Immunodeficiency Diseases

Low-dose methotrexate as salvage therapy for refractory graft-versus-host disease after reduced-intensity conditioning allogeneic stem cell transplantation

This study investigated the use of low dose methotrexate (MTX) (5 mg/m²/infusion) for the treatment of graft-versus-host disease (GVHD), after failure of corticosteroids. Twelve patients had refractory acute GVHD, while eight received MTX for severe chronic GVHD and/or the side effects of corticosteroids. Thirteen patients responded to MTX with six complete remissions and little toxicity, suggesting that low dose MTX is beneficial in refractory GVHD and deserves further investigations.

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Although reduced intensity conditioning regimens for allogeneic stem cell transplantation are associated with a relatively low early rate of transplant-related mortality, graft-versus-host disease remains a matter of concern. Of note, corticosteroid-resistant GVHD is associated with high morbidity.[1] Furthermore, elderly patients are more exposed to the side effects of long-term corticosteroids (CS).[2] Thus, therapeutic options are usually limited for these patients. Methotrexate is a well established modality for prophylaxis of GVHD. The aim of this analysis was to investigate low dose methotrexate as salvage therapy for GVHD.

Twenty consecutive patients with severe or refractory GVHD received intravenous infusions of low doses of methotrexate (5 mg/m²/infusion) at weekly intervals. Patients were scheduled to receive at least four infusions. Reasons for administering methotrexate were: refractory

Table 1A. Patients' characteristics, transplant procedures, GVHD features and outcome.

Case n°	Age	Diag.	Conditioning regimen/stem cell source	GVHD prophylaxis	Day of GVHD diagnosis stage	Organ involvement	Prior ther.	Day of methotrexate administration (first dose) Total N. of infusions	Reason for administration of methotrexate	Response to methotrexate	Follow up (day): from onset of GVHD/from last infusion of methotrexate	Status (follow up from allogeneic SCT)	Comments: CS dose 1 month after last methotrexate administration % of change from baseline CS dose
1	33	NHL	FBT/ PBSC	CSA/MMF	25 3	Gut: stage 3 Skin: stage 2	CS 2 Leukotac	32/3 doses	Refractory acute GVHD	CR	565/537	Alive at day 590, mo GVHD	Limited cutaneous chronic GVHD at day + 100. CS taper at day +194
2	22	AML	CFT/CB	CSA/MMF	15 3	Gut: stage 3 Skin: stage 2 Liver: stage 1	CS 2 mg/kg ATG 5 mg/kg	32/4 doses	Refractory GVHD	CR	280/235	Alive at day 295, continuing response; CS=5 mg/d	
3	59	MM	FBATG/ PBSC	CSA	28 3	Gut: stage 3 Skin: stage 3 Liver: stage 2	CS 2 mg/kg	35/5 doses*	Refractory acute GVHD	NR	37/1	Death on day 65 from acute GVHD	
4	53	Colon carc.	FBT/ PBSC	CSA	30 3	Gut: stage 3 Skin: stage 3 Liver: stage 2	CS 2 mg/kg ATG 5 mg/kg	41/4 doses	Refractory acute GVHD	NR	57/28	Death on day 87 from acute GVHD	
5	53	NHL	FBT/ PBSC	CSA/MMF	40 4	Gut: stage 4 Skin: stage 1 Liver: stage 1	CS 2 mg/kg	54/2 doses	Refractory acute GVHD	NR	40/8	Death on day 80 from acute GVHD	
6	54	NHL	FT/ PBSC	CSA/MMF	40 2	Skin: stage 3	CS 2 mg/kg doses +	57/3 complications	Refractory acute GVHD	PR	480/442	Alive at day 520, CS=15 mg/d	Extensive chronic GVHD at day 100 (skin and oral mucosa)
7	51	Renal carc.	FBT/ PBSC	CSA	49 4	Gut: stage 4 Skin: stage1	CS 2 mg/kg ATG 5 mg/kg	58/4 doses	Refractory acute GVHD	CR	51/21	Death on day 100 from disease progression	
8	57	MM	FT/ PBSC	CSA/MMF	58 4	Gut: stage 4 Skin: stage 2	CS 2 mg/kg ATG 5 mg/kg	70/4 doses	Refractory acute GVHD	CR	222/182	Death on day 280 from disease progression	Extensive chronic GVHD at day+100 (gut, liver)
9	49	NHL	FBT / PBSC	CSA/MMF	45 2	Gut: stage 2 Skin: stage 1 Liver: stage 1	CS 2 mg/kg	64/1 dose	Refractory acute GVHD	CR	356/330	Alive at day 401, no chronic GVHD, no CS	Discontinuation of methotrexate because of cytopenia grade 3 (Hb=6.8 g/dL)
10	38	Breast carc.	FBT / PBSC	CSA/MMF	71 3	Gut: stage 2 Skin: stage 2	CS 2 mg/kg	86/1 dose	Refractory acute GVHD	PR	37/22	Death on day 108 from disease progression	

Table 1B. Patients' characteristics, transplant procedures, GVHD features and outcome.

Case n°	Age	Diag.	Conditioning regimen/stem cell source	GVHD prophylaxis	Day of GVHD diagnosis staging	Organ involvement	Prior ther.	Day of MTX administration (first dose)/ Total Nb of infusions	Reason for administration of MTX	Response to MTX	Follow up (day): from onset of GVHD/from last infusion of MTX	Status (follow up from alloSCT)	Comments: CS dose one month after last MTX administration % of change from baseline CS dose
11	45	MDS	FBATG/PBSC	CSA	29 2	Skin: stage 3	CS 2 mg/kg	93/4 doses	Refractory acute GVHD	NR	157/65	Death on day 186 from acute GVHD	Chronic liver GVHD
12	41	MM	FT/PBSC	CSA/MMF	70 2	Skin: stage 2 Liver: stage 1	CS 2 mg/kg	108/1 dose	Progressive acute GVHD	NR	380/335	Alive at day 450, cutaneous GVHD controlled by ECP	
13	60	MM	FT/PBSC	CSA/MMF	100 extensive	Skin, Liver	CS 1 mg/kg	148/5 doses*	chronic GVHD + CS complications (diabetes)	CR	364/281	Alive at day 464, continuing response (CS=5 mg/d every two other days)	15 mg ↓ 75%
14	54	MM	FBATG/PBSC	CSA	132 extensive	Skin, Oral mucosa, Eyes, Liver	CS 1 mg/kg	182/5 doses*	chronic GVHD + CS complications (myopathy, diabetes)	PR: Skin, Oral mucosa, Eyes: CR, Liver: PR	465/380	Alive at day 597, continuing response CS= 10mg/d	10 mg ↓ 80%
15	51	MM	FT/PBSC	CSA/MMF	160 extensive	Skin, Oral mucosa, Eyes	CS 1 mg/kg	202/4 doses	chronic GVHD + CS complications (severe myopathy)	NR	332/262	Alive at day 492, GVHD controlled by rituximab	
16	53	WD	FBATG/PBSC	CSA	131 extensive	Skin, Oral mucosa, Liver	CS 1 mg/kg	204/4 doses	chronic GVHD exacerbation after CS taper	PR: Skin, Liver	284/183	Alive at day 415, continuing response CS taper at day 370	20 mg ↓ 60%
17	50	AML	FBATG/PBSC	CSA	100 extensive	Skin, Eyes, Liver, Pulmonary	CS 1 mg/kg	210/1 dose	Resistant chronic GVHD	PR: Liver, Skin, Eyes, Pulmonary	545/428	Alive at day 645, no signs of GVHD CST = 7.5mg/d	20 mg ↓ 30%
18	51	Breast carc.	FBATG/PBSC	CSA	187 extensive	Skin, oral mucosa Liver	CS 1 mg/kg	252/4 doses	Resistant chronic GVHD	PR Liver: CR Skin, Oral mucosa : PR	325/232	Alive at day 512, continuing response CS= 2.5 mg/d	2.5 mg ↓ 50%
19	45	CML	FBATG/PBSC	CSA	28 extensive	Skin, Eyes, Oral mucosa, Liver	CS 1 mg/kg	286/4 doses	chronic GVHD+CS complications (diabetes)	PR: Skin, oral mucosa, Eyes	540/254	Alive at day 568, continuing response (CS=10mg/d)	15 mg ↓ 25%
20	54	Renal carc.	FBT/PBSC	CSA/MMF	27 extensive	Skin, Oral mucosa, Eyes, Liver	CS 2 mg/kg	300/4 doses	Resistant chronic GVHD	NR	633/332	Alive at day 633	

** These patients received one supplementary MTX infusion as per the attending physician decision (in addition to the initially scheduled 4 infusions). NHL: non Hodgkin's lymphoma; AML: acute myeloid leukemia; MM: multiple myeloma; Carc.: carcinoma; MDS: myelodysplastic syndrome; WD: Waldenström's disease; CML: chronic myeloid leukemia; FBT: fludarabine, busulfan, total lymphoid irradiation; CFT: cyclophosphamide, fludarabine, total body irradiation 2 Gy; FBATG: fludarabine, busulfan, anti-thymocyte globulin; FT: fludarabine, total body irradiation 2 Gy; PBSC: peripheral blood stem cell; CB: cord blood (all other patients received allogeneic stem cells from an HLA-identical sibling); CSA: cyclosporine A; MMF: mycophenolate mofetil; ECP: extracorporeal photopheresis; CR: complete response; NR: no response; PR: partial response.*

acute GVHD (after one week of 2 mg/Kg/day CS; n=12), CS-refractory chronic GVHD (n=3), chronic GVHD exacerbation after tapering CS (n=1), or CS side effects (CS-induced diabetes, severe metabolic or psychiatric disorders, toxic myopathy; n=4). The response to methotrexate was assessed 1 month after the last infusion. For acute GVHD, complete response was defined as resolution of all manifestations in involved organs, while partial response was defined as a one grade decrease according to the Glucksberg staging system. In chronic GVHD, partial response was defined as a change from extensive to limited stage. The benefit in chronic GVHD was also evaluated in terms of a decrease in CS use. The CS dose received was assessed 1 month after the last administration of methotrexate, and compared with the previous CS dose received at the time of the first infusion of methotrexate: a decrease of at least 50% in CS use was considered to signify a partial response in chronic GVHD. Failure was defined as the absence of a complete or partial remission.

The patients' characteristics, GVHD features and outcome are summarized in Table 1. With a median follow-up of 272 days (range, 22-558) from first the infusion of methotrexate (median number of infusions: 4), no hepatic, renal or pulmonary toxicity was observed. However, seven patients received less than four doses (three doses, n=2; two doses, n=1; one dose, n=4), because of: (i) toxicity (grade 2 cytopenia, patient #1; grade 3 cytopenia, #9; grade 4 cytopenia, #6); (ii) early death from acute GVHD (#5); (iii) disease progression (#10); (iv) progressive GVHD without evidence of methotrexate efficacy (#12); and (v) patient's non-compliance (#17). Overall, 13 patients responded to methotrexate administration (65%; 95%CI, 44-86%) with six achieving complete responses. In responding patients, improvement was observed as soon as after the first

methotrexate infusion. Among the 12 patients treated for acute GVHD, seven responded (58%), of whom five had complete responses (42%). Five out of eight patients with grade 3-4 acute GVHD responded (four complete responses and one partial response). Four patients did not respond and died from refractory acute GVHD, while three responding patients died of disease progression. Among the eight patients treated for chronic GVHD, six responded (75%). In addition, methotrexate allowed a significant reduction of CS dosage (range, 25-80%). In a median follow-up of 267 (range, 183-428) days after the last infusion of methotrexate, no increase of CS therapy had been necessary among the six patients with chronic GVHD who responded. With a median follow-up of 329 days from the onset of GVHD (range, 37-633), the actuarial survival rate was 60% at 1 year after the first methotrexate infusion.

Currently, there is no standard *second line* therapy for acute or chronic GVHD. Several candidate drugs have been already tested.[3-7] In addition to age, which is a significant risk factor for GVHD, elderly patients are likely to have several comorbidities, and thereby be more likely to suffer from the side effects of CS. Therefore, strategies aimed at minimizing the duration of CS treatment are obviously needed. In our study, the global response rate to methotrexate was impressively high (65%) if considered in terms of salvage therapy for cases including grade 3-4 acute GVHD. Furthermore, the toxicity was low. Intravenous infusions were preferred to oral administration, as allogeneic stem cell transplant recipients usually suffer from clinical or sub-clinical gut GVHD.[8] Our results are in line with those reported by Giaccone *et al*.[9] for chronic GVHD. However, Giaccone *et al*.[9] reported a lower toxicity associated with oral administration of low dose methotrexate, but also lower efficacy, likely related to reduced absorption of the drug. From a mechanistic point of view, recent studies suggested that low doses of methotrexate, in addition to having anti-mitotic effects, can induce a sustained suppression of T-cell activation and expression of adhesion molecules, supporting its use in GVHD therapy.[10] In conclusion, low dose methotrexate appears to be a well-tolerated, likely steroid-sparing agent worthy of further investigations in refractory GVHD, but also as frontline therapy in combination with CS.

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