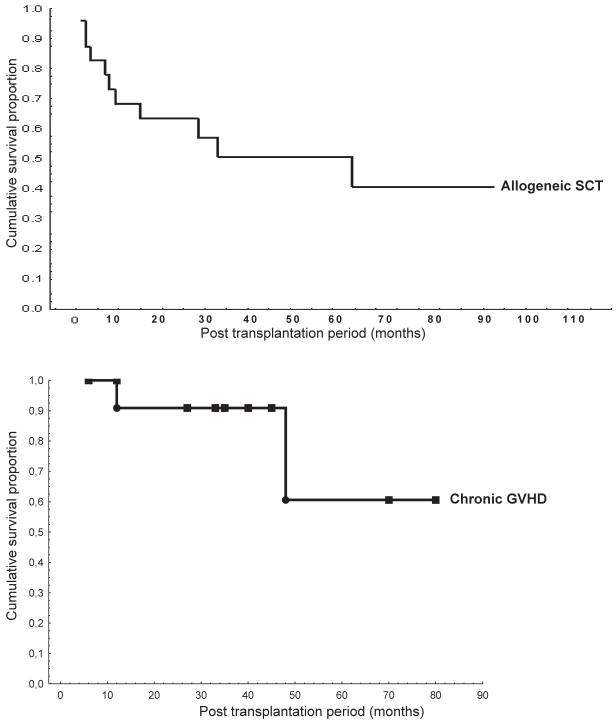

Fig.1: Cumulative proportion of survival in patients treated with SCT.

day, Tacrolimus 0.03 mg/kg b.w, or Psoralen Ultraviolet light A therapy were introduced. We describe clinical and patohistological findings and influence of chronic GVHD on survival in allogeneic stem cell recipients.

Results

From 40 patients treated with allogeneic SCT, 12 patients presented chronic GVHD. According to diagnosis: 8 patients were transplanted with AML, 3 with CML and 1 with Primary myelofibrosis. 6 patients were transplanted in complete remission, 3 in active disease, and 3 patients in chronic phase of underlying disease. In 4 cases we have female donor with male recipient, in 5 cases male donor with female recipient, in 2 cases male donor with male recipient, in 1 case female donor with female recipient. According to Schulman grading system 6 patients were with extensive, while 6 patients with limited form of chronic GVHD. Ten patients are alive from 9 to 80 months after transplantation. Two patients died. The first one (patient with CML transplanted in active disease) died 12 months after transplantation with cytomegalovirus pneumonia, and the second one (patient with AML transplanted in active disease) died 48 months after transplantation because of complications of chronic GVHD (pulmonal fibrosis, pleural effusion, cardiomyopathia and sicca syndrome). From all patients treated with allogeneic stem-cells transplantation 40% lived 80 months, while 60% with chronic GVHD live 80 months (Fig. 1).

The histopathologic findings in the early stage of cGVHD, are very similar to the findings in aGVHD. This so-called lichenoid stage is characterised by vacuolar alteration of keratinocytes and the presence of necrotic keratinocytes, most often located in the lower portion of the epidermis (Fig. 3a). The superficial, perivascular lymphocytic infiltrate varies from mild to dense. Melanophages can be found in variable numbers. The absence of a hyperplastic epidermis, the absence of wedge-shaped hypergranulosis and the presence of parakeratosis can distinguish cGVHD from lichen planus. In lichen planus the cornified layer

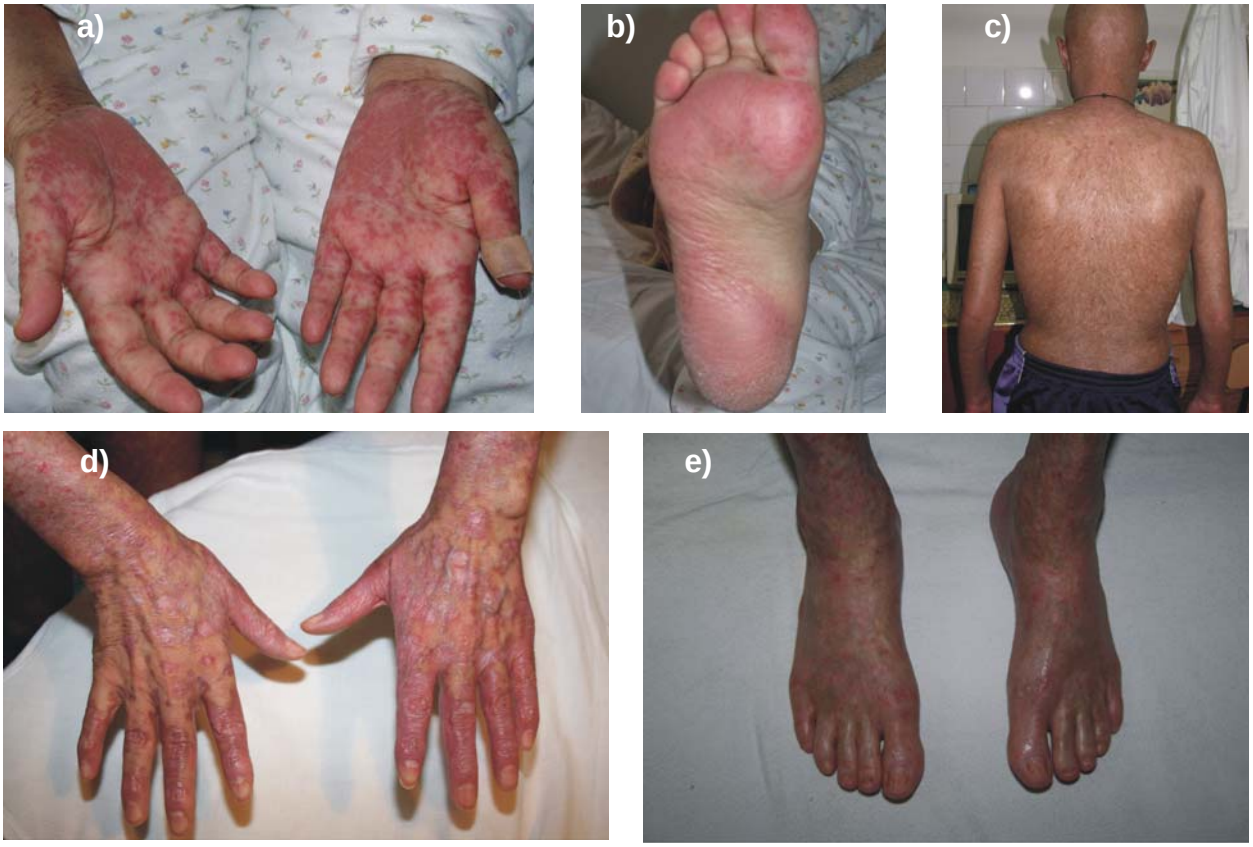

Fig. 2: *Cutaneus findings in the patients with GVHD. a) palmar erythema in acute GVHD (day +30); b) soles erythema in acute GVHD (day +30); c) chronic GVHD - indurative phase- (day+320); d) abnormal pigment distribution in cGVHD; e) chronic GVHD (scleroderma-like) (day +460).*

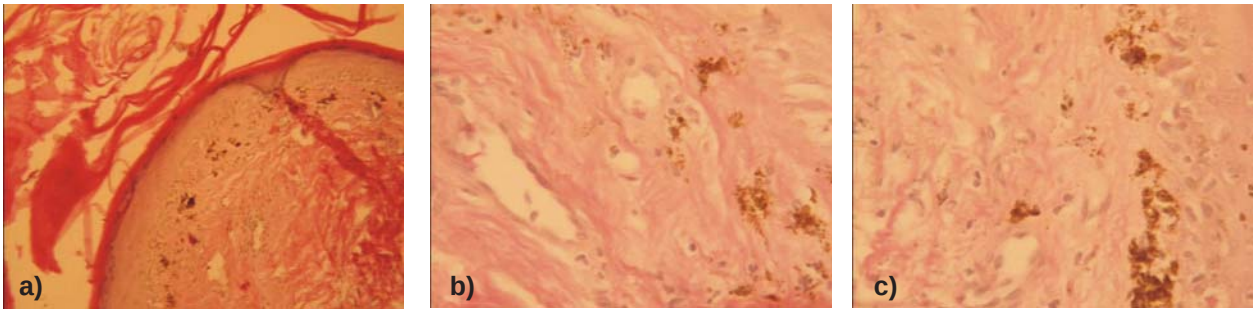

Fig. 3: Histopathology in chronic GVHD. a) Vacuolar alteration of keratinocytes and the presence of necrotic keratinocytes, most often located in the lower portion of the epidermis; b) A mononuclear perivascular infiltrate in dermis, consisting of lymphocytes, some plasma cells, and dispersed melanophages as a marker of melanin incontinence; c) Presence of a lichenoid infiltrate, still present in a small number of patients with late stage cGVHD.

solely shows compact orthokeratosis and the infiltrate is more dense. When the biopsy contains a hair follicle with necrotic keratinocytes, the histology may resemble lichen planopilaris. Markedly thickened collagen bundles within the reticular dermis cause the sclerodermoid changes. These bundles are often arranged parallel to the skin surface. As in systemic scleroderma, the collagen spreads into the fibrous septa of the subcutaneous fat. A slight infiltrate, consisting mostly of lymphocytes and some plasma cells, can be found around dermal vessels (Fig. 3b). Sometimes only a few melanophages are present. Skin appendages are often atrophic. Without clinical information, it is usually not possible to distinguish the features of the sclerodermoid phase of GVHD from morphoea or systemic sclerosis. Only the presence of a lichenoid infiltrate, which is still present in the late stage in a small number of patients with cGVHD, can help in assigning the diagnosis (Fig. 3c). In a few patients, secondary cutaneous mucinosis is reported. Secondary dermal mucinosis is a common finding in connective tissue diseases. These patients developed nodular lesions on different sites of the body.

Discussion

Of long-term survivors after marrow transplantation, 30 to 50% develop chronic GVHD (9). Chronic GVHD was reported to occur more frequently in survivors transplanted for aplastic anemia compared to patients with acute leukemia (p=0,0018). Manifestations may be subclinical or clinical, limited or extensive. Treatment is not necessary for limited cGVHD, which often resolves spontaneously. Recurrent infections, weight loss, sicca syndrome, mucositis, or other clinical problems were not observed. Extensive cGVHD necessitates immunosuppressive treatment; if untreated, it may progress and debilitate the patient by complications such as contractures, malabsorption, blindness, etc. Additional grading systems for extensive disease are desirable, since this may involve anything from localized skin involvement or liver dysfunction combined with mild sicca syndrome to advanced disease affecting multiple target organs. Because of the complex and dynamic nature of cGVHD, the Karnofsky score has often been used to estimate the overall performance status of the patient. With the increasing numbers of patients surviving peritransplant complications of BMT, the incidence of cGVHD has increased too. This is also due to the use of allogeneic peripheral blood as a source of hematopoietic stem cells and the increased use of non-related donors.

Risk factors associated with the development of cGVHD are: previous aGVHD, prior cytomegalovirus infection, recipient age over 18 years, use of a matched unrelated donor (MUD) and the combination of a male recipient with a female donor (10). Also a donor lymphocyte infusion given after SCT increases the risk to develop cGVHD. The skin is involved in almost all patients with cGVHD. It can also involve the eyes, mouth, genitalia, oesophagus, liver, respiratory tract and the muscles. The skin lesions can be localised or generalised. It usually, but not always, follows aGVHD.

Classically, cGVHD is arbitrarily subdivided into an early, lichenoid stage and a late sclerodermoid stage. The early phase is characterised by a slowly progressive rash, becoming lichenoid and resembling lichen planus. It involves the skin and the oral mucosa. In the late stage the patient develops sclerodermoid plaques on the trunk, buttocks and thighs and in severe cases also on the extremities. Skin-involvement of cGVHD localised to an area of piebaldism has been reported. Linear lesions have been reported both following Blaschko's lines and in a dermatomal distribution.

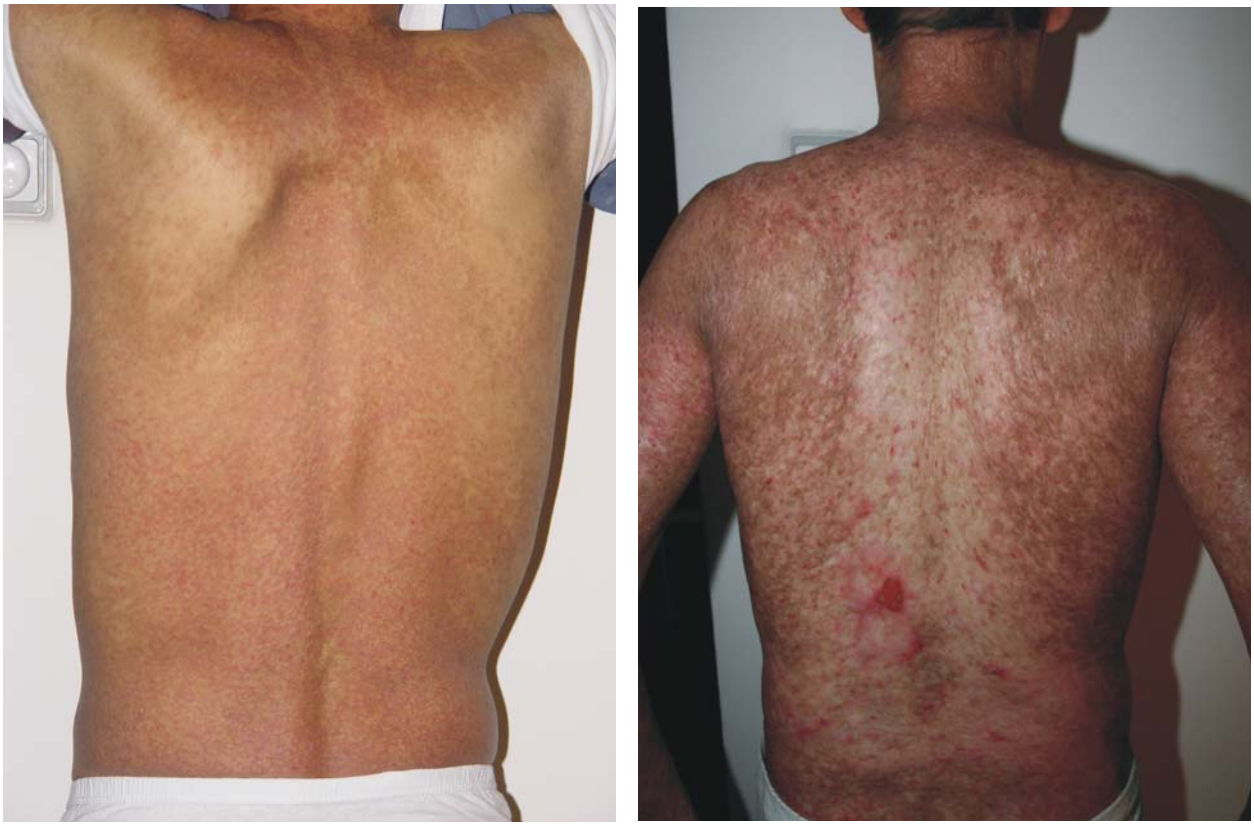

Fig. 4: Early lichenoid stadium in cGVHD (day+154) on the left, and late sclerodermoid stadium in cGVHD (day +425) on the right.

Besides lichenoid and sclerodermoid lesions, patients with cGVHD can show clinical and histological lesions resembling ichthyosis vulgaris. Also hyperpigmentation and vitiliginous depigmentation can be present. Patients may complain on a dry mouth and alopecia. Sclerotic changes in the oesophagus can cause problems with swallowing. When liver-involvement is present, liver-enzymes are elevated. Keratoconjunctivitis sicca develops in about 60% of all BMT-patients, partly due to cGVHD, partly due to total body irradiation. Other ocular manifestations of cGVHD are cicatricial lagophthalmos, sterile conjunctivitis, corneal epithelial defects and ulceration. A rare form of cGVHD is fasciitis, leading to functional disability. The pathologic changes vary from mild septal fibrosis to severe fibrosis of the fascia. There is usually a mild lymphohistiocytic infiltrate in the early stage and a severe lymphocytic infiltrate in older lesions. Another rare manifestation of cGVHD is polymyositis. This can even present as a respiratory muscle weakness. Although cGVHD can stimulate the beneficial graft-versus-leukaemia effect, it now becomes the most important determinant for long-term morbidity and mortality in SCT-patients (11). A combination of CsA and Prednisolone has been the started front line therapy for chronic GVHD for almost 20 years. The initial dose of steroid ranges from 1-1,5mg/Kg day for at least two weeks than the dose is slowly tapered, according to response. Duration of therapy is also determined by response, but is prolonged usually for close to 12 months, even in patients achieving complete resolution (12). The morbidity associated with steroid therapy is significant including avascular necrosis, glucose intolerance requiring administration of Insullin, infections, hypertension, changes in body habitus, cutaneous atrophy, cataracts, osteoporosis, and growth retardation in children. So far, the addition of other immunosuppressive drug to standard upfront therapy, such as thalidomide, has not lead to any significant difference in cGVHD response rate of survival (13). There is a need for randomized prospective trials to investigate the addition of other immunosuppressive drugs to upfront therapy in an effort to improve outcomes and ameliorate steroid-related side-effects.

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References

1. Ferrara JL. Cytokine dysregulation as a mechanism of graft versus host disease. Curr Opion Immunol. 1993;5:794-799. doi:10.1016/0952-7915(93)90139-J PMID:8240742

2. Antin JH, Ferrara JL. Cytokine dysregulation and acute graft-versus-host disease. Blood. 1992;80(12):2964-8. PMID: 1467511

3. Okunewick JP, Kociban DL, Machen LL, Buffo MJ. Comparison of the effects of CD3 and CD5 donor T cell depletion on graft-versus-leukemia in a murine model for MHC-matched unrelated-donor transplantation. Bone Marrow Transplant. 1994;13(1):11-7. PMID: 7517254

4. Soiffer RJ, Ritz J. Selective T cell depletion of donor allogeneic marrow with anti-CD6 monoclonal:rationale and results. Bone Marrow Transplant. 1993;12 Suppl 3:S7-10. PMID: 8124262

5. Blazar BR, Taylor PA, Snover DC, Bluestone JA, Vallera DA. Nonmitogenic anti-CD3F(ab')2 fragments inhibit lethal murine graft-versus-host disease induced across the major histocompatibility barrier. J Immunol. 1993 Jan 1;150(1):265-77. PMID: 8417127

6. Hale G, Waldmann H. CAMPATH-1 monoclonal antibodies in bone marrow transplantation. J Hematother. 1994;3(1):15-31. PMID: 7922004

7. Cobbold SP, Hale G,Clark MR,Waldmann H. Purging in auto-and allografts: Monoclonal antibodies which use human complement and other natural effector mechanisms.Prog Clin Biol Res. 1990;333:139-151. PMID: 2308978

8. Shulman HM, Sullivan KM, Weiden PL, McDonald GB, Striker GE, Sale GE, Hackman R, Tsoi MS, Storb R, Thomas ED. Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. Am J Med. 1980;69(2):204-17. doi:10.1016/0002-9343(80)90380-0 PMID:6996481

9. Wagner JE, Thompson JS, Carter SL, Kernan NA; Unrelated Donor Marrow Transplantation Trial. Effect of graft-versus-host disease prophylaxis on 3-year disease-free survival in recipients of unrelated donor bone marrow (T-cell Depletion Trial): a multi-centre, randomised phase II-III trial. Lancet. 2005;366(9487):733-41. doi:10.1016/S0140-6736(05)66996-6 PMID: 16125590

10. Kim SS. Treatment options in steroid-refractory acute GVHD following hematopoetic stem cell transplantation. The Annals of Pharmatherapy. 2007;41:1436-1444. doi:10.1345/aph.1K179 PMID:17684033

11. Bacigalupo A. Management of acute GVHD. Br J Haematol. 2007;137:87-98. doi:10.1111/j.1365-2141.2007.06533.x PMID:17391488

12. Fraser CJ,Baker KS. The management and outcome of chronic graft-versus host disease. Br J Haematol. 2007;138:131-145. doi:10.1111/j.1365-2141.2007.06652.x PMID:17593020

13. Vogelsang GB. How i treat chronic graft-versus host disease.Blood. 2001;97:1196-1201. doi:10.1182/blood.V97.5.1196 PMID:11222360