Current issues in chronic graft versus host disease

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Introduction:

Chronic GVHD is a pleomorphic syndrome with “autoimmune” features that sometimes resemble clinical findings in scleroderma and Sjögren syndrome [1]. The prevalence and severity of chronic graft-versus-host disease (GVHD) have increased during the past 2 decades in association with the increasing use of hematopoietic stem cell transplantation (HCT) for treatment of older age patients, the widespread use of mobilized blood cells instead of marrow for grafting, and improvements in survival during the first several months after allogeneic HCT [2-7]. The prevalence varies from 25–80% in long-term survivors [8].Chronic GVHD causes significant late morbidity and mortality and affects quality of life, survival and other transplant outcomes [9]. Recent advances have been made in understanding the pathophysiology of chronic GVHD as well as in establishing precise criteria for the diagnosis and classification of disease manifestations. These advances will, it is hoped, pave the way to improving both the prophylaxis and treatment of chronic GVHD [10].

Pathophysiology of chronic GVHD:

There are six hallmarks that are unique to chronic GVHD syndrome. These include damage to the thymus associated with the conditioning regimen and more importantly, occurrence of acute GVHD earlier in the post-HCT course resulting in decreased negative selection of alloreactive CD4+ T cells [11]; Th2 cytokine pattern deviation resulting in release of fibrogenic cytokines such as interleukin (IL)-2, IL-10 and transforming growth factor (TGF)-β [12]; macrophage activation followed by tissue fibroblasts proliferations and activation through release of TGF-β and platelet derived growth factor (PDGF) from macrophages [13,14] ; lower T regulatory (Treg) levels [15] and finally, dysregulation of B-cells leading to emergence of autoreactive B-cells and

production of autoreactive antibodies [16]. It is suggested that the latter maybe due to excessive presences of B cell activating factor (BAFF) in the lymphoid microenvironment [17]. Recent studies have suggested that Th1 and Th17 cells contribute also in the pathogenesis of chronic GVHD [18,19]. All these will results in autoimmune-like systemic syndrome mostly associated with fibroproliferative changes that can occur in almost any organ in body but primarily affecting oral and ocular mucosal surfaces and the skin, lung, kidneys, liver and gut [20]. The pathophysiology of chronic GVHD is illustrated in figure 1 [20].

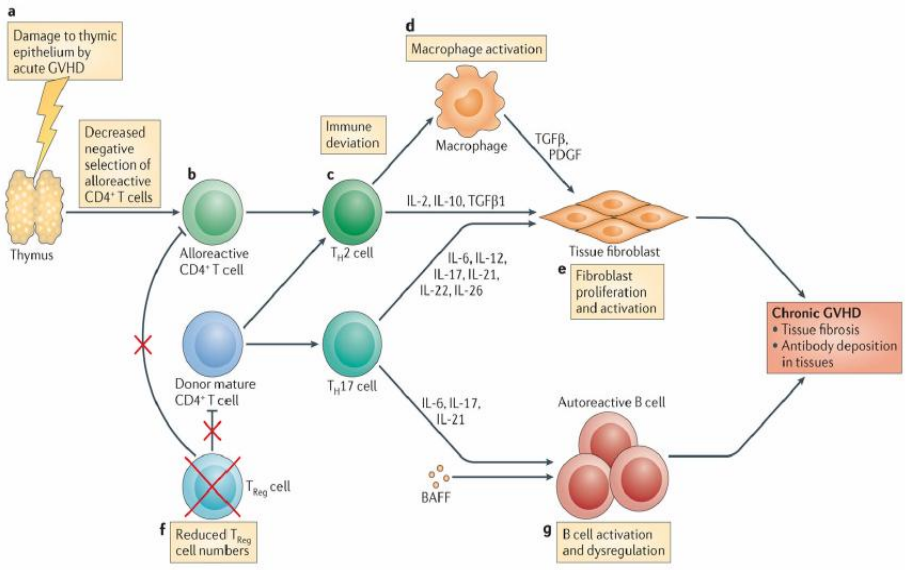

Figure 1: Pathophysiology of chronic graft versus host disease [20].

Diagnosis of chronic GVHD:

In the past, chronic GVHD included any clinical manifestations of GVHD that occurred beyond 100 days after transplantation. This definition was clearly imprecise and became inadequate. In 2005 a group of experts met under the auspices of the National Institutes of Health (NIH), USA in a consensus meeting. The goals of this NIH consensus working group on the diagnosis and staging of GVHD were: (i) to establish criteria for diagnosis of the disease, emphasizing the distinction between acute and chronic GVHD; (ii) to define criteria for scoring the severity of clinical manifestations in affected organs; and (iii) to propose categories describing the overall severity of the disease and the indications for treatment [21]. The NIH consensus conference classified chronic GVHD

into: classic chronic GVHD, presenting with manifestations that can be ascribed only to chronic GVHD. Chronic GVHD also includes an overlap syndrome, which has diagnostic or distinctive manifestations of chronic GVHD together with features typical of acute GVHD (Table 1) [22]. The NIH consensus conference proposed a new global chronic severity score establishing mild, moderate and severe forms of chronic GVHD based on a numerical scoring system for individual organs to calculate a summary scale (Table 2) [21]. Although the NIH global score was developed through expert opinion, several studies have shown that the global score at onset of chronic GVHD is associated with risk of subsequent mortality [23-26].

To identify perceived areas of controversy, Inamoto et al. conducted an international survey on diagnosis and scoring of chronic GVHD. There was agreement in the need for modifying criteria in 6 situations:

- 2 or more distinctive manifestations should be enough to diagnose chronic GVHD
- symptoms not due to chronic GVHD should be scored differently,
- active disease and fixed deficits should be distinguished
- a minimum threshold body surface area of hidebound skin involvement should be required for a skin score 3
- asymptomatic oral lichenoid changes should be considered a score 1
- lung biopsy should be unnecessary to diagnose chronic GVHD in a patient with bronchiolitis obliterans as the only manifestation.

The survey also identified 26 points of controversy [9]. In 2014, the NIH Conference was reconvened, and revisions are under consideration to update the recommendations based on available evidence and insights from clinical application of the original recommendations [27].

Table 1: Clinical manifestations of chronic GVHD [22]:

Organ/site	Diagnostic	Distinctive (insufficient for diagnosis)	Features seen in both acute and chronic GVHD	Other
Skin	Poikiloderma Lichen planus-like Sclerosis Morphea-like, Lichen sclerosis-like	Depigmentation Papulosquamous	Erythema Maculopapular Pruritis	
Nails		Dystrophy Onycholysis Nail loss Pterygium unguis		
Scalp/body hair		Alopecia (scarring or nonscarring) Scaling		
Mouth	Lichen planus-like	Xerostomia Mucoceles Mucosal atrophy Pseudomembranes or ulcers*	Gingivitis Mucositis Erythema Pain	
Eyes		New dry, grity or painful eyes (sicca) Keratoconjunctivitis sicca Punctate keratopathy		
Genitalia	Lichen planus-like Lichen sclerosis-like **Female:** Vagina scarring or stenosis Clitorial or labial agglutination **Male:** Phimosis,Urethral scarring or stenosis	Erosions* Fissures* Ulcers*		
GIT	Esophageal web Esophageal stricture		Diarrhea Anorexia Nausea or emesis Failure to thrive Weight loss	
Liver		Total bilirubin, alkaline phosphatase or ALT > 2 x ULN		
Lung	Bronchiolitis obliterans diagnosed by biopsy**			Cryptogenic organizing pneumonia*** Restrictive lung disease***
Muscles, fascia, joints	Fasciitis Joint stiffness or contractures due to sclerosis	Myositis Polymyositis		
Hematopoietic and immune				Thrombocytopenia Eosinophilia Hypo- or hypergamm-aglobulinemia Autoantibodies Raynaud phenomenon
Others				Effusions**** Nephrotic syndrome Myasthenia gravis Peripheral neuropathy

ALT = alanine aminotransferase, GIT = gastrointestinal tract, ULN = upper limit of normal
*In all cases infection, drug effect, malignancy, endocrine causes must be excluded as applicable.
** can be diagnostic for lung chronic GVHD only, if distinctive feature present in another site.
***These pulmonary manifestations are under investigation or unclassified.
****Pericardium, pleural, or ascites.

Table 2: Grading of severity of chronic GVHD [21]:

Severity	Mild	Moderate	Severe
Number of affected organ systems	1 to 2	>2	>2
Severity of organ manifestations	Mild (excluding lung)	Mild-moderate (lung: mild only)	Severe (lung: moderate or severe)

Biomarkers of chronic GVHD:

There has recently been considerable research effort devoted to the discovery and validation of GVHD relevant biomarkers [28]. At the first meeting of the NIH biomarker consensus group in 2006, the ideal chronic GVHD biomarker was formally defined [29,30]. Several inflammatory markers and cytokines like transforming growth factor-β1 (TGF-β1), tumour necrosis factor (TNF) and interferon- γ (IFN-γ), that are increased in acute GVHD, have been identified as candidate biomarkers for chronic GVHD, but none have been developed for clinical use. Currently there are no validated biomarkers for chronic GVHD [28]. Ease of measurement, accessibility of plasma samples from patients and preliminary data suggesting that significant elevation of BAFF preceded chronic GVHD development make soluble BAFF a tempting biomarker [31]. Pidala et al. classified biomarkers according to: risk for subsequent chronic GVHD development, association with established chronic GVHD diagnosis, chronic GVHD severity, chronic GVHD phenotype, response to therapy and prognosis (table 3) [32]. Genetic markers of chronic GVHD development have also been proposed, such as MHC class I chain-related protein A (MICA)-129 genotype and the negative regulator of T cell costimulation CTLA-4 +49 A/G*GG genotype, but the significance of these remains unknown [33,34]

Table 3: Biomarkers of chronic graft versus host disease [32]:

Biomarkers			Risk
Risk of subsequent GVHD development	Donor-recipient non-HLA genetic polymorphism	IL-1, IL-6, IL-10, TNF-α	Increased
	Immune cell populations	Donor allograft nucleated cell, $CD34^+$ cell dose, Plasmacytoid DC cell dose, NK cell dose, Treg cells	Decreased
	Inflammatory and immunoregulatory mediators	TNF-α, IL-10, BAFF	Increased
		TGF-β, IL-15	Decreased
Association with established chronic GVHD diagnosis	Immune cell populations	$CD3^+$ T cells, Th17, $CD4^+$ effector memory cells, $CD8^+$ effector memory cells, Monocytes, CD86 expressing monocytes, CD86 expressing B cells	Increased
		Total $CD19^+$ B cells, NK cells, Treg, naive $CD8^+$ T cells	Decreased
	Inflammatory and immunoregulatory mediators	TNF-α, IL-6, IL-1β, IL-8, sIL-2Rα, BAFF, sCD13, Elafin, CXCL9, anti-dsDNA Ab	Increased
	Other markers	anti-PDGF receptor Ab, CD28 upregulation, PI3K, expression of several genes, e.g. CXCR7	Increased
Chronic GVHD severity	Immune cell populations	naive B cells. non-class-switched memory B cells, Treg cells	Decreased
	Inflammatory and immunoregulatory mediators	TNF-α, IL-6, IL-1β	Increased
		FoxP3 expression, TGF-β	Decreased
	Other markers	caspase-3, CD8, CD3, CD68, KI-67, IL-15, MxA	Increased
Phenotype		CXCL9, CXCR3	Conjunctival, oral
		CXCL10	Conjunctival
		T-bet^+ effector T cells, STAT1phosphorylation	Oral
		CC16, $CD19^+CD21^{low}$ B cells	Bronchiolitis obliterans
		CCR9 genotype 926AG	Cutaneous
		Low $CD34^+$ $CD133^+$ VEGF-R2 endothelial progenitor cells	Sclerodermatous
		sBAFF	Hepatic, joint, lichenoid skin rash, pulmonary, sclerodermatous
		sCD13	Hepatic
		anti-dsDNA Ab	Ocular, joint, sclerodermatous
		Anticardiolipin Ab	Ocular
		IL-6, MCP-1	Joints
		anti-PDGF receptor Ab	Cutaneous, pulmonary
Response to therapy		decrease in sIL-2R levels	Response
		lower ratio of sBAFF at start of therapy to 2-month level	Response
		naive B-cell reconstitution	Response to anti-CD20 therapy
		decreased BAFF/B-cell ratios	Response to anti-CD20 therapy
		decreased ratio of $CD21^-$ immature/ transitional B cells to $CD27^+$ memory B cells	Partial response
Prognosis		Polymorphism in MADCAM-1	Decreased overall survival
		Progressive sIL-2R increase	Increased mortality

GVHD = graft versus host disease; HLA = human leukocyte antigen; IL = interleukin; TNF= tumour necrosis factor; CD = cluster of differentiation; DC = dendritic cell; NK = natural killer; Treg = T regulatory; BAFF = B cell activating factor; sCD13 = soluble CD13; TGF = transforming growth factor; Th17 = T helper cells 17; sIL-2Rα = soluble α chain of interleukin-2 receptor; CXCL9 = chemokine(C-X-C motif) ligand 9; anti-dsDNA Ab = anti double stranded DNA antibody; anti-PDGF receptor Ab = anti-platelet derived growth factor receptor antibody; PI3K = phosphatidylinositol-4,5-bisphosphate 3 kinase; CXCR7 = chemokine (C-X-Cmotif)receptor type 7; foxP3 = forkhead box P3; KI-67 = proliferation-related Ki-67 Ag; MxA = IFN-induced MxA GTPase; STAT1 = signal transducer and activator of transcription 1; CC16 = clara cell secretory protein; CCR9 = chemokine (C-C motif) receptor 9, VEGF-R2 = vascular endothelial growth factor-receptor 2; MCP-1 = monocyte chemoattractant protein 1, MADCAM-1 = mucosal addressin cell adhesion molecule-1

Treatment of chronic GVHD:

Treatment of chronic GVHD is intended to produce a sustained benefit by reducing symptom burden, controlling objective manifestations of disease activity, and preventing damage and disability, without causing disproportionate toxicity or harms related to the treatments themselves. The long-term goal of GVHD treatment is the development of immunologic tolerance, indicated by successful withdrawal of all immunosuppressive treatment without recurrence or clinically significant exacerbation of disease manifestations [27]. Symptomatic mild chronic GVHD is often treated with topical therapies alone (Table 4). Topical agents may also be used as adjuncts to systemic therapy to improve and accelerate local response [35].

Systemic therapy for at least 1 year is generally indicated for patients who meet criteria for moderate-to-severe disease according to the NIH consensus criteria (Table 2) [21]. Systemic treatment is also generally indicated for patients with less severe disease if high-risk features such as thrombocytopenia, hyperbilirubinemia, or onset during corticosteroid treatment are present [36]. Primary systemic management of chronic GVHD has relied on corticosteroids as the mainstay of treatment of >3 decades. Combination therapy with other immunosuppressive agents is often considered in hopes of minimizing toxicity caused by prolonged corticosteroid treatment [36]. Randomized trials, however, showed no benefit from adding azathioprine, thalidomide, mycophenolate mofetil, or hydroxychloroquine to initial treatment of chronic GVHD [27]. A trial comparing cyclosporine plus prednisone vs prednisone alone showed no statistically significant differences in survival or the duration of treatment. The incidence of avascular necrosis was lower in the cyclosporine plus prednisone arm, suggesting that cyclosporine could have had a steroid-sparing effect [37].

Approximately 50% to 60% of patients with chronic GVHD require secondary treatment within 2 years after initial systemic treatment [38]. Indications for secondary treatment include worsening manifestations of chronic GVHD in a previously affected organ, development of signs and symptoms of chronic GVHD in a previously unaffected organ, absence of improvement after 1 month of standard primary treatment, inability to decrease prednisone below 1 mg/kg per day within 2 months, or significant treatment-related toxicity [39]. Options for secondary treatment have been recently reviewed and are summarized in Table 5 [39].

Table 4: Topical treatment options for chronic GVHD [35]:

Organ	Drug	Recommendation grade	Evidence level	Response rate	Side effects	Comments
Skin	Topical steroids	C-1	III-1	n/a	Skin atrophy	Trunk and extremities: medium- and high potency steroids; face: hydrocortisone 1%
	Tacrolimus/ pimecrolimus	C-1	III-1	~70%	Increased long-term risk of cutaneous malignancies	Applied twice daily
	PUVA	C-1	III-1	~75%	Phototoxicity, increased long-term risk of cutaneous malignancies	Must not be combined with phototoxic drugs
	UVA	C-1	III-1	~60 to 70%	Phototoxicity, increased long-term risk of cutaneous malignancies	No UV protection needed after treatment, must not be combined with phototoxic drugs
	UVB	C-1	III-2	~60%	Phototoxicity, increased long-term risk of cutaneous malignancies	Lack of efficacy in cutaneous sclerosis
GI	Topical steroids	C-1	III-1	~60 to 70%		Budesonide or beclomethasone
Lung	Topical steroids	B	III-2	~50%		Can be combined with betamimetics
Oral mucosa	Topical tacrolimus/ cyclosporine	C-2	III-1	~60%	Potential long-term risk of malignant disease of the oral mucosa	Systemic drug levels possible, with associated risk of renal toxicity
	Topical steroids	C-1	III-1	~60 to 80%	Risk of local infections (fungal, viral)	Best results with budesonide
	Topical PUVA	C-2	III-2	~60 to 70%	Phototoxicity, long-term risk of oral malignancy	Important option for refractory oral cGVHD
Eyes	Topical steroids	C-1	III-1	~60 to 75%	Risk of atrophy of the cornea and infectious keratitis	Better short-term tolerability, not for long term therapy
	Topical cyclosporine	C-1	III-1	~60%	Local burning and stinging sensation	Fewer long-term side effects, higher longterm efficacy than steroids
Vagina	Topical steroids	B	III-3	n/a	Increased risk of local infections and atrophy	Topical estrogen therapy and antifungal prophylaxis recommended
	Topical tacrolimus/ cyclosporine/ pimecrolimus	B	III-3	n/a	Burning	Poorer tolerability, higher long-term efficacy

B: should generally be used; C-1: use in first-line therapy justified; C-2: use after failure of second-line therapy justified; III-1: several reports from retrospective evaluations or small uncontrolled clinical trials; III-2: only one report from small uncontrolled clinical trial or retrospective evaluations; III-3: only case reports available
GI: gastrointestinal; PUVA: psoralen plus UVA; n/a: not available

Table 5: second-line treatment for chronic GVHD [39]:

Treatment	Recom m-endatio n grade	Evid-ence level	Response rate	Side effects in more than 25% of treated patients	Comments
Steroids	B	III-1	n/a	Osteoporosis,osteo ne-crosis, diabetes mellitus	Of central importance
Photopheresis	C-1	II	~60 to 70% ~30% CR	Infections of the central venous access (if applicable)	Venous access required, steroid-saving effect, good tolerability
mTOR inhibitors (sirolimus, everolimus)	C-1	III-1	~60% ~20% CR	Transplant-associated microangiopathy, hyperlipidemia, hematotoxicity	Increased risk of micro-angiography when combined with CNI, regular examination of blood levels required
MMF	C-1	III-1	~50% ~10% CR	GI SEs, risk of infection (viral) and increased risk of relapse	Steroid sparing activity
CNIs (cyclosporine, tacrolimus)	C-1	III-1	n/a	Renal toxicity, hypertension	Reduces steroid use, examination of blood levels required
MTX	C-2	III-1	~50% ~10 to 20% CR	Hematotoxicity	Best results in mucocutaneous GVHD, reduces steroid use, contraindicated in the presence of pleural effusions or ascites
High-dose steroid	C-2	III-2	50 to 75% (PR only)	Risk of infection	Rapid control of GVHD
Thoracoabdo minal radiation	C-2	III-2	~50% ~25% CR	Hematotoxicity	Best results for fasciitis and mucocutaneous GVHD
Hydroxychlor oquine	C-2	III-2	~25% ~10% CR	GI side effects	Best results for fasciitis and mucocutaneous GVHD
Clofazimine	C-2	III-2	~50% (PR only)	GI side effects, hyperpigmentation	Best results for mucocutaneous GVHD
Pentostatin	C-2	II	~50% ~10% CR	Hematotoxicity, risk of infection	Best results in children
Rituximab	C-2	II	~50% ~10% CR	Risk of infection	Effective in manifestations associated with autoantibodies and sclerodermoid cutaneous involvement
Imatinib	C-2	III-1	~50% ~20% CR	Fluid retention	Efficacy demonstrated mainly in sclerodermoid GVHD and bronchiolitis obliterans
Thalidomide	C-3	II	~20 to 30% (PR only)	Neurotoxicity, drowsiness, constipation	Treatment for simultaneous GVHD and recurrent multiple myeloma
Azathioprine	C-3	III-1	n/a	Hematotoxicity, risk of infection	Increased risk of malignant disease of the oral mucosa
Retinoids	C-3	III-2	~60% (PR only)	Skin toxicity, hyperlipidemia	Effective in sclerodermoid cutaneous involvement
Alemtuzumab	C-4	III-3	n/a	Risk of infection	Last resort for refractory GVHD
Etanercept	C-4	III-3	n/a	Risk of infection	May be used to treat mixed acute and chronic GVHD or GI manifestations of GVHD

B: should generally be used; C-1: use in second-line therapy justified; C-2: use after failure of second-line therapy justified; C-3: should only be used in specific circumstances, due to unfavorable risk profile; C-4: experimental, should only be used in clinical trials and individual cases; III-1: several reports from retrospective evaluations or small uncontrolled clinical trials; III-2: only one report from small uncontrolled clinical trial or retrospective evaluations; III-3: only case reports available

MMF: mycophenolate mofetil; CNI: calcineurin inhibitors; MTX: methotrexate; CR: complete remission; PR: partial remission; GI: gastrointestinal; SE: side effect; n/a: not available

Future perspectives

Participation in a clinical trial represents the first option to consider for eligible patients with chronic GVHD. Novel strategies directed toward depleting or modulating B cells, expanding T or B regulatory cells, and targeting the processes implicated in fibrosis are under active investigation and could lead to future advances in treatment of chronic GVHD. Progress toward decreasing the impact of chronic GVHD after HCT will be made not only through improved treatment but also through development of prevention strategies that do not impair the immunological activity of donor cells against malignant cells in the recipient. In the absence of specific interventions to decrease the risk of chronic GVHD, marrow should be preferred over mobilized blood as a source of stem cells for HCT with myeloablative conditioning regimens [27].

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