Infliximab treatment for steroid-refractory acute graft-versus-host disease

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A B S T R A C T

Background and Objectives. Tumor necrosis factor α is one of the principal cytokines involved in the pathogenesis of acute graft-versus-host-disease (GVHD). Infliximab is an antibody to this cytokine.

Design and Methods. We performed a retrospective analysis to evaluate the activity of infliximab in 32 patients with severe steroid-refractory acute GVHD. The patients received a median of 3 weekly courses of infliximab. The main organs involved in the patients were skin (n=2) liver (n=1), bowel (n=19), liver and bowel at the same stage (n=10).

Results. Nineteen out 32 patients (59%) responded to infliximab with 6 (19%) complete and 13 (40%) partial responses. Age younger than 35 years, intestinal involvement and a longer time between hematopoietic stem cell transplantation and infliximab administration were factors predicting a favorable response. Infective episodes developed in 23/32 (72%) patients. All the 13 unresponsive patients died of GVHD shortly after infliximab. Thirteen of 19 responsive patients were alive at a median follow-up of 449 days (range 155-842) after infliximab, with no signs of chronic GVHD (n=5), limited (n=5) or extensive involvement (n=3). Six patients who responded subsequently died, one of chronic lung GVHD, the others of vascular complications or infections (2 fungal diseases).

Interpretation and Conclusions. We conclude that infliximab is active in the treatment of severe steroid-refractory acute GVHD, particularly when the intestine is involved. Infections commonly followed its administration. The clinical activity of infliximab and the possibility that it increases the risk of infections are worth investigating in prospective trials.

Key words: acute GVHD, TNF-α, infliximab.

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Transplantation with allogeneic marrow or peripheral blood hematopoietic stem cells (HSCT) has been increasingly performed for a wide variety of hematologic diseases in the last two decades. Acute graft-versus-host disease (GVHD) is a common cause of significant morbidity and mortality after allogeneic HSCT, especially in patients in whom first-line treatment with high-dose steroids has failed. Insights into the pathophysiology of GVHD have indicated that the gastrointestinal tract is not only a major target organ of GVHD, but also that it has a critical role in initiating and amplifying the inflammatory process.[1] Both inflammatory cytokines and cells from the donor and the host contribute to the tissue damage of GVHD. Tumor necrosis factor α (TNF-α) emerged as a key cytokine in the inflammatory cascade of acute GVHD. In fact, this cytokine, secreted together with interleukin-1 by the macrophages of the host gastrointestinal tract damaged by drugs of the conditioning regimen, leads to donor T-cell proliferation and secretion of interleukin-2 and interferon-α, with further amplification of T-lymphocytes and mononuclear phagocyte responses. Damage to the intestinal mucosa allows translocation of lipopolysaccharides of the normal bowel flora and other immuno-stimulatory molecules from the intestinal lumen to the circulation, propagating the cytokine storm characteristic of acute GVHD.

Serum levels of TNF-α and soluble TNF-α receptors I and II increase in patients with acute GVHD and have been shown to be sensitive and specific parameters for this complication.[2-4] Some clinical experience has suggested that infliximab, an antibody against TNF-α, can have beneficial effects in patients with steroid-refractory acute

GVHD.[5-12] Infliximab, already approved for clinical use in rheumatoid arthritis and Crohn's disease, is a chimeric mouse/human IgG$_{1\kappa}$ antibody that binds with high affinity to soluble and transmembrane forms of human TNF-α. Binding to soluble TNF-α results in the neutralization of its activity, whereas binding to the transmembrane form of TNF-α causes lysis of the affected cells by activation of complement and induction of antibody-mediated cellular toxicity.[13] In a mouse model, blocking LIGHT (a T-cell co-stimulatory molecule belonging to the TNF-α superfamily), with monoclonal antibodies led to a persistent anergy of CD4 T lymphocytes and prevented acute GVHD after infusion of immuno-competent allogeneic donor cells.[14]

Based on these findings, we conducted a retrospective analysis to evaluate the activity of infliximab in steroid-refractory acute GVHD.

Design and Methods

Patients

Thirty-two patients receiving an allogeneic HSCT for a neoplastic disease between June 2000 and December 2004 were retrospectively analyzed. Clinical and laboratory data of the patients were collected through a questionnaire sent to 26 major Italian Transplant Units for children and adult patients. Eight Hematology Centers replied that they had some experience with infliximab treatment and sent the data on 32 patients. All subjects met the following eligibility criteria: (i) diagnosis of hematologic or solid tumor; (ii) non-T-cell-depleted transplant from an HLA-matched sibling or unrelated donor (fully matched for HLA-A,-B, -DR by serological and molecular testing); (iii) development of acute GVHD ≥ grade II refractory to prednisone therapy; (iv) salvage treatment with infliximab (Remicade™).

The patients' characteristics are shown in Table 1. The median age at transplant was 39 years (range 2-66). Seven patients were younger than 14 years. Twenty-two of the 32 patients were male (69%). The hematologic diseases necessitating transplantation were acute leukemia (n=14), chronic myeloid leukemia (n=6), lymphoma (n=4), multiple myeloma (n=5) Fanconi's anemia (n=1) renal carcinoma (n=1), and prostatic carcinoma (n=1). The median time between diagnosis and transplant was 16 months (range 4-68). Nine out the 32 patients (28%) were transplanted in a late phase of disease after heavy pre-treatment (previous autologous stem cell transplantation and/or ≥ 3 chemotherapy regimens). All the patients provided written informed consent to the allogeneic HSCT and infliximab administration under studies approved by the local Ethical Committees.

Table 1. Clinical characteristics of the patients treated with infliximab.

Clinical characteristics	No. of patients	(%)
Age at HSCT (years)		
Median (range)	39	(2-66)
Sex		
Male	22	69
Female	10	31
Diagnosis		
Acute myeloid leukemia	9	28
Acute lymphocytic leukemia	5	16
Chronic myeloid leukemia	6	18
Lymphoma	4	13
Myeloma	5	16
Solid tumor	2	6
Fanconi's anemia	1	3
Heavy pretreatment	9	28
Time diagnosis-HSCT		
Median (range)	16	4-68
Donor		
Sibling	18	56
Unrelated	14	44
Conditioning regimen		
TBI	9	28
BU±CY± melphalan	10	31
Reduced intensity	13	41
Source of stem cells		
BM	19	59
PB	13	41
GVHD grade		
II	4	12%
III	8	25%
IV	20	63%
Main organ(s) involved		
Skin	2	6%
Liver	1	3%
Bowel	19	60%
Liver + bowel	10	31%
GVHD treatment prior to infliximab		
Cyclosporine A	26	81%
Prednisone	32	100%
ATG	12	38%
Other drugs	11	34%

(HSCT: hematopoietic stem cell transplantation; TBI: total body irradiation; BU: busulfan; CY: cyclophosphamide; BM : bone marrow; PB: peripheral blood; GVHD: graft-versus-host disease; ATG: anti-human T lymphocyte serum).

Transplant-related data

Stem cells were harvested from sibling donors for 18 patients (56%) and from unrelated donors for the other 14 patients (44%). Thirteen patients (41%) received

marrow cells and 19 (59%) peripheral blood stem cells. The conditioning regimen used conventional myeloablative doses for 19 patients (59%), who received total body irradiation and cyclophosphamide (n=9) or busulfan plus cyclophosphamide or melphalan (n=10) and was of reduced intensity for 13 patients (41%), who were treated with fludarabine, thiotepa, cyclophosphamide (n=10), fludarabine plus cyclophosphamide (n=1), fludarabine, thiotepa, melphalan (n=1), or 2 Gy total body irradiation (n=1).

Antibiotic prophylaxis with levofloxacin was administered after HSCT until complete neutrophil recovery. An antifungal drug, fluconazole or itraconazole, was used until all immunosuppressive drugs were withdrawn. In addition, trimethoprim-sulphamethoxazole was used to prevent *Pneumocystis carinii* pneumonia. Cytomegalovirus (CMV) infection was monitored weekly by CMV antigenemia. If CMV antigenemia tests became positive, patients were treated with ganciclovir or foscarnet.

GVHD prophylaxis included cyclosporine A (CyA) and methotrexate for 24 patients (75%), CyA alone for 5 patients (16%), tacrolimus for 1 patients (3%) and CyA plus mycophenolate mofetil (MMF) for the other 2 patients (6%). Anti-human T lymphocyte rabbit serum (ATG) was administered in association with CyA and methotrexate in 12 patients (37%) receiving unrelated transplants.

The assessment and grading of acute and chronic GVHD were primarily based on clinical findings and followed the commonly accepted diagnostic criteria.[15,16] Diagnosis was supported by skin, liver or gut biopsies, whenever indicated and clinically possible. Patients with diarrhea who could not undergo colonoscopy had stool cultures negative for bacteria, fungi and protozoa.

Response was assessed for each organ involved 7 days after the initiation of infliximab. A complete response was defined as resolution of all manifestations of acute GVHD in all evaluable organs, a partial response was defined as a decrease in organ stage by 1 in at least one evaluable organ without deterioration of others. Unresponsive patients had an increase of 1 in organ stage in at least one evaluable organ or absence of any change after infliximab.

All patients developing grade II-IV acute GVHD received 2-5 mg/kg/day methylprednisolone i.v. for 7 days; if no partial or complete resolution of symptoms occurred, they were considered steroid-refractory and proceeded to infliximab treatment. The dose of infliximab was 10 mg/kg/day i.v. weekly and at least 2 courses were given.

Statistical analysis

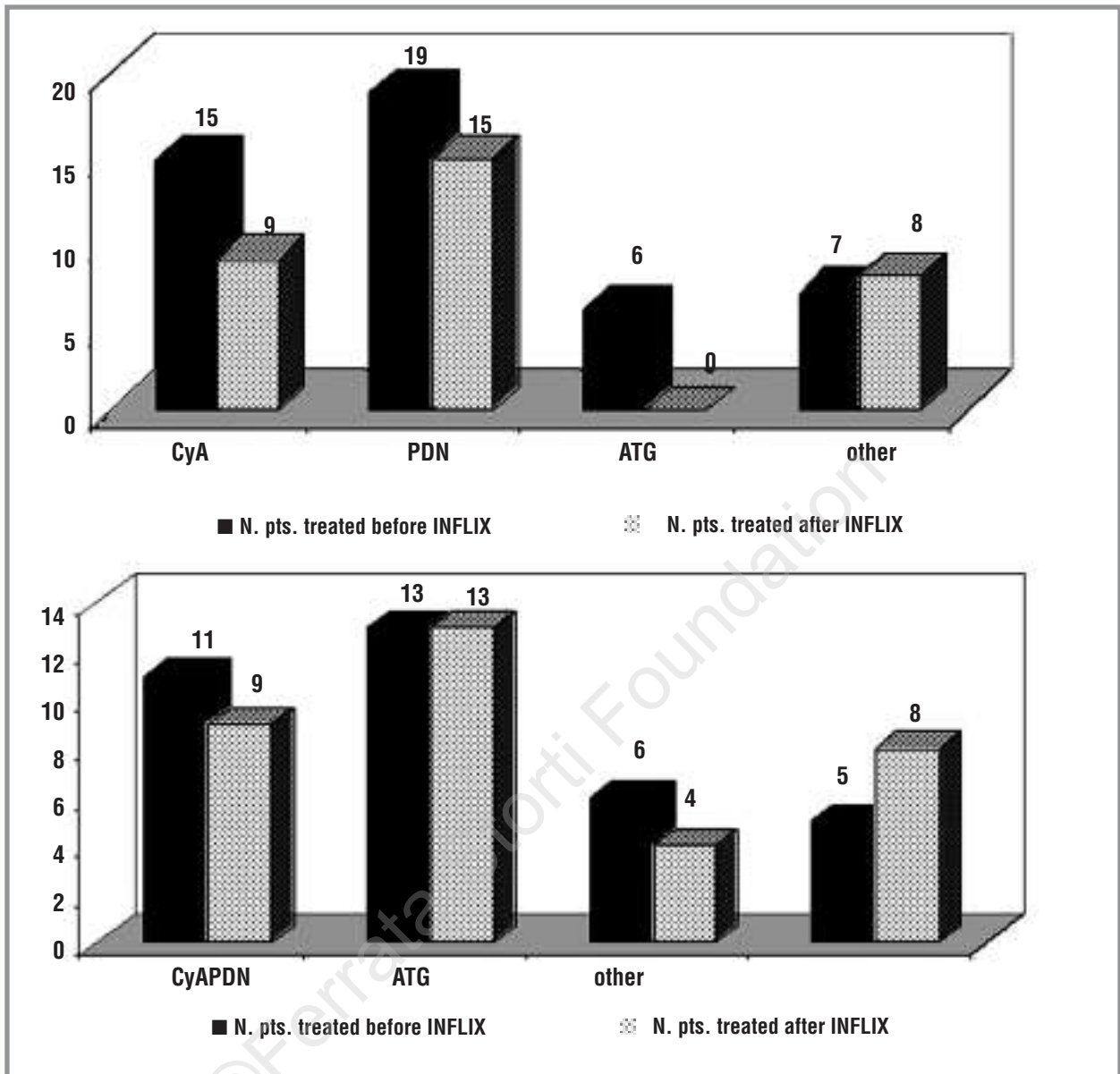Patients responsive and unresponsive to infliximab treatment were compared on the basis of several of their clinical characteristics and features of the graft. Categorical variables were compared using χ^2 statistics or Fisher's exact test and continuous values were compared with the two-sided Student's test. A *p* value <0.05 was considered to be statistically significant. Survival was estimated by the Kaplan-Meier product limit method and curves from different groups were compared by the log-rank test.

Results

The median time between the allogeneic HSCT and diagnosis of acute GVHD was 22 days (range 9-161). All but 2 cases were taking some form of prophylaxis (27 Cy, 1 prednisone, 2 tacrolimus) at the moment of GVHD onset. The 2 patients who were not receiving any prophylaxis were being given donor leukocyte infusions for disease relapse. All the patients showed clinical features of acute GVHD: the main organs involved were the skin in 2 cases (6%), liver in 1 case (3%), bowel in 19 cases (60%), liver and bowel at the same stage in 10 patients (31%). The diagnosis of GVHD was confirmed by 7 intestinal, 2 cutaneous and 1 hepatic biopsies. GVHD was overall classified as grade 2 in 4 patients (12%), grade 3 in 8 patients (25%) and grade 4 in 20 patients (63%).

First-line therapy for the acute GVHD was 2-5 mg/kg/day methylprednisone i.v. in all patients, in association with CyA (n=26) or tacrolimus (n=1) or MMF (n=5). All the patients were steroid-refractory, since they had no improvement after at least 7 days of treatment. Twelve patients were subsequently treated with ATG, 2 with methotrexate, 3 with photophoresis and 1 with cyclophosphamide without any improvement, before receiving infliximab. Overall, infliximab courses were administered to 14 patients (44%) as second-line therapy, after failure of prednisone and CyA (or tacrolimus or MMF) and as third-line therapy to another 18 patients (56%) who did not respond to second-line therapy.

The median time between HSCT and infliximab administration was 47 days (range 19-262); 27 of the 32 patients (84%) were treated within 30 days after the onset of GVHD, the remaining 5 patients received infliximab after 70, 129, 134, 220 and 240 days. Infliximab was administered at the dose of 10 mg/kg/day i.v. weekly for a median of 3 courses (range 2-8) without infusion-related side-effects. Steroid doses were maintained between 1-2 mg/kg/day for all patients during the therapy with infliximab. Nineteen of the 32 patients (59%) responded to infliximab: responses were complete in 6 patients (19%) and partial in 13 patients (40%). Fifteen out of the 19 responsive patients had predominant intestinal involvement, whereas 4 had liv-

Figure 1. Modification of immunosuppressive therapy in patients responsive (1A) and unresponsive (1B) to infliximab treatment. (CyA: cyclosporine A; PDN: prednisone; ATG: anti-human T lymphocyte serum; other: tacrolimus, mycophenolate mofetil, cyclophosphamide, methotrexate or photophoresis).

er involvement with significantly increased bilirubin levels, associated with skin or bowel disease. The median level of serum birilubin of these cases was 5.5 mg/dL before infliximab and 1.7 mg/dL after treatment. After infliximab, Cy A and prednisone were gradually tapered down and then definitively stopped in 6 and 4 responsive patients, respectively. No patient who responded received ATG whereas 8 patients received other immunosuppressive therapies, such as tacrolimus (n=3), MMF (n=2), cyclophosphamide (n=1), or photophoresis (n=1) (Figure 1A). In the group of unresponsive patients, steroids were not suspended in any patients: 4 received

ATG and 8 received other treatments, which were tacrolimus (n=2), MMF (n=2), methotrexate (n=2), cyclophosphamide (n=2) (Figure 1B).

Twenty-two out 32 patients (72%) developed one or more infectious episodes after infliximab: there were 7 cases of septicemias (5 Gram-positive and 2 Gram-negative), 2 cases of septic shock, 2 cases of pneumonia of bacterial origin and 5 caused by an undetermined etiologic agent, 4 cases of infectious enteritis, 1 case of encephalitis, 13 cases of CMV reactivation (documented by positive antigenemia) and 2 proven invasive mycotic infections (candidemia and pulmonary

aspergillosis). All the 13 unresponsive patients died of GVHD at a median time of 43 days (range 14-450) after its onset. Thirteen of the 19 responsive patients (68%) were still alive at a median follow-up of 630 days (range 300-1170) after HSCT and 449 days (range 155-842) after the infliximab treatment. Five out of the 19 (26%) responsive patients had no signs of chronic GVHD, 5 (26%) had limited and 3 (16%) had extensive chronic GVHD. Six out the 19 (32%) responsive patients died: 3 because of infections (mycotic pneumonia, candidemia, encephalitis), 1 of thrombotic thrombocytopenic purpura, 1 of ischemic stroke with concomitant evidence of sepsis and 1 of respiratory failure due to lung GVHD. Four patients (12%) had evidence of disease relapse or progression after HSCT (3 lymphomas and one chronic myeloid leukemia transplanted in blastic phase). Survival curves after infliximab are shown in Figure 2. Patients who did or did not respond to infliximab were compared on the basis of several clinical features (sex, age, disease diagnosis, phase of disease, previous treatment), characteristics of their transplantation (myeloablative or reduced-intensity treatment, source of stem cells, type of donor) and GVHD features (time between HSCT and GVHD onset or first dose of infliximab, main organ involvement, infliximab as second or third-line treatment for GVHD), The analysis showed that age under 35 years, a predominant intestinal involvement (with no liver GVHD) and a longer time between HSCT and infliximab administration were associated with a favorable response to infliximab, as shown in Table 2. Patients with concomitant hepatic and intestinal GVHD had a significant adverse outcome after infliximab treatment.

Discussion

We conducted a multicenter retrospective study of 32 patients with steroid-refractory grade II-IV acute GVHD. More than half of them had grade IV GVHD and all but 3 had gastrointestinal involvement. Patients had a uniform conventional first-line treatment with high-dose prednisone and were evaluated as unresponsive after a 7-day course. Infliximab was added as a second-line drug in 14 patients whereas it was used as third-line salvage treatment in 18 patients. We report an encouraging response rate: 19% complete and 40% partial responses according to standard clinical criteria. It is worth noting that the amount of GVHD therapy could be reduced in patients responding to infliximab, whereas no drugs could be tapered down or withdrawn in the unresponsive patients and sometimes new therapies, such as ATG, had to be added.

That infliximab was clinically effective in acute GVHD had been suggested by a few case-reports and small
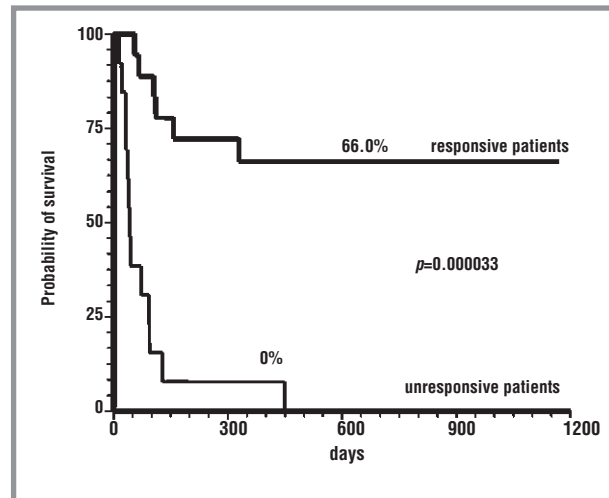
Figure 2. Comparison of probability of overall survival between patients responsive and unresponsive to infliximab therapy.

series reported in the literature. Kobbe *et al.*[5] described that 3 of 4 patients with acute stage III or IV intestinal GVHD refractory to high-dose steroids improved after infliximab administration. The MD Anderson group[6,8] reported the largest experience in acute (37 patients) and chronic GVHD (26 patients), with an overall response rate of 70% and the greatest benefit in cases with lower gastrointestinal tract involvement. Recently, Couriel *et al.*[12] confirmed these data in 21 patients in whom infliximab was added as a single agent to tacrolimus and corticosteroids for the initial treatment of steroid-resistant acute GVHD: complete responses were obtained in 62% with the best responses occurring in patients with gastrointestinal and cutaneous GVHD. Other groups[7,9-11,18] observed some responses in series between 5 and 11 cases with acute or chronic GVHD unresponsive to steroids and several other drugs, such as ATG, daclizumab, MMF and tacrolimus. The majority of these studies did not allow definitive conclusions to be drawn because the groups of patients were small, quite heterogeneous, pre-treated with several conventional and experimental immunosuppressants and had a very short follow-up. The dose of infliximab was 10 mg/kg/day in the majority of the studies and was repeated weekly for a few courses: this schedule was drawn from clinical experience in rheumatology, but adequate pharmacokinetic studies are lacking in the field of HSCT. Other clinically promising but not conclusive data came from the experimentation of etanercept, a genetically engineered TNF-α receptor, which inhibits TNF-α activity by binding the soluble form of this cytokine. Clinical responses were reported in a pediatric case of chronic intestinal GVHD,[17] in 15 patients with obstructive or restrictive patterns of lung injury associated with extensive chronic GVHD,[19] and in

Table 2. Univariate analysis of possible differences in clinical characteristics between patients responsive and unresponsive to infliximab treatment.

Clinical characteristics	Responsive	(%)	Unresponsive	(%)	p value
Age at HSCT (years)					
Mean±SD	36.8±18.2		46±14.6		0.03
<35	13/19	68	1/13	8	0.04
Sex: male	15/19	79	7/13	54	0.24
Previous diagnosis:					
Acute leukemia	8/19	42	6/13	46	0.9
Previous heavy pretreatment	7/19	37	2/13	15	0.35
Time between diagnosis and HSCT (months)					
Mean±SD	21.6±13.6		24.2±21.8		0.60
≤12	9/19	47	4/13	31	0.57
Full conditioning regimen	12/19	63	7/13	54	0.87
Sibling donor	12/19	63	6/13	46	0.55
Source of stem cells:					
BM	11/19	58	6/13	46	0.77
Time between HSCT and GVHD development (days)					
Mean±SD	35.8±37.3		27.7±22.4		0.96
≤ 30	13/19	62	9/13	69	0.73
Time between HSCT and 1st infliximab dose (days)					
Mean±SD	89.2±72.3		43.9±24.1		0.009
≤ 60	9/19	47	11/13	85	0.06
Organs involved by GVHD:					
Predominant bowel involvement	15/19	74	4/13	31	0.01
Bowel and liver at the same stage	2/19	11	8/13	62	0.005
Infliximab as second-line treatment for GVHD	6 /19	31	8/13	42	0.56

HSCT: hematopoietic stem cell transplantation; BM: bone marrow GVHD: graft-versus-host disease; SD: standard deviation; ns: not significant.

20 patients who received 2 mg /kg/day prednisone and etanercept as first-line treatment of acute GVHD.[20]

We observed that the strongest predictive factor for a favorable outcome after infliximab was when the intestine was the organ involved by GVHD; moreover, more frequent responses were reported in younger patients and those in whom the time between HSCT and infliximab administration was longer. The MD Anderson experience suggested that infliximab was most active on low intestinal tract GVHD.[6,8,12] This observation was confirmed in our series; moreover, we found that patients who had predominant hepatic involvement or concomitant severe hepatic and intestinal involvement had a significantly lower response rate. It could be hypothesized that TNF-α is the principal mediator of intestinal GVHD, but that other cytokines, such as interleukin-6 and interleukin-10, are involved in skin and liver GVHD and that these are partially independent from infliximab inhibition of the TNF-α cascade.[21-22] Patients who had greater benefit from infliximab had received it at a longer interval after HSCT; these patients included a few who developed GVHD late in the 3-month period after HSCT or after immune manipulation (donor leukocyte infusion or CyA withdrawal) and a few who had had GVHD conventionally treated in the first 3-month period and then received infliximab for a

recurrence of GVHD with clinical features of the acute form (namely diarrhea and erythema) more than 100 days after the HSCT. The correlation between shorter time after transplantation and worse response might be explained by the conditioning regimen having damaged the intestinal mucosa,[23] thus releasing large amounts of endotoxins, which primed GVHD. The shorter interval between the conditioning and the GVHD could produce very high levels of TNF-α that might not be effectively inhibited by anti-TNF-α therapy.

The outcome of responsive and unresponsive patients was dramatically different. In fact, all 13 unresponsive patients died of GVHD shortly after infliximab administration, while only one out the 19 patients who had some response died of chronic GVHD of the lung. Other causes of death in responsive patients were infections, vascular complications, disease recurrence or progression alone or in association in 5 out 19 patients. The 13 surviving patients were followed up for a median of 15 months after the infliximab treatment and the majority had no signs of chronic GVHD or only limited involvement. In our series we observed several infectious episodes: the etiological agents varied greatly (bacterial, viral, mycotic and unknown) and the infections were frequently localized to organs such as lungs, brain, and bowel, as was to be expected in a population of severely immunosuppressed patients. It should be emphasized that 2 patients who responded to infliximab died of proven invasive fungal infections. Moreover, there were 5 cases of pneumonia caused by an undetermined etiologic agent, which could have been a fungus. In other series, fungal infections were reported in 38 to 54% of patients treated with infliximab for acute GVHD.[5-6,8,10-12] Recently, Jacobsohn et al.[11] reported that all 6 out 11 patients who developed invasive mycosis after infliximab treatment had a fatal outcome. In these small retrospective series of patients treated with high-dose steroids and combinations of anti-GVHD drugs, it is difficult to assess how much the infliximab influenced the inability to control the fungal disease. However, it has also been reported that patients who received infliximab for treatment of Crohn's disease or rheumatoid arthritis have an unusually high rate of extrapulmonary and disseminated tuberculosis or histoplasmosis, possible due to defective granuloma formation and absence of anti-TNF-α mediated macrophage apoptosis.[24-25] The only controlled study in transplanted patients is that by Marty et al.,[26] who demonstrated that patients who received infliximab to control severe GVHD had a significantly higher risk and a shorter time to diagnosis of non-Candida invasive fungal infections than did conventionally treated patients and that these differences were independent of the cumulative dose of steroids received.

Our study has several limitations due to its retrospective nature: the collection of data from 8 different centers, where infliximab was prescribed at the discretion of the attending physicians, and the heterogeneity of the additional immunosuppressive agents administered to the patients.

However, infliximab produced clinical responses in 59% of the largest series of patients with steroid-refractory acute GVHD ever reported in the literature. The majority of these responses were obtained in patients with gastrointestinal involvement and many were durable: only 4 out of 19 responsive patients progressed to develop extensive (3 cases) or fatal (1 patient) chronic GVHD. Infective episodes occurred in 72% of the patients and 3 patients who responded to infliximab died of infections, 2 of them with proven fungal diseases. The clinical activity of infliximab and the possibility of an increased risk of infections are worthy of investigation in prospective trials. Given the suspicion of an increased risk of fungal infection, pre-emptive systemic antimycotic therapy should be considered in the study design for patients who develop severe GVHD and are treated with infliximab.

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