Evaluation of prognostic factors among patients with chronic graft-*versus*-host disease

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ABSTRACT

Background

Chronic graft-*versus*-host disease (cGVHD) is a major complication after allogeneic stem cell transplantation with an adverse effect on both mortality and morbidity. In 2005, the National Institute of Health proposed new criteria for diagnosis and classification of chronic graft-*versus*-host disease for clinical trials. New sub-categories were recognized such as late onset acute graft-*versus*-host disease and overlap syndrome.

Design and Methods

We evaluated the prognostic impact of the new sub-categories as well as the clinical scoring system proposed by the National Institute of Health in a retrospective, multicenter study of 820 patients undergoing allogeneic stem cell transplantation between 2000 and 2006 at 3 different institutions. Patients were retrospectively categorized according to the National Institute of Health criteria from patients' medical histories.

Results

As far as the new sub-categories are concerned, in univariate analysis diagnosis of overlap syndrome adversely affected the outcome. Also, the number of organs involved for a cut-off value of 4 significantly influenced both cGVHD related mortality and survival. In multivariate analysis, in addition to NIH score, platelet count and performance score at the time of cGVHD diagnosis, plus gut involvement, significantly influenced outcome. These 3 variables allowed us to develop a simple score system which identifies 4 subgroups of patients with 84%, 64%, 43% and 0% overall survival at five years after cGVHD diagnosis (score 0: HR=15.96 (95% CI: 6.85-37.17), $P<0.001$; score 1: HR=5.47 (95% CI: 2.6-11.5), $P<0.001$; score 2: HR=2.8 (95% CI: 1.32-5.93), $P=0.007$).

Conclusions

In summary, we have identified a powerful and simple tool to discriminate different subgroups of patients in terms of chronic graft-*versus*-host disease related mortality and survival.

Key words: cGVHD, prognostic factors, NIH classification, overlap syndrome, delayed acute GVHD.

*Citation: Pérez-Simón JA, Afram G, Martino R, Piñana JL, Caballero-Velazquez T, Ringden O, Valcarcel D, Caballero D, Remberger M, de Paz Y, Sierra J, San Miguel J, and Hagglund H. Evaluation of prognostic factors among patients with chronic graft-*versus*-host disease. Haematologica 2012;97(8):1187-1195. doi:10.3324/haematol.2011.055244*

Manuscript received on September 21, 2011. Revised version arrived on January 12, 2012. Manuscript accepted February 3, 2012.

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Introduction

Chronic graft-*versus*-host disease (cGVHD) is a major complication following allogeneic hematopoietic stem cell transplantation (HSCT) which impairs patients' quality of life and hampers long-term survival.[1] Historically, cGVHD has been classified as 'limited' or 'extensive' on the basis of the results from a small retrospective study.[2] This system was developed primarily to distinguish between patients requiring systemic immune suppression from those for whom local care might suffice. Nevertheless, a majority of patients experience extensive stage cGVHD[3] that constitutes an extremely heterogeneous population. In addition, diagnosis of cGVHD was classically based on the presence of any manifestation of GVHD beyond 100 days after allogeneic transplantation. Based on expert opinion, in 2005 the National Institute of Health (NIH) proposed new criteria for the diagnosis and classification of cGVHD based on the clinical manifestations and not on the time of onset.[4] According to this proposal, new subcategories were recognized both for acute (classic aGVHD and late-onset aGVHD) and for cGVHD (classic chronic and overlap syndrome). As far as the prognostic value of these categories is concerned, few reported studies have analyzed the impact of delayed aGVHD on outcome,[5] and no study has evaluated the outcome of patients with overlap syndrome in a large series of patients.

Also, the NIH proposed a new clinical scoring system for the global assessment of cGVHD severity based on the number of organs involved (with an arbitrary cut-off value of involvement of more or less than 3 organs to distinguish between mild *versus* moderate) and the degree of functional impairment in the affected organs (mild, moderate or severe) which should allow patients requiring a purely topical approach *versus* systemic immune suppression to be identified, as well as facilitating decisions regarding the timing and intensity of therapy. Nevertheless, few studies have been performed to validate this scoring system in a large series of patients.[3,6] Furthermore, this scoring system is quite time-consuming which makes it difficult to satisfy all the requirements during a routine out-patient consultation.[4]

In this current study, we evaluated the prognostic impact of the new sub-categories as well as the clinical scoring system proposed by the NIH Consensus Development Project. We also tried to identify the most important variables predicting outcome in a series of 747 patients undergoing allogeneic stem cell transplantation in 3 different institutions in order to build up a simplified scoring system.

Design and Methods

Patients' characteristics

Eight hundred and twenty patients undergoing allogeneic stem cell transplantation in 3 different institutions from January 2000 to December 2006 were included in the analysis. The analysis was restricted to those 747 patients surviving more than 100 days after transplantation. The study protocol was approved by the ethics committee at Karolinska Institutet and was performed in accordance with the declaration of Helsinki. Written informed consent was obtained from all patients in Salamanca and Sant Pau. Patients were retrospectively categorized according to the NIH scoring system based on the data collected from patients' medical histories. The specified organ involvement and grading was also categorized according to the classical limited *versus* extensive classification. Characteristics of the patients who developed cGVHD are summarized in Table 1.

Median age at the time of transplantation was 50 years. Median follow up was 41 months. The most common diagnosis was acute myeloid leukemia in 24% of patients, acute lymphoblastic leukemia in 11% and myelodysplastic syndrome in 11%. Twenty-seven percent of patients were in 1st or subsequent complete remission at the time of transplant. Eighty-four percent received hematopoietic stem cells from a related donor and 71% received reduced intensity conditioning regimens. Sixty percent of the patients received cyclosporine plus methotrexate and 27% received *in vivo* T-cell depletion.

The first-line treatment of extensive cGVHD was based on CsA or tacrolimus plus prednisone at 1 mg/kg/day that was switched to alternate days after four weeks of treatment. The disease response was generally evaluated five weeks after the introduction of steroid and then every three months until the end of treatment.

All patients received antibacterial, antifungal and antiviral prophylaxis according to standard procedures.

Table 1. Patients' characteristics.

Characteristics of the patients	N=336 N (%)
Age	
Median (range)	50 (1-69)
Diagnosis (%)	
Acute myeloid leukemia	81 (24)
Acute lymphoblastic leukemia	37 (11)
Chronic myeloid leukemia	30 (9)
Myelodysplastic syndromes	37 (11)
Multiple myeloma	31 (9)
Non-Hodgkin's lymphoma	37 (11)
Chronic lymphocytic leukemia	20 (6)
Others	63 (19)
Disease status at transplant (%)	
1st CR	91 (27)
2nd or subsequent CR	64 (19)
PR	60 (18)
Progressive / relapsed disease	71 (21)
Others	50 (15)
Sex (%)	
Male / female	(63%) / (37%)
Sex mismatched	148 (44)
HLA matched (%)	302 (90)
Source of progenitor cells (%)	
Peripheral blood	279 (83)
Bone marrow	54 (16)
Cord blood	3 (1)
Type of donor (%)	
Related	282 (84)
Unrelated	54 (16)
Type of conditioning	
Myeloablative	97 (29)
RIC	239 (71)
GVHD prophylaxis (%)	
CsA-Tacro plus MTX	202 (60)
CsA-Tacro plus MMF	37 (11)
T-cell depletion	91 (27)
Others	6 (2)

Definitions

According to the NIH scoring system, mild cGVHD was diagnosed when only one or 2 organs or sites (except the lung) were involved, with no clinically significant functional impairment (maximum score 1 in all affected organs or sites). Moderate cGVHD involved at least one organ or site with clinically significant impairment but no major disability (maximum score 2 in any affected organ or site), or 3 or more organs or sites with no clinically significant functional impairment (maximum score 1 in all affected organs or sites). A lung score of 1 was also considered moderate cGVHD. Severe cGVHD was indicated by a major disability caused by cGVHD (score 3 in any organ or site). A lung score of 2 or over was also considered 'severe cGVHD'.[4]

Patients who were receiving prednisone, or who were still on a therapeutic dose of cyclosporine due to prior aGVHD that had evolved into cGVHD without the resolution of symptoms, were considered as having 'progressive cGVHD'. Patients who were on cyclosporine taper with a resolution of symptoms, or who were free from immunosupression at the time of diagnosis, were categorized 'quiescent', while those without a prior history of aGVHD were diagnosed with '*de novo* cGVHD'. Otherwise, acute and limited *versus* extensive chronic GVHD were graded by established criteria.[2] Assignment of the patients to the different categories for the different classifications was established according to the organ involvement observed within the first month of cGVHD diagnosis.

Statistical analysis

Mean and median values, as well as their 95% confidence intervals (CI) and ranges, were calculated for each continuous variable. The χ^2 or Fisher's exact test was used to establish differences in the distribution of discontinuous variables, whereas Student's t-test or Mann-Whitney's U test was applied to compare continuous variables. All reported *P* values are two-sided. *P*=0.05 was considered significant. Overall survival was calculated using the Kaplan-Meier estimate. The log rank test was used for univariate comparisons.

Patients who survived more than 100 days were evaluable for cGVHD. The incidences of cGVHD and its different subtypes were calculated from the time of transplantation using cumulative incidence estimates. Non-relapse mortality (NRM) was defined as "death due to causes unrelated to the underlying disease" and relapsing patients were censored at the time of relapse. GVHD related mortality (cGVHD-RM) was calculated from the time to cGVHD onset until cGVHD related death, defined as "death due to causes directly related to GVHD according to primary physician criteria". More specifically, among patients diagnosed with cGVHD, those deaths attributed to complications or failure in cGVHD-target organs, as well as deaths related to immunosupression such as infectious complications, in patients requiring treatment for cGVHD were considered as cGVHD related mortalities.

Progression of the underlying malignancy and non-relapse mortality (NRM) without prior cGVHD were competing events for cGVHD. The starting point (Day 0) for the landmark analysis of the incidence of cGVHD-RM and NRM was the time of onset of cGVHD, and the competing events were disease progression and death not related to cGVHD. Patients who were still alive and progression-free at the time of analysis were censored at the last follow up or at five years post transplant. Univariate analyses of the variables that influenced cGVHD-RM and NRM were performed using proportional hazards models for competing risks (Gray's test). The variables that showed at least a trend in univariate analysis (*P*<0.1) were used in a multivariate Cox's proportional hazards regression analysis, checking for the assumption of proportional hazards over time for each tested variable.

To analyze the incidences and probabilities of later outcomes, and determine their risk factors in patients who developed cGVHD, we performed a landmark analysis including only patients who developed cGVHD. For the analysis of later outcomes, the starting point for follow up was the day of onset of cGVHD and not the day of transplant. The methods used for generating landmark cumulative incidence estimates, survival probabilities, and univariate and multivariate landmark analyses were identical to those previously described, but of course using the cGVHD landmark database.

Overall survival (OS) was calculated from transplant until death from any cause, and surviving patients were censored at the last follow up. In addition, overall survival from cGVHD onset (OS-cGVHD) was also calculated from the time of cGVHD diagnosis until death from any cause.

All factors that significantly or marginally (*P*<0.1) influenced the incidence or outcome of cGVHD in the univariate analysis were included in a multivariate analysis using a forward step Cox's regression model. The statistical analyses were performed using SPSS version 18.0 (SPSS, Chicago, IL, USA), with the exception of the cumulative incidence plots which were carried out with NCSS 2004 (Number Cruncher Statistical System, Kaysville, UT, USA) and the univariate Gray's test which was carried out using the Cmprsk package R software (The R Foundation, Vienna, Austria).

Results

Incidence and characteristics of graft-versus-host disease

First of all, we confirmed that results were similar among the 3 centers in terms of survival (53%, 52% and 49% at five years, respectively) and NRM (26%, 21% and 30% at five years, respectively). The cumulative incidence of cGVHD was 48%. Respective values were 26% and 22% for limited and extensive cGVHD, and 14%, 22% and 17% for mild, moderate and severe cGVHD, respectively. Fourteen percent of patients developed overlap syndrome while 13% developed delayed aGVHD. Out of 54 cases of delayed aGVHD, 12 achieved complete remission after first-line treatment, one progressed and died while the remaining patients either progressed to cGVHD or had recurrent delayed aGVHD.

Cumulative incidences of *de novo*, quiescent and progressive cGVHD were 18%, 22% and 8%, respectively. As far as organ involvement at time of onset is concerned (Table 2), the most commonly involved organ was oral mucosa in 65% of patients followed by liver in 56% and skin in 55% of patients. At the time of cGVHD diagnosis, 36% had a performance score of 0 according to the ECOG scale and 44% had ECOG 1. Finally, 51% of patients had involvement of 3 or more organs.

As far as the characteristics of cGVHD among patients with classic *versus* overlap cGVHD is concerned (Table 3), a significantly higher percentage of patients with overlap syndrome had a progressive type of onset. In fact, a high correlation was found between overlap syndrome and progressive onset (r=0.44, *P*<0.001). Finally, considering NIH criteria, a higher number of patients displayed features of severe cGVHD among those with overlap syndrome compared to those with classic cGVHD.

Prognostic factors for cGVHD-related mortality

Causes of NRM included cGVHD-RM in 21 patients, fungal infection in 10, viral in 3, respiratory failure in 3,

brain hemorrhage in 2, TTP in one and heart failure in one. As previously specified, in addition to deaths due to causes directly attributed to complications or failure in cGVHD target organs, deaths related to immunosupression such as infectious complications were also considered cGVHD related mortalities. Variables that significantly influenced cGVHD-RM are summarized in Table 4. Type of onset, limited *versus* extensive cGVHD, as well as NIH score system significantly influenced cGVHD-RM. Among variables included in the NIH score, skin, gut, liver and lung involvement had the highest impact on mortality together with performance status. As far as the number of organs involved is concerned, both a cut-off value of 3 and 4 significantly affected the outcome, but the cut-off value of 1-3 *versus* 4 or more organs involved had a better predictive value. Thus, cGVHD-RM was 8% *versus* 18% for patients with 1-2 *versus* 3 or more organs involved (P=0.04) while these figures were 9% *versus* 24% for patients with 1-3 *versus* 4 or more organs involved (P=0.01). Also, platelet count at the time of cGVHD diagnosis significantly influenced cGVHD-RM for a cut-off value of 100×10^9/L. Finally, patients with overlap syndrome had a significantly higher mortality compared to those without (23% *vs.* 8% cGVHD-RM for patients with and without overlap syndrome, respectively; P=0.01). In multivariate analysis, the development of severe cGVHD according to NIH significantly influenced outcome (HR=4.4 (95% CI: 1.3-14.6), P=0.01). When the variables included in the NIH score system were entered in the multivariate analysis, performance status at the time of cGVHD diagnosis [HR=7.9 (3.4-19), P<0.001] and platelet count [HR=5.1 (1.9-14), P=0.01], together with severe gut involvement [HR=6.2 (1.8-22), P=0.01] significantly influenced mortality (Figure 1). Based on these findings, a new variable was developed with a score of 0 or 1 for platelets over or below 100×10^9/L plus score 0, 1 or 2 for ECOG 0, 1 or 2 or over. A score of 3 was given for severe gut involvement irrespective of platelets and ECOG. This variable allowed different subgroups of patients to be differentiated in terms of cGVHD related mortality (Figure 2). Furthermore, when this variable was included in multivariate analysis it had a higher impact on outcome (score 2: HR=6.9 (95% CI: 2-24), P=0.002; score 3: HR=26.3 (95% CI 7.4-93) P<0.001 for score 3. In fact, the same patients with the poorest outcome (score 3) could be identified by just combining ECOG plus platelets.

Table 2. Chronic GVHD organ involvement.

Characteristics	N=336 (%)
Performance status score	
ECOG 0	71 (36)
ECOG 1	85 (44)
ECOG ≥ 2	40 (20)
Oral mucosa	
No	104 (35)
Mild	100 (33)
Moderate	92 (31)
Severe	3 (1)
Liver	
No	133 (44)
Mild	66 (22)
Moderate	65 (22)
Severe	35 (12)
Skin	
No	140 (45)
Mild	69 (22)
Moderate	50 (16)
Severe	51 (17)
Ocular involvement	
No	214 (71.5)
Mild	39 (13)
Moderate	44 (15)
Severe	2 (0.5)
Gastrointestinal tract involvement	
No	220 (74)
Mild	40 (13)
Moderate	35 (12)
Severe	4 (1)
Lung	
No	244 (82)
Mild	26 (9)
Moderate	21 (7)
Severe	7 (2)
Musculoskeletal involvement	
No	288 (96)
Mild	3 (1)
Moderate	8 (3)
Severe	
N. of organs involved	
1 or 2	145 (49)
3 or 4	122 (41)
5 or more	30 (10)

Prognostic factors for OS-cGVHD

The cut-off value of 1-2 *versus* 3 or more organs involved did not significantly affect survival while the cut-off value of 4 retained a significant influence in univariate analysis (Figure 3). Concerning OS-cGVHD (Table 5), the same variables affecting cGVHD-RM significantly affected the outcome (Figure 4). In addition, delayed acute GVHD did not influence survival while patients with overlap syndrome displayed a poorer survival as compared to those with classic cGVHD (Figure 3). In multivariate analysis, again NIH classification significantly affected survival (HR=2.48 (95% CI: 1.38-4.45), P=0.002) for those patients

Table 3. Characteristics of cGVHD among patients with classic *versus* overlap syndrome.

Characteristics N=336	Classic cGVHD N=227	Overlap syndrome N=61	*P*
Type of onset (%)			0.001
De novo	46.5	14	
Quiescent	46	39	
Progressive	7.5*	46	
N. of organs involved at onset (%)		0.19	
1 or 2	49	55	
3 or more	5	45	
Limited / extensive cGVHD (%)			0.087
Limited	58	57	
Extensive	42	43	
NIH score (%)			0.11
Mild	33	36	
Moderate	36	28	
Severe	31	36	
Platelet count (%)			0.2
Mean x 10^9/L (SD)	79 (112)	104 (85)	

Table 4. Univariate and multivariate analysis for cGVHD related mortality.

Characteristics N=336	cGVHD related mortality*	*P* univariate	HR (95% CI)	*P* multivariate
Type of onset (%)[a]		<0.001		
De novo	4			
Quiescent	15			
Progressive	29			
Overlap syndrome		0.01		
No	8			
Yes	23			
Limited / extensive cGVHD (%)		<0.001		
Limited	4			
Extensive	22			
NIH score (%)[b]		<0.001		0.01
Mild	4			
Moderate	9			
Severe	28		4.4 (1.3-14.6)	
Organ involvement (%)				
Skin 0/1/2/3	5/16/17/27	0.01		
Gut 0/1/2/3[c]	7/11/39/75	0.001	6.2 (1.8-22)	0.01
Liver 0/1/2/3	9/10/19/21	0.007		
Lung 0/1/2/3	10/15/27/30	0.04		
Performance status 0 / 1 / ≥ 2[d]	4/12/38	< 0.001	7.9 (3.4-19)	< 0.001
N. of organs involved at onset (%)		0.01		
1 to 3	9			
4 or more	24			
Platelet count (x10^9/L)				
< 100	35	0.001	5.1 (1.9-14)	0.01
≥ 100	10			

**Landmark analysis from cGVHD onset to death. [a]significant differences for the comparison between de novo or quiescent versus progressive type of onset; [b]significant differences for the comparison between mild or moderate versus severe NIH scale; [c]significant differences for the comparison between 0 and 1 versus 2 versus 3 in univariate analysis and HR shown for 0, 1 or 2 versus 3 in multivariate analysis; [d]significant differences for the comparison of 0 versus 1 versus 2 and HR shown for 0 or 1 versus 2 in multivariate analysis.*

Table 5. Univariate and multivariate analysis for OS-cGVHD.

Characteristics N=336	Overall survival 5 years after cGVHD onset	*P* univariate	HR (95% CI)	*P* multivariate
Type of onset (%)[a]		0.025		
De novo	66			
Quiescent	57			
Progressive	50			
Overlap syndrome		0.02		
No	68			
Yes	52			
Limited / extensive cGVHD (%)		0.008		
Limited	70			
Extensive	57			
NIH score (%)[b]		0.001		
Mild	68			
Moderate	66	2.48 (1.38-4.45)	0.002	
Severe	48			
Organ involvement (%)				
Skin 0/1/2/3	74/60/52/48	0.003		
Gut 0/1/2/3[c]	67/65/33/0	<0.001	29.48 (4.9-175)	<0.001
Liver 0/1/2/3	67/64/64/19	0.051		
Lung 0/1/2/3	64/59/55/19	0.026		
Performance status 0 / 1 / ≥ 2[d]	79/61/33	<0.001	4.1 (2.14-7.83)	*P*=0.005
N. of organs involved at onset (%)		0.035		
1 to 3	65			
4 or more	54			
Platelet count (×10^9/L)		<0.001		
< 100	36			
≥ 100	69		2.75 (1.71-4.42)	<0.001

[a]Significant differences for the comparison between de novo versus quiescent or progressive type of onset; [b]significant differences for the comparison between mild or moderate versus severe NIH scale; [c]significant differences for the comparison between 0 and 1 versus 2 versus 3 in univariate analysis and HR shown for 0, 1 or 2 versus 3 in multivariate analysis; [d]significant differences for the comparison of 0 versus 1 versus 2 and HR shown for 0 or 1 versus 2 in multivariate analysis.

with features of mild or moderate *versus* severe cGVHD. In order to analyze which variables had an impact on survival within those included in the NIH score, we included the different organs involved in multivariate analysis. According to this, ECOG (ECOG 1: HR=2.08 (1.11-3.87), *P*<0.001; ECOG 2 or more: HR=4.1 (2.14-7.83), *P*=0.005) and a platelet count less than 100×10^9/L (HR=2.75 (1.71-4.42), *P*<0.001) at the time of cGVHD diagnosis, together with gut involvement (HR=24.06 (4.21-137.4), *P*<0.001) significantly affected outcome. Variables with a closer correlation to performance status were skin (r=0.16, *P*=0.012), gut (r=0.25, *P*<0.001) and lung involvement (r=0.29, *P*<0.001).

Finally, we combined ECOG, platelets and gastrointestinal involvariable allowed 4 subgroups of patients to be clearly identified in terms of outcome (Figure 5); overall survival was 84% for patients with a combined score of 0 (n=74), 64% for patients with a combined score of 1 (n=93), 43% for patients with a combined score of 2 (n=58) and 0% for patients with a combined score of 3 or more (n=17). Furthermore, in multivariable involvement using the previously mentioned score system and again the new variable analysis, the variable obtained had the highest impact on survival (score 0: HR=15.96 (95% CI: 6.85-37.17), *P*<0.001; score 1: HR=5.47 (95% CI: 2.6-11.5), *P*<0.001; score 2: HR=2.8 (95% CI: 1.32-5.93), *P*=0.007).

As for transplant related mortality, the combination of ECOG plus platelets also identified the same subgroup of patients with the worst outcome (score 3). Furthermore, the combined variable ECOG plus platelets also identified different subgroups of patients in terms of survival within the different NIH categories. Therefore, for patients with mild cGVHD, the combined variable identified patients with 90%, 61%, 57% and 25% OS (*P*<0.001); the corresponding values for patients with moderate cGVHD were 93%, 65%, 44% and 0% OS (*P*=0.001), and the respective values for severe cGVHD were 66%, 58%, 38% and 0% OS (*P*<0.001), respectively.

Discussion

Chronic GVHD (cGVHD) is the major cause of morbidity and mortality in long-term survivors after allogeneic stem cell transplantation (allo HSCT). However, cGVHD is also correlated with a strong graft-*versus*-malignancy effect, as previously reported in different hematologic malignancies.[7-9] This complicated relationship between increased risk for non-relapse mortality and decreased risk of relapse highlights the importance of defining the group

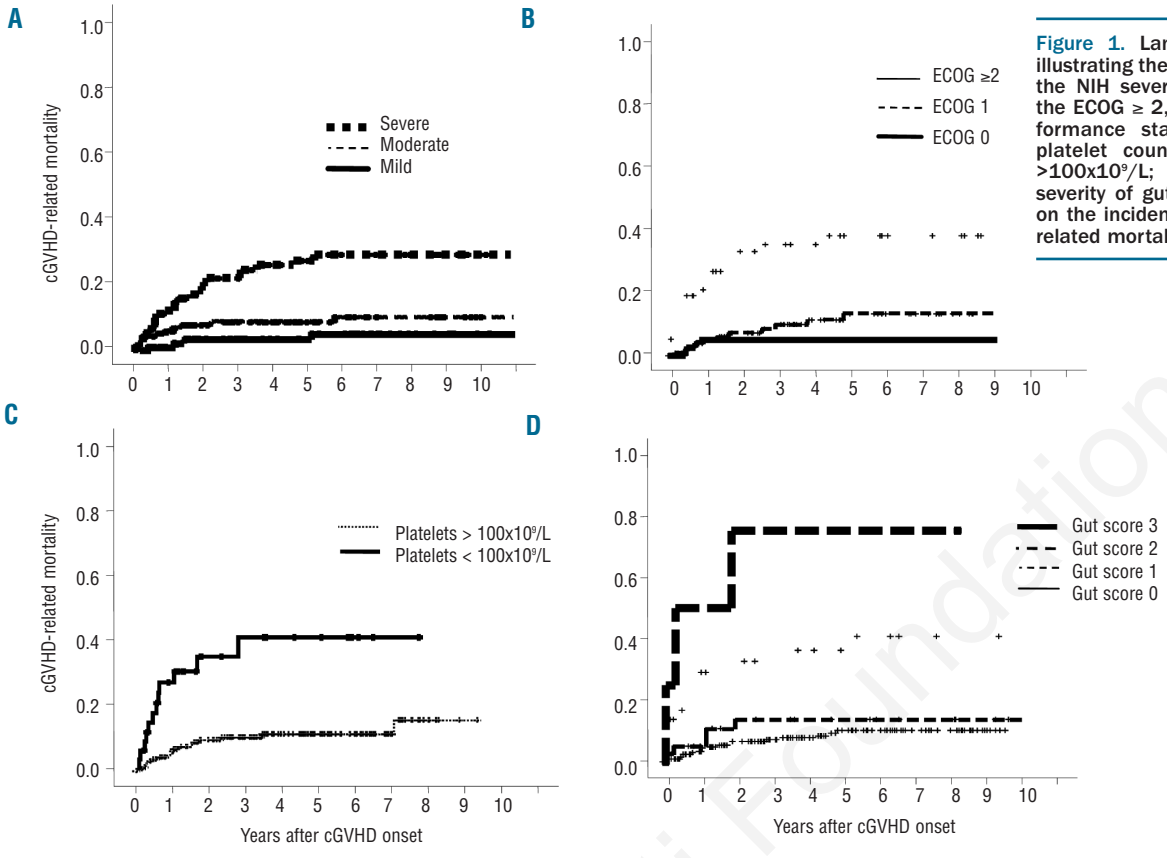

Figure 1. Landmark plots illustrating the impact of (A) the NIH severity score; (B) the ECOG ≥ 2, 1 and 0 performance status; (C) the platelet count <100 and >100x10⁹/L; and (D) the severity of gut involvement on the incidence of cGVHD-related mortality.

of patients who may benefit from systemic immune-suppression *versus* those who may just require topical or local treatment.

The first classification of cGVHD into limited or extensive forms was based on 20 patients and was aimed at separating patients who needed systemic rather than local or topical therapy.[2] Unfortunately, most patients are finally categorized as having extensive cGVHD, especially among those receiving peripheral blood progenitor cells.[10] In 2005, the NIH published consensus criteria for diagnosis of cGVHD that establish the diagnosis of acute or chronic GVHD on the basis of the clinical manifestations and not the time of onset.[4] Furthermore, the NIH established a scoring system in an attempt to identify those patients who may require systemic treatment or those who may benefit from topical treatment only. Although this classification has been evaluated in several studies,[3,5,6,11] its prognostic value has not been evaluated in a multicenter study in a large series of patients. Furthermore, few studies have evaluated the prognostic value of the new entities, delayed acute and overlap syndrome, proposed by the NIH with contradictory results.[3,6,12]

Therefore, we aimed to evaluate the prognostic value of the published NIH consensus criteria on cGVHD, focusing on developing a simple scoring system, and evaluation of the prognostic value of the new entities proposed by the NIH.

The incidence of cGVHD in this study was 48% which is in line with previous reports, although the reported incidence varies between 6% and 80%.[1,10,13,14] The reasons for this disparity are multi-factorial and include diagnosis,

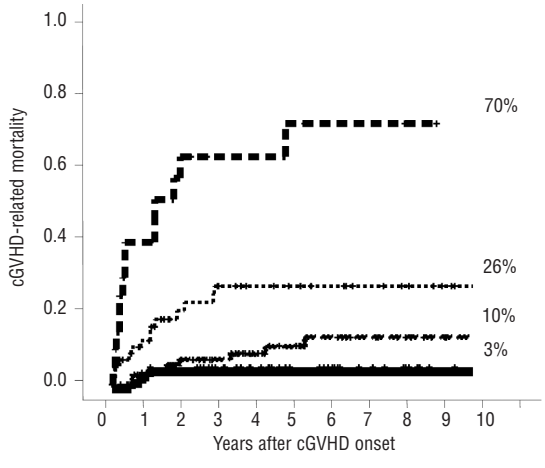

Figure 2. Landmark plot illustrating the impact of the new composite variable on the incidence of cGVHD-related mortality. The composite variable has a value of 0 to 3 based on the sum of platelet count < 100x10⁹/L (1 point) and the ECOG performance status (0, 1 or 2 points for ECOG of 0, 1 o ≥2). In addition, patients with severe gut involvement were assigned 3 points irrespective of platelets and ECOG.

type of donor,[15] conditioning regimen, GVHD prophylaxis,[7] stem cell source,[14] graft manipulation,[16] use of donor leukocyte infusion (DLI),[17] etc. In addition, the lack of standardized diagnostic criteria is also likely to be an important reason for this heterogeneity in the incidence of cGVHD.

Our retrospective study included patients from 3 differ-

ent European centers. Medical records of 336 patients who developed cGVHD were evaluated and organ involvement was categorized according to NIH criteria if the medical records contained documentation showing unequivocal manifestations. Transplant related mortality and overall survival was similar for all 3 centers indicating a homogeneous study population.

As far as the new sub-categories proposed by the NIH are concerned, we can confirm that delayed acute cGVHD has no adverse effect on outcome. This is in agreement with conclusions of Vigorito *et al.*[5] In addition to these data, in the current study we were able to evaluate the outcome of delayed aGVHD and observed that a significant proportion of patients subsequently developed recurrent delayed acute or chronic GVHD. These data would suggest that, in terms of immune suppressive treatment, these patients should be initially managed as aGVHD but treatment should be maintained for a longer period of time.

Contrary to delayed aGVHD, overlap syndrome is an

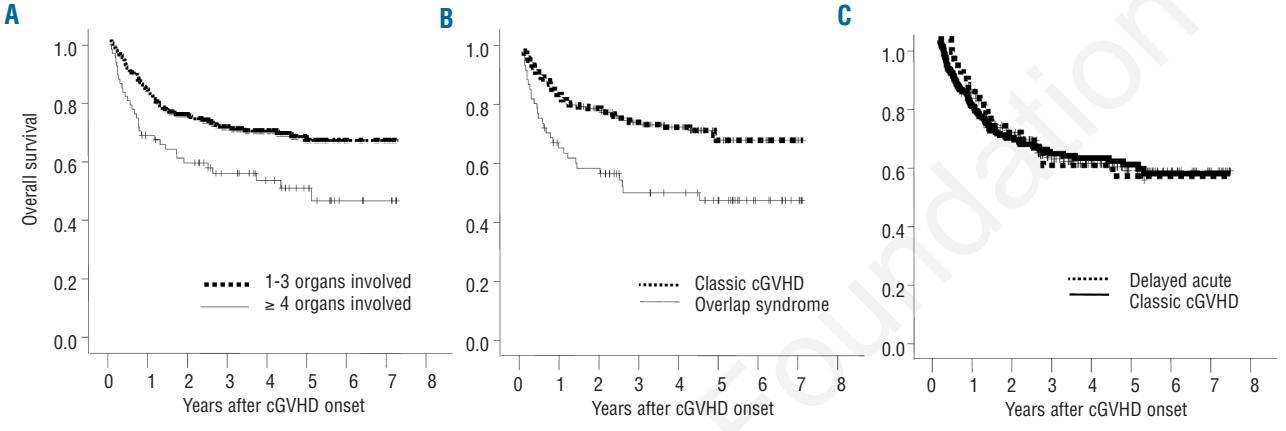

Figure 3. (A) Overall survival from cGVHD diagnosis for patients with 1-3 *versus* ≥ 4 organs involved (B) for patients with classic cGVHD *versus* overlap syndrome and (C) for patients with delayed aGVHD *versus* classic cGVHD.

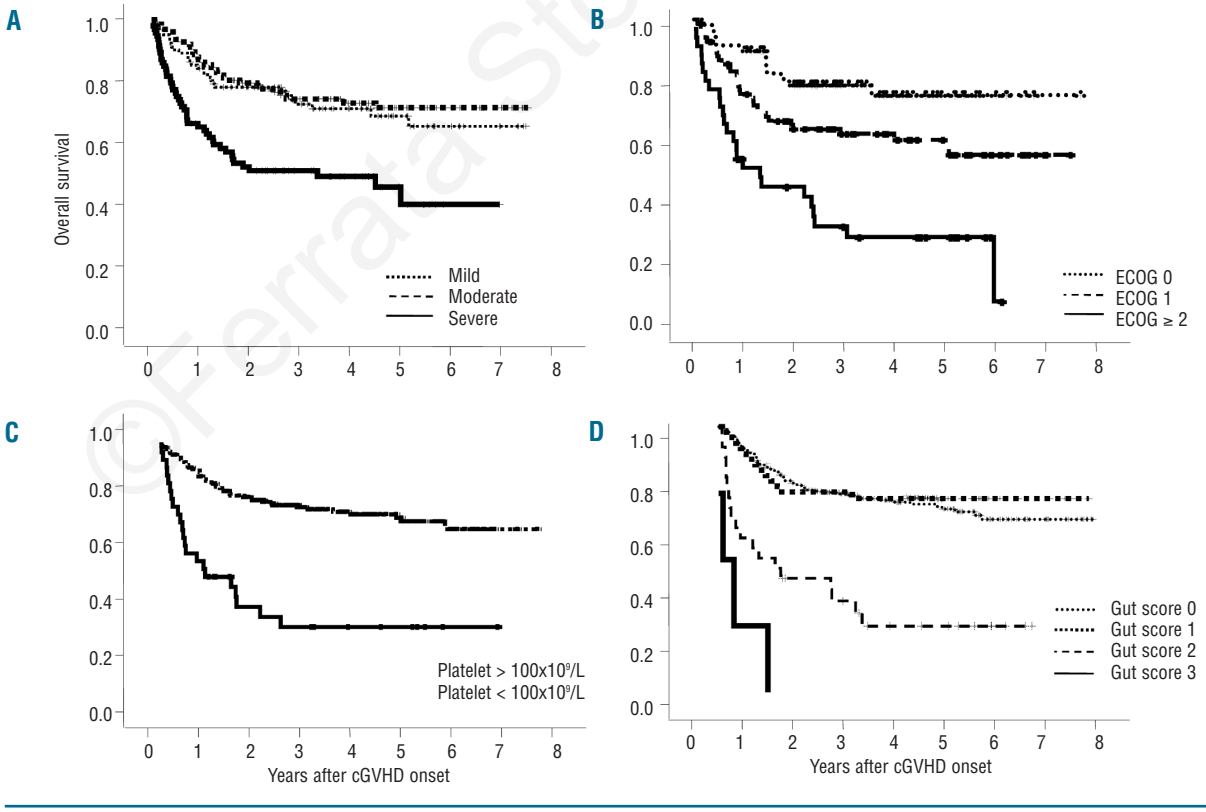

Figure 4. (A) Overall survival from cGVHD diagnosis for patients with mild, moderate or severe cGVHD (B) for patients with ECOG 0, 1 and ≥ 2 (C) for patients with platelets <100 and >100x10⁹/L and (D) for patients with gut score 3, 2, 1 and 0.

adverse prognostic factor. This finding has been reported previously.[11] It is worth mentioning that a high percentage of patients categorized as having overlap syndrome had a progressive type of onset so that both variables were highly correlated. In this retrospective analysis, we carefully tried to differentiate between patients who had signs or symptoms of aGVHD that were resolving at the moment in which the signs or symptoms of cGVHD appeared, i.e. classic cGVHD with progressive type of onset from those who developed both active signs or symptoms of acute and chronic GVD, i.e. true overlap syndrome. Nevertheless, these data must be confirmed in prospective studies.

We confirmed that NIH criteria are the most important variables in predicting outcome in multivariate analysis. Nevertheless, only severe forms can be clearly differentiated in terms of outcome while mild and moderate disease had rather similar outcomes. Among those variables included in the NIH, performance status according to ECOG score and platelet counts at the time of cGVHD had the highest impact on outcome, both in terms of cGVHD-RM and survival. In addition, gastrointestinal involvement significantly influenced outcome in multivariate analysis while skin, liver and lung involvement also affected outcome in univariate analysis. By contrast mouth, eyes and musculoskeletal involvement did not influence outcome. In fact, skin, liver and lung involvement had a high correlation with ECOG and, accordingly, are responsible for the performance score of the patients at the time of cGVHD.

According to these data we created a new score system which, in fact, was much more powerful than NIH in predicting outcome and far easier to perform. Thus, greater ECOG score in combination with a low platelet count together with severe gastrointestinal involvement are strong prognostic factors for cGVHD-RM and OS. Interestingly, the simple combination of ECOG plus platelet count allowed us to discriminate the same subgroups of patients suggesting that this combination has prognostic impact and could be applied irrespective of the organs involved. Larger studies with a higher number of patients with severe involvement of skin, liver or lung are required to confirm these data and also to confirm the prognostic value of this score within the different NIH subgroups. Interestingly, Lee S *et al.*[18] reported that Karnofsky performance score, diarrhea, weight loss, and cutaneous and oral involvement are independent prognostic factors among patients with cGVHD. While we did not find cutaneous or oral involvement to be independent prognostic factors, the prognostic value of performance score and gastrointestinal involvement (manifested as diarrhea and/or weight loss) are confirmed in the current study. By contrast, we also found platelet count to be an independent prognostic factor, similar to the study by Akpek *et al.*[19] which described extensive skin involvement, progressive type of onset and thrombocytopenia as independent prognostic factors. While we also found these variables to have a prognostic value in univariate analysis, only thrombocytopenia was confirmed in multivariate analysis. In a recent large retrospective study, Arora *et al.*[20] developed a new score system among patients with cGVHD which, similar to the current study, also identifies platelet count and performance status as the most important prognostic factors at the time of cGVHD diagnosis. In contrast to the current manuscript, their study is based on registry data so that no specific analysis can be provided regarding either the impact of organ involvement or the NIH classification on outcome. By contrast, Arai *et al.*[21] have recently reported a prospective study which confirms the impact of the NIH classification on outcome. Interestingly, the percentage of patients diagnosed with mild cGVHD is much lower than the current study although, as discussed by the authors, the requirement of systemic treatment in order to enroll the patients in the cohort could be a reason why the incidence of cGVHD was underestimated. It is worth mentioning the similarities in terms of cGVHD related mortality in the different NIH subgroups in both studies. By contrast, the survival reported by Arai *et al*. is better for the different NIH subgroups than the current study. Median follow up in their series is 18 months while the current series of patients were transplanted between 2000 and 2006; this could at least in part justify the difference.

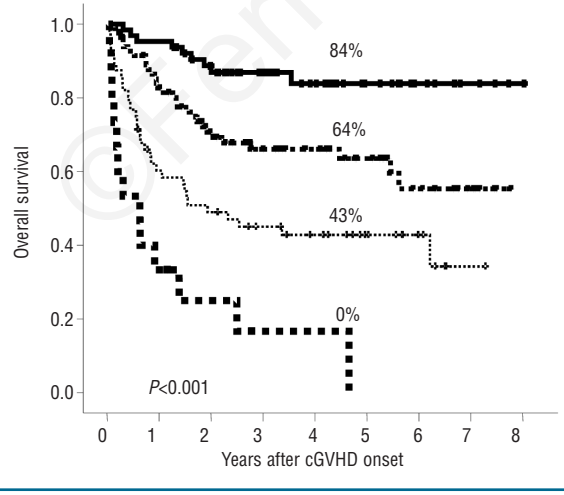

Figure 5. Overall survival from cGVHD diagnosis depending on the variable which resulted from combining ECOG, platelets and gastrointestinal involvement. The four graphs show overall survival for patients with a score of 0 (84%), 1 (64%), 2 (43%) and ≥3 (0%).

Interestingly, NIH proposed a cut off of involvement of 3 organs in order to distinguish mild from moderate cGVHD[4] so that, according to this classification, systemic immune suppression is recommended for patients with involvement of 3 organs. Since ours is a retrospective study, we cannot draw any conclusions about the best treatment for patients with involvement of 3 organs but, according to our data, these patients had a similar survival to those with 1-2 organs involved and a significantly better survival than those with 4 or more organs involved. Thus, further studies will be required to confirm the best cut off for systemic treatment.

In conclusion, we have identified a simple prognostic tool that does not take too long to identify different subgroups of patients in terms of cGVHD-RM and survival. Regarding the new entities proposed by NIH, delayed acute GVHD did not influence outcome while overlap syndrome did. Finally, involvement of 4 or more organs with moderate scores according to NIH criteria is required before systemic immune suppression should be started.

Authorship and Disclosures

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

Financial and other disclosures provided by the authors using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are also available at www.haematologica.org.

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