

ORIGINAL ARTICLE

HLA AND RISK OF ACUTE GRAFT VERSUS HOST DISEASE IN ALLOGENEIC BONE MARROW TRANSPLANTATION FROM AN HLA-IDENTICAL SIBLING

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Abstract

Background-Recent reports have shown a probable association between certain HLA antigens and acute graft versus host disease (GVHD) after allogeneic bone marrow transplantation (BMT).

Methods-Medical records of 162 patients who had undergone allogeneic BMT from an HLA-identical sibling in the Hematology, Oncology and Bone Marrow Transplantation Center of Shariati Hospital in Tehran between 1991 and 1999, were studied and analyzed by univariate and multivariate analyses. All factors including HLA antigens, age, sex and diagnosis were examined jointly using a logistic regression analysis. The relationship between HLA antigens and acute GVHD was re-examined within the regression setting.

Results- The diagnosis was thalassemia in 81 (50%), CML in 27 (16.7%), AML in 16 (9.9%), ALL in 6 (3.7%) and aplastic anemia in 22 (13.6 %) cases. Overall, 36 (22.2%) patients developed clinical Grade III and IV acute GVHD. Univariate analysis confirmed an increased risk of severe acute Grade III and IV GVHD, which was associated with the diagnosis of AML, donor to recipient sex mismatch and probably with the allele HLA-DR7 ($p=0.01$, 0.051 and 0.07 , respectively). The risk of GVHD was reduced in the presence of the HLA-B35 allele ($p=0.04$). Multivariate analysis confirmed a decreased risk of acute GVHD associated with HLA-B35 ($p=0.01$), while the risk was increased in patients with AML ($p=0.009$) and in those with a sex-mismatched donor.

Conclusion-In this study, AML, donor-to-recipient sex mismatch and probably HLA-DR7 were found to be probable risk factors associated with GVHD, and HLA-B35 was found to be a protective factor against severe acute GVHD. These data might be useful as prognostic factors in predicting the risk of GVHD and also in defining risk-related methods for GVHD prophylaxis.

Keywords • Graft versus host disease (GVHD) • human leukocyte antigen (HLA) • bone marrow transplantation (BMT)

Introduction

Graft versus host disease (GVHD), the most important and serious complication of allogeneic bone marrow transplantation, is the result of an immunologic reaction of donor cells against host antigens. There

are some identified risk factors associated with GVHD, such as age and sex differences between the donor and host. Recent reports have suggested various risk factors, among which, some have noticed probable association between certain HLA antigens and increased or decreased rate of acute GVHD.¹⁻⁴ However some have reported no change in the risk of acute GVHD due to HLA antigens.⁵

In spite of exclusive serologic and cellular

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histocompatibility testing, GVHD is still the most important cause of mortality after BMT. Although the donor and the host are usually identical at the major histocompatibility complex (MHC), a disparity at the minor histocompatibility antigens is probably responsible for the large number of patients who develop GVHD. Minor histocompatibility antigens are endogenous peptides that occupy the cleft between the α -helices of MHC molecules. All individuals have about seven minor histocompatibility loci, which encode antigens that cause GVHD.⁵ Hence, for acute GVHD to occur, there must be a non-HLA antigen difference between the patient and the sibling donor, and also the grafted marrow must contain or produce cells able to mount the immune reaction against the non-HLA antigens of the host. The wide range of GVHD activity from mild (assuring grade I and II) to severe (life threatening grade III and IV) might be due to the degree of disparity of these antigens, and to differences in the ability of the donor lymphocytes to react against recipient antigens.¹ On the other hand, certain classes of MHC molecules may not be able to present any peptides as minor histocompatibility antigens that cause GVHD, and alternatively the variety of minor histocompatibility antigens could be constrained by the repertoire of polymorphic peptides encoded by minor histocompatibility loci, so that certain MHC molecules might not present any peptides that initiate GVHD.⁵ Therefore, certain HLA alleles could be associated with reduced risk of GVHD, while others may be associated with increased risk.

In the current study, we have analyzed our center's experience in HLA-identical sibling donor allogeneic BMT, to define the possible association between certain HLA antigens and acute GVHD. The results may prove useful in predicting the risk of GVHD after allogeneic BMT as well as in modifying techniques for GVHD prophylaxis.

Materials and Methods

Patients

The clinical records of 162 recipients of HLA-identical sibling donor, allogeneic bone marrow transplants, which had been performed in Hematology, Oncology and Bone Marrow Transplantation Center of Shariati Hospital in Tehran between 1991 and 1999, were studied. The diagnoses were thalassemia in 81 (50%), CML in 27 (16.7%), AML in 16 (9.9%), ALL in 6 (3.7%),

Table 1. Patients' characteristics.

Characteristic	No. of Patients (%)
Sex	
Male	89 (54.9%)
Female	73 (45.1%)
Disease:	
Thalassemia	81 (50%)
CML	27 (16.7%)
AML	16 (4.4%)
ALL	6 (3.7%)
Aplastic anemia	22 (13.6%)
Other	10 (6.1%)
Donor: recipient sex mismatch	68 (60.5%)

aplastic anemia in 22 (13.6%) cases and other diseases (3 with Fanconi's anemia, 2 with dyskeratosis congenita, one with Chediak-Higashi, one with paroxysmal nocturnal hemoglobinuria and one with myelodysplastic syndrome) in the remaining cases. The mean age was 14.25 ± 10.49 years (range 2 to 40 years) and 73 cases were females (45.1%). The patients' characteristics are shown in Table 1.

Pre-transplant conditioning included cyclophosphamide plus busulfan in most patients. Prophylaxis against acute GVHD included cyclosporine and methotrexate, according to standard methods. All patients were nursed in isolated rooms, and all received oral ciprofloxacin, acyclovir and fluconazole. In presence of fever, the patients were treated empirically with broad-spectrum antibacterial agents.

HLA typing

HLA typing was done for the patients, sibling bone marrow donors, and parents. Serologic typing was performed using the microcytotoxicity method. Donor selection criteria required full matching for the serologically defined HLA alleles. The categorization of HLA antigens and the number of patients with each antigen are summarized in Table 2.

Assessment of GVHD

The diagnosis of acute GVHD was made clinically, and the grading was performed according to the standard Seattle Criteria with additional modifications.⁶ The severity of GVHD was evaluated by grading of organ involvement, including the skin, liver and gastrointestinal tract and final grades ranging from 0 to 4 were assigned to each patient as follows: no reaction (0),

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Table 2. Relationship between HLA types and severe GVHD.

HLA-A* locus	n†	Severe‡ GVHD	p value	HLA-B locus	n	Severe GVHD	p value	HLA-DR locus	N	Severe GVHD	p value
A1	31	8	0.5	B4	1	0		DR1	18	3	0.5
A2	56	13	0.8	B5	51	11	0.8	DR2	51	12	0.7
A3	35	8	0.9	B7	10	1	0.3	DR3	25	6	0.8
A5	1	0		B8	14	2	0.4	DR4	28	5	0.5
A9	11	2	0.7	B12	3	0		DR5	2	1	
A10	2	0		B13	8	4	0.05	DR7	18	7	0.07
A11	29	7	0.7	B14	6	2	0.5	DR9	2	0	
A13	2	1	0.3	B15	6	2		DR11	3	1	
A19	2	0		B15	3	0		DRw1	1	0	
A20	1	0		B16	2	0		DRw6	5	2	
A24	39	7	0.4	B17	16	4	0.7	DRw8	4	2	
A26	11	3	0.6	B18	14	4	0.5	DRw9	1	0	
A28	7	2	0.6	B21	21	4	0.7	DRw10	1	0	
A29	12	2	0.6	B22	1	0		DRw11	41	11	0.4
A30	3	1		B24	2	1	0.2	DRw13	3	0	
A31	2	0		B27	5	6					
A32	4	1		B35	49	0	0.04				
A33	2	0		B39	2	6					
Aw33	1	0		B40	10	3	0.5				
A23	1	0		B44	8	1					
A25	1	0		B51	2	1					
		0		B70	4	1					
				Bw22	1	0					
				Bw73	8	3					
					1	0					

*Only individual alleles expressed at one or both loci in more than 10 patients were tested and allele subtypes in fewer than 10 cases were excluded, †Number of patients expressing the alleles shown at either or both loci, ‡Number of patients with acute grade 3 or 4 GVHD.

insignificant (I), mild (II), moderate (III) and severe (IV). In this study grades 0, I and II were considered as mild, and grades III and IV were severe reaction.⁶

Statistics

The endpoint of this study was the development of acute severe (grade III and IV) GVHD. The association between HLA antigens and acute severe GVHD was examined by univariate and multivariate analysis, using an SPSS package. The HLA antigens, which were found in less than 10 patients, were excluded from the multivariate analysis. First, each of the probable risk factors, including HLA antigens, age, sex mismatch, donor's sex, recipient's sex and the diagnoses, was examined individually for possible relationships with acute GVHD. In order to do that, we used the *t* test, to compare GVHD incidence in the presence of each particular HLA antigen to that in the absence of the antigen and *p* value of less than 0.05 was considered significant. Then, all those factors were re-examined jointly in the multivariate analysis using a stepwise logistic regression model, to find independent risk factors for severity of GVHD.

Results

GVHD incidence

Among the 162 HLA-matched sibling donor allogeneic transplants performed at our center during 8 years, 36 (22.2%) developed severe acute GVHD (23 grade III and 13 grade IV), 55 (34%) grade II acute GVHD and 31 (19.1%) grade I acute GVHD. The difference between mean survival of patients with severe GVHD and that patients with mild GVHD was significant ($p=0.003$). Overall, 118 cases (72.8%) were still alive while 44 (27.2%) had expired; the reason of death being GVHD in 18 (43.9%), sepsis in 7 (17.1%), multiple organ failure in 2 (4.9%), hemorrhagic cystitis in 2 (4.9%), relapse in 5 (12.2%), ARDS in 4 (9.8%) and other reasons in 6 cases.

Univariate analysis

The variables tested in this study for probable association with risk of development of GVHD included: recipient age, recipient's sex, donor's sex, recipient to donor sex mismatch, the diagnosis and HLA alleles, including HLA-A, B and DR alleles.

Overall, 50% of our patients were less than 10

Table 3. Results of univariate analysis.

	No. of patients	No. of patients with severe GVHD	Risk of severe of GVHD	P value
Diagnosis of AML	16	9	Increased	0.01
Donor: recipient sex mismatch	68	20	Increased	0.05
HLA-B35	49	7	Decreased	0.04
HLA-DR7	18	7	Probably increased	0.07

years. The difference in GVHD occurrence and its severity was similar in patients aged less than 10 and older than 10 years ($p=0.4$). The diagnosis appeared to show a relationship with GVHD, and 56% (9 of 16) of AML patients experiencing GVHD, compared to 18% of thalassemic patients, 25% of CML patients and 13% of patients with aplastic anemia ($p=0.03$). The association between diagnosis of AML and severe acute GVHD was significant ($p=0.01$).

Neither recipient's sex nor donor's sex, predicted different rates of GVHD, but donor recipient sex mismatch was associated with an increased risk of severe acute GVHD ($p=0.05$). The results of univariate analysis have been summarized in Table 3.

As shown in Table 2, among all alleles tested (when expressed in groups with more than 10 patients), HLA-DR7 was probably associated with an increased risk of acute GVHD ($p=0.07$), while the risk was significantly reduced in the presence of HLA-B35 ($p=0.04$) and the incidence of acute GVHD in the presence of HLA-B35 is estimated to be about half that in the absence of the allele.

Multivariate analysis

A summary of the stepwise logistic regression results has been shown in Table 4. Using the regression model, recipient's age, recipient's sex and donor's sex were not recognized as important in predicting the risks of GVHD. The dominant factor significantly associated with reduced risk of GVHD, was HLA-B35 ($p=0.01$).

The relationship between the diagnosis and acute GVHD was re-examined within the regression setting, and multivariate analysis confirmed a relationship between diagnostic categories and acute GVHD ($p=0.01$). Patients

with AML and those with a sex-mismatched donor were at significantly higher risk of acute severe GVHD ($p=0.009$ and $p=0.05$, respectively).

Discussion

Previous studies have tested relationships between HLA antigens and risk of GVHD. Storb et al¹ found that among the 130 patients with aplastic anemia, there was a significant relationship between risk of severe acute GVHD and HLA-B subtypes, but no relationship existed between its risk and HLA-A antigens. In that study, it was claimed that the incidence of severe GVHD in patients with HLA-B18 was nearly three times higher than that in other patients, and the incidence in patients with HLA-B8 or HLA-B35 was about half in the absence of these alleles. Bross et al² reported a significantly higher risk of severe acute GVHD occurrence in older patients, in those with ALL as well as those with a sex-mismatched donor. In addition, in their study, the presence of HLA-CW4 or HLA-BW21 was associated with an increased risk of severe acute GVHD, while the presence of HLA-A19 and probably HLA-B17 was associated with a decreased risk.² Weisdorf et al³ claimed that HLA-A26 and HLA-DR3 were associated with increased and decreased risk of severe acute GVHD, respectively. Also, they found that the most dominant factor associated with severe acute GVHD in their study was patient's age more than 18 years.³ Martin et al⁵ found no significant relationship between HLA antigens and risk of acute GVHD.

In the current study, we found HLA-B35 to be associated with a decreased risk of severe acute GVHD (about half that in patients without HLA-B35). Interestingly, this finding is concordant with

Table 4. Results of multivariate analysis.

	No. of patients	Risk of severe GVHD	p value
HLA-B35	49	Decreased	0.01
AML	16	Increased	0.009
Donor: recipient sex mismatch	68	Increased	0.05

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data reported by Storb et al.¹ In contrast, we found no association between HLA-B8 and severe acute GVHD. On the other hand, similar to Bross², we found that donor: recipient sex mismatch is associated with an increased risk of GVHD, but our results suggesting an increased risk of GVHD associated with AML, are in contrary to their results (in which they reported the same result, but for ALL). In fact, we found no association between ALL and risk of GVHD, although it should be considered that the number of patients with ALL in our study was low. In addition, in contrast to the study of Bross et al.² and that of Weisdorf et al.³, we found no relationship between patient's age and risk of severe GVHD. This finding may be due to the fact that our patients were mostly young (50% were less than 10 years old). Some previous reports have claimed that the risk of GVHD occurrence is lower in female recipients³, but in our study neither recipient's sex nor donor's sex predicted different rates of GVHD. No association between HLA-A antigens and risk of GVHD, were found in our study whereas Bross et al.² have suggested a decreased rate of GVHD associated with HLA-A19, and Weisdorf et al.³ have suggested an increased risk associated with HLA-A26.

These differences may be due to several reasons, for example, our conditioning regimen is different from those of other centers, and also total body irradiation is not included in our programs. In addition, the prevalence of certain HLA antigens (such as HLA-B18) was very low among our patients (probably due to racial difference). In some studies² Grade II GVHD was considered severe, while in our analysis only Grade III and IV were considered, as severe GVHD.⁶

It should be kept in mind that until present, there has been no confirming evidence for the above-mentioned relationships. However, the evidence strongly suggested, the HLA alleles could be the primary determinants in the development of

GVHD², and in conjunction with diagnosis and sex mismatch (and age according to previous reports), may influence the prognosis. Currently, there is no certain explanation for this observation, but it is possible that minor transplantation antigens, interact differentially with HLA-specificity to contribute to incidence and severity of GVHD.²

In this study, we suggest HLA-B35 as a protective factor against GVHD, perhaps because of associated suppressor genes and weaker immune response to non-HLA antigens. The probable association between HLA-B35 and reduced risk of acute GVHD has possible implications for the management of immunosuppression in bone marrow transplant recipients. The usual immunosuppression may not be needed in such patients and its side effects could thus be avoided. These data might be useful in predicting the risk of severe acute GVHD in order to use modified techniques for GVHD prophylaxis.

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