Association between acute graft versus host disease and lung injury after allogeneic haematopoietic stem cell transplantation

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Objective: To investigate the characteristics of chest high-resolution computed tomography (HRCT) and pathogenesis of acute graft versus host disease (acute GVHD)-induced lung injury after allogeneic haematopoietic stem cell transplantation (allo-HSCT).

Methods: A study of 47 patients with acute GVHD of grades II–IV describes the clinical manifestations and characteristics of chest HRCT of acute GVHD-induced lung injury. Detection of serum interferon γ (IFNγ) and tumour necrosis factor α (TNFα) were performed before the treatment for acute GVHD. Transbronchial biopsy was performed in four patients whose chest HRCT did not recover completely after treatment for acute GVHD. Pulmonary function was measured in patients who survived more than 6 months in every 3 months.

Results: Chest HRCT scans were performed in 47 cases and 20 cases showed abnormal in which 17 cases were suspected of acute GVHD-induced lung injury. In 17 patients with acute GVHD-induced lung injury, HRCT revealed diffused interstitial infiltrate in five cases, diffused interstitial and alveolar infiltrate in seven cases, diffused interstitial and segmental lobar alveolar infiltrate in five cases accompanied by bilateral pleural effusion and hydropericardium in nine patients. There was no statistical significance between the levels of serum IFNγ and TNFα in cases with and without lung injury, but the levels of serum IFNγ and TNFα in patients were significantly higher than the healthy group (IFNγ: p=0·000, TNFα: p=0·000). The histopathology of the lung tissue was characterized by disorganization, epithelial cell damage, interstitial fibroplasias and interstitial T lymphocyte or macrophage infiltrate. Forty-seven cases all attained the treatment for acute GVHD, and the total effective rate and the rate of completely remission (CR) were 74·47 and 55·32%, respectively. The total effective rate and the rate of CR in the treatment for acute GVHD-induced lung injury were 94·12 and 58·82%, respectively. The effective rate of treatment for acute GVHD-induced lung injury positively correlated with that for acute GVHD (r=0·771, p=0·001). Three cases in nine cases with lung injury and three cases in 15 cases without lung injury who survived more than 6 months developed late-onset non-infectious lung injury. Eleven patients of 24 patients who survived more than 6 months had abnormal pulmonary function, including seven patients in nine patients with acute GVHD-induced lung injury and four patients in 15

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patients without acute GVHD-induced lung injury. There was no difference in the incidence of late-onset non-infectious lung injury, but significance in the incidence of abnormal pulmonary function between cases with and without lung injury (p=0·033, cross-tabs).

Conclusions: These results suggested that the lung might be one of the target organs of acute GVHD and participation of T lymphocyte, macrophage and cytokines such as IFNγ and TNFα might play a role in the pathogenesis of acute GVHD-induced lung injury. Acute GVHD-induced lung injury may progress to late-onset non-infectious lung injury.

Keywords: allogeneic haematopoietic stem cell transplantation, acute graft versus host disease, lung injury

Introduction

Allogeneic haematopoietic stem cell transplantation (allo-HSCT) has been used with increasing frequency for the malignant or non-malignant haematological diseases. Acute graft versus host disease (acute GVHD) and lung injury remain common complications and are significant causes of mortality after allo-HSCT. Acute GVHD usually involves the liver, skin and gut. In recent years, other organs such as thymus and lung have also been reported to be involved by acute GVHD.[1–3] In 1978, Beschorner *et al.*[4] reported a group of patients who developed lymphocytic bronchitis associated with acute GVHD after allo-HSCT. Although many studies reported that early lung injury was related to acute GVHD, whether the lung is one of the target organs of acute GVHD remains controversial.[1,2] The pathogeneses of acute GVHD-induced lung injury is unknown. The mechanisms of T-cell axis and gut–liver–lung inflammatory cytokine axis were proposed. Some studies proposed that cytokines such as interferon γ (IFNγ) and tumour necrosis factor α (TNFα) might play a critical role in the process of acute GVHD-induced lung injury.[3,5,6] Clinical reports of imaging characteristics of lung at the onset of acute GVHD-induced lung injury are rare.

In this report, the characteristics of chest high-resolution computed tomography (HRCT) and pathogenesis of acute GVHD-induced lung injury were investigated in 47 patients with acute GVHD of grades II–IV after allo-HSCT. Chest HRCT of the cases suspected of acute GVHD-induced lung injury was characterized by diffused interstitial, alveolar and segmental lobar infiltrates. The histopathology of lung tissue showed T lymphocyte or macrophage infiltrates. Increased levels of serum IFNγ and TNFα were found in patients with acute GVHD. The association between acute GVHD and lung injury, and the association between acute GVHD-induced lung injury and late-onset non-infectious lung injury, are discussed.

Patients and methods

Patients

Forty-seven patients with a median age of 32 years (range: 14–49 years) who were diagnosed of acute GVHD (grades II–IV) after allo-HSCT from February 2003 to June 2007 were enrolled in this study. The clinical characteristics of the cases are shown in Table 1.

Conditioning regimens

Twenty-four patients received total-body irradiation (TBI) and cyclophosphamide conditioning regimens (TBI + CY) and 19 patients were treated with busulfan, cyclophosphamide and cytarabine conditioning regimens (modified BUCY). Four patients received increased intensity conditioning regimens (fludarabine: 50 mg once daily i.v. on −10 to −6 day; cytarabine 1·0 to 2·0 g once daily i.v. on −10 to −6 day; TBI 4·5–5·0 GY/day, on −5, −4 day; cyclophosphamide 50 mg/kg once daily i.v. on −3 to −2 day).[7]

Diagnosis criteria of acute GVHD-induced lung injury

Diagnosis criteria of acute GVHD-induced lung injury are as follows: (1) patients have manifestations of acute GVHD involving at least one organ, e.g. skin, liver and gut;[8] (2) patients have abnormal chest HRCT; (3) lung injury induced by infection and heart diseases can be excluded; (4) chest HRCT scans are improved after treatment for acute GVHD.

Treatment and therapeutic effect

Methylprednisolone at a dose of 2 mg/kg/day was used for the treatment of acute GVHD. Antihuman thymocyte globulin (ATG) or ATG combined with CD25 monoclonal antibody and other immunosuppressants were used for steroid-resistant acute GVHD cases. Acute GVHD-induced lung injury was treated according to acute GVHD protocols. Therapeutic effect was stratified into complete remission (CR), partial response (PR) and non-remission (NR) (CR: chest HRCT scans recover completely; PR: chest HRCT improved after treatment; NR:

Table 1 The clinical characteristics of the 47 cases

Case	Age (years)	Gender	Diagnosis	Donor	Stem cell source	HLA-matching (serology)	HLA-matching (genotype)	Conditioning regimens	Acute GVHD	Chronic GVHD	GVHD prophylaxis	Lung injury before HSCT	CMV	Survival time
1	34	Male	CML-CP -NR	Unrelated	BMT	Matched	1 genetic locus mismatched	Modified BUCY	III	−	MTX + CsA + ATG	−	+	86 d
2	47	Female	MDS	Related	PBSCT	2 antigen mismatched	−	Modified BUCY	II	−	MTX + CsA	−	−	>3 years
3	28	Female	AML-CR	Unrelated	BMT	Matched	Matched	Modified BUCY	II	−	MTX + CsA + ATG	−	+	82 days
4	48	Male	CML-BP -PR	Related	PBSCT	Matched	−	Over intensity	II	Intensive	MTX + CsA	−	+	273 days
5	29	Male	CML-CP -PR	Related	PBSCT	1 antigen mismatched	−	Improved BUCY	II	Limited	MTX + CsA + ATG	−	+	149 days
6	22	Male	ALL-CR	Related	PBSCT	1 antigen mismatched	−	Modified BUCY	III	Limited	MTX + CsA + ATG	−	−	170 days
7	33	Female	AML-CR	Related	PBSCT	Matched	−	TBI + CY	II	Limited	MTX + CsA	−	+	916 days
8	29	Female	AML-CR	Related	PBSCT	Matched	−	Modified BUCY	II	Intensive	MTX + CsA	−	+	811 days
9	49	Male	AML-CR	Related	PBSCT	Matched	−	TBI + CY	III	−	MTX + CsA + ATG	−	+	167 days
10	41	Male	AML-CR	Unrelated	PBSCT	Matched	2 genetic locus mismatched	TBI + CY	II	Intensive	MTX+CsA +ATG	−	+	762 days
11	36	Male	AML-NR	Unrelated	BMT	Matched	2 genetic locus mismatched	Modified BUCY	III	Limited	MTX + CsA + ATG + MMF	−	+	58 days
12	32	Female	AML-CR	Related	PBSCT	Matched	−	Modified BUCY	II	Limited	MTX+CsA	−	−	659 days
13	45	Male	CML-BP	Unrelated	PBSCT	Matched	1 genetic locus mismatched	TBI + CY	II	Limited	MTX+CsA +ATG+MM	−	+	613 days
14	29	Male	CML-BP	Related	BMT	3 antigen mismatched	−	TBI + CY	II	−	MTX + CsA + ATG + MMF	IFI	+	80 days
15	39	male	CML-CP -NR	Unrelated	PBSCT	Matched	matched	Modified BUCY	IV	−	MTX + CsA + ATG + MMF	−	+	74 days
16	21	Female	ALL-CR	Related	PBSCT	matched	−	TBI + CY	II	Limited	MTX + CsA	−	−	>3 years
17	34	Female	AML-CR	unrelated	BMT	Matched	2 genetic locus mismatched	Over intensity	II	Intensive	MTX + CsA + ATG	−	−	855 days
18	32	Female	AUL-NR	Related	PBSCT	2 antigen mismatched	−		IV	−	CsA + ATG	Pulmonary tuberculosis	−	32 days
19	18	Female	AML-CR	Unrelated	BMT	Matched	1 genetic locus mismatched	TBI + CY	II	−	MTX + CsA + ATG + MMF	IFI	+	86 days
20	14	Male	ALL-NR	Unrelated	PBSCT	Matched	3 genetic locus mismatched	TBI + CY	IV	Limited	MTX + CsA + ATG	−	+	65 days
21	17	Male	ALL-CR	Unrelated	BMT	Matched	1 genetic locus mismatched	TBI + CY	IV	Limited	MTX + CsA + ATG	−	+	177 days
22	36	Male	ALL-CR	Related	PBSCT	Matched	−	TBI + CY	II	Intensive	MTX + CsA	−	+	3 years

Table 1 Continued

Case	Age (years)	Gender	Diagnosis	Donor	Stem cell source	HLA-matching (serology)	HLA-matching (genotype)	Conditioning regimens	Acute GVHD	Chronic GVHD	GVHD prophylaxis	Lung injury before HSCT	CMV	Survival time
23	22	Female	AML-CR	Unrelated	PBSCT	Matched	1 genetic locus mismatched	Modified BUCY	II	Limited	MTX + CsA + ATG	IFI	+	105 days
24	39	Female	AML-NR	Related	PBSCT	Matched	−	TBI + CY	IV	−	MTX + CsA	−	−	76 days
25	14	Female	ALL-NR	Related	PBSCT	Matched	−	TBI + CY	III	Intensive	MTX + CsA	−	+	202 days
26	19	Male	AML-CR	Unrelated	BMT	Matched	matched	Over intensity	II	−	MTX + CsA + ATG	−	−	117 days
27	45	Female	CML-CP -NR	Related	PBSCT	Matched	−	Modified BUCY	III	−	MTX + CsA + ATG	−	−	3 years
28	22	Male	CML-CP	Unrelated	BMT	Matched	1 genetic locus mismatched	Modified BUCY	II	limited	MTX + CsA + ATG	−	+	938 days
29	38	Male	AUL-CR	Related	PBSCT	Matched	−	TBI + CY	III	Limited	MTX + CsA	−	−	255 days
30	14	Male	AML-CR	Related	PBSCT	1 antigen mismatched	−	Modified BUCY	III	Intensive	MTX + CsA + ATG	−	+	906 days
31	41	Female	ALL-CR	Related	PBSCT	2 antigen mismatched	−	TBI + CY	II	−	MTX + CsA + ATG	−	−	59 days
32	32	Male	CML-CP-PR	Unrelated	BMT	Matched	1 genetic locus mismatched	Modified BUCY	II	Intensive	MTX + CsA + ATG + MMF	−	−	855 days
33	28	Male	CML-CP	related	PBSCT	matched	−	Modified BUCY	II	Intensive	MTX + CsA	−	+	854 days
34	40	Male	AML-NR	Related	PBSCT	Matched	−	Modified BUCY	IV	−	MTX + CsA	−	+	166 days
35	20	Male	ALL-NR	Unrelated	PBSCT	Matched	matched	Over modified	IV	Limited	MTX + CsA + ATG	−	+	122 days
36	22	female	CML-CP-PR	Unrelated	PBSCT	Matched	3 genetic locus mismatched	TBI + CY	IV	Intensive	MTX + CsA + ATG + MMF	−	−	682 days
37	17	Female	CML-CP	Related	PBSCT	1 antigen mismatched	−	Modified BUCY	III	−	MTX + CsA + ATG + MMF	−	+	672 days
38	16	Male	ALL-CR	Unrelated	BMT	Matched	1 genetic locus mismatched	TBI + CY	II	Limited	MTX + CsA + ATG + MMF	−	+	652 days
39	36	Male	AML-NR	related	PBSCT	Matched	−	TBI + CY	III	−	MTX+CsA	−	−	46 days
40	24	Female	AML-PR	Unrelated	BMT	Matched	1 genetic locus mismatched	TBI + CY	III	Limited	MTX + CsA + ATG	−	+	245 days
41	32	Male	AUL-PR	Related	PBSCT	Matched	−	TBI + CY	III	Intensive	MTX + CsA	−	−	572 days
42	34	Male	MDS	Related	PBSCT	Matched	−	TBI + CY	II	Limited	MTX + CsA	−	+	558 days
43	42	male	CLL-NR	Related	PBSCT	Matched	−	TBI + CY	III	−	MTX + CsA	−	+	58 days
44	43	Male	CML-BP	Unrelated	PBSCT	Matched	2 genetic locus mismatched	TBI + CY	II	Intensive	MTX + CsA + ATG + MMF	−	+	375 days
45	18	Male	CML-CP-NR	Related	BMT	2 antigen mismatched	−	Modified BUCY	II	−	MTX + CsA + ATG + MMF	−	+	500 days
46	15	Male	CML-BP-NR	Unrelated	PBSCT	Matched	−	TBI + CY	IV	0	MTX + CsA + ATG + MMF	−	+	28 days
47	36	Male	AML-CR	Related	PBSCT	Matched	−	Modified BUCY	II	−		−	−	428 days

CML-CP=chronic myelogenous leukaemia-chronic phase; CML-BP=chronic myelogenous leukaemia-blastic phase; AML=acute myeloid leukaemia; ALL=acute lymphoblastic leukaemia; AUL=acute undifferentiated leukaemia; MDS=myelodysplastic syndrome; CLL=chronic lymphatic leukaemia; NR=non-remission; CR=complete remission; PR=partial remission; PBSCT=peripheral blood stem cell transplantation; BMT=bone marrow transplant; HLA=human leucocyte antigen; MTX=methotrexate; CsA=ciclosporin A; ATG=antihuman thymocyte globulin; MMF=mycophenolate mofetil; CMV=cytomegalo virus; IFI=invasive fungal infection.

chest HRCT aggravates or has no improvement after treatment).

Chest HRCT

Chest HRCT scans were performed in 45 patients within 3 days after the onset of symptoms of acute GVHD. Another two patients had chest HRCT scans after the body temperature was over 38·5°C for over 12 h and the occurrence of dyspnea, respectively.

Histopathology and immunohistochemistry

Transbronchial biopsy was performed in four patients whose chest HRCT did not recover completely after treatment for acute GVHD. Samples of lung tissue were obtained using fibreoptic bronchoscopy and immersed in 10% neutral formalin. Formalin-preserved specimens were then embedded in paraffin, cut into 1–2 μm thick sections, and stained with haematoxylin and eosin for histopathology examination. For immunohistochemistry examination, paraffin-embedded sections were dewaxed, rehydrated and incubated with 0·5% hydrogen peroxide in methanol to quench endogenous tissue peroxidase. Sections were incubated with pepsin for 45 min for antigen retrieval. After blocking nonspecific sites with 1% BSA in PBS, sections were treated with primary anti-CD3 and anti-CD68 (DAKO) and with appropriate horseradish peroxidase-conjugated secondary antibodies for 90 and 45 min, respectively.

Pulmonary function

Pulmonary function was measured three times monthly in 24 patients who survived more than 6 months. A decrease of less than 80% of the total lung capacity was defined as a new restrictive physiological defect. A new obstructive defect was defined by a 15% or more reduction in forced expiratory volume in one-second with a concomitant decrease in the forced expiratory volume in one-second to forced vital capacity ratio. A certified technician performed all pulmonary function tests.

The levels of serum IFNγ and TNFα

The levels of serum IFNγ and TNFα were measured with enzyme linked immunosorbent assay (ELISA) in 47 patients before the treatment for acute GVHD. Venous peripheral blood from each patient was drawn into tubes containing EDTA and into anticoagulant-free tubes. After centrifugation, serum samples were stored at −20°C until required. ELISA was performed according to the manufacturer's instruction (IFNγ and TNFα ELISA kit, Jingmei Corporation, China). The healthy group consisted of 10 healthy adults (five males and five females) who were enrolled in the study.

Prophylaxis of infection

All patients were treated with compound sulfamethoxazole and norfloxacin by oral administration. Ganciclovir was used as the fundamental prophylaxis for cytomegalovirus (CMV) infection. Antifungal agents were used 5 days before allo-HSCT. Patients who did not have invasive fungal infection (IFI) history took fluconazole at a dose of 0·3 g once daily until peripheral white blood cells count was over $2{\cdot}0 \times 10^9$/l. Patients who had IFI history used itraconazole at a dose of 0·4 g once daily or voriconazole at a dose of 0·4 g once daily or AmBisome at a dose of 2 mg/kg once daily by intravenous administration. Oral application of itraconazole and voriconazole were started after peripheral white blood cells count was over $2{\cdot}0 \times 10^9$/l and discontinued for 90 days, except one case with Mucor infection receiving AmBisome by intravenous administration intermittently.

Results

Onset time and characteristics of acute GVHD

In 47 patients, 25 had acute GVHD of grade II, 13 had acute GVHD of grade III and nine had acute GVHD of grade IV. The onset time of acute GVHD was from +17 to +122 days with the median day of +31 days after allo-HSCT. Thirty-two patients had skin, liver and gut involved, 10 patients had two organs involved and five patients had only skin or liver involved. At the onset of acute GVHD, 42 patients had fever, 28 patients had dry cough and 11 patients presented with shortness of breath. Two patients had respiratory symptoms and abnormal chest HRCT without skin, liver and gut involved early at the onset of acute GVHD-induced lung injury. The first patient had fever and her HRCT showed diffuse interstitial and alveolar infiltrate accompanied by bilateral pleural effusion. Anti-infection treatment was discontinued and methylprednisolone was added when her clinical condition and chest HRCT worsened after anti-infection for 1 week. Respiratory symptoms were improved and the temperature returned to normal by the third day after methylprednisolone was added. The repeat chest HRCT showed pleural effusion and hydropericardium disappeared with lung diffuse infiltrate improved obviously after anti-GVHD for 10 days (Fig. 1A–F). Rechecked chest HRCT showed thickening and deranged interstitial markings 95 days after the onset (Fig. 1G). The second had only

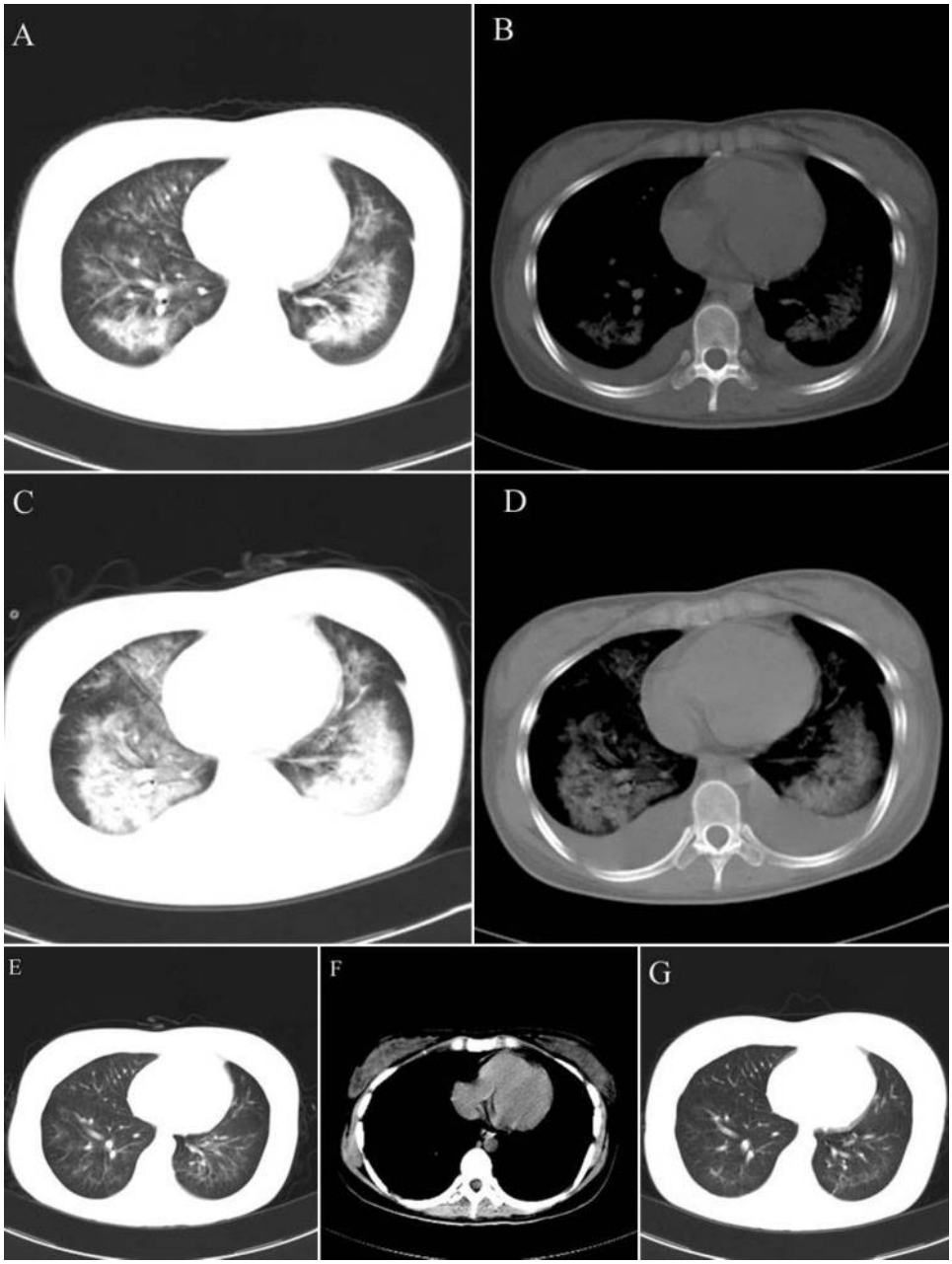

Figure 1 **This patient had only fever, respiratory symptoms and abnormal chest HRCT without skin, liver and gut involvement on +89 days post-transplantation. Chest HRCT showed diffused interstitial and alveolar infiltrate (A) and bilateral pleural effusion accompanied by hydropericardium (B) at the onset. Chest HRCT showed diffused interstitial and alveolar infiltrate worsened (C) and worsened bilateral pleural effusion (D) after anti-infection for 1 week. Chest HRCT showed that intraparenchymatous infiltrate was obviously improved (E), hydropericardium and pleural effusion disappeared (F) after anti-GVHD for 10 days. Chest HRCT showed thickening interstitial markings and deranged vascular shadow after anti-GVHD for 95 days (G)**

respiratory symptoms without fever when chest HRCT showed diffuse interstitial accompanied by bilateral pleural effusion and hydropericardium. Thoracentesis was performed and pathogen culture of hydrothorax was negative. Her cough and shortness of breath resolved and rechecked chest HRCT showed that interstitial infiltrate and hydropericardium were obviously improved and pleural effusion disappeared after anti-GVHD for 11 days (Fig. 2A–D). Chest HRCT recovered completely 88 days after the onset (Fig. 2 E). These two patients presented with manifestations of acute GVHD (grade II) on skin and liver 9 and 11 days after the occurrence of respiratory symptoms, respectively, and evolved to limited chronic GVHD of skin and liver, respectively.

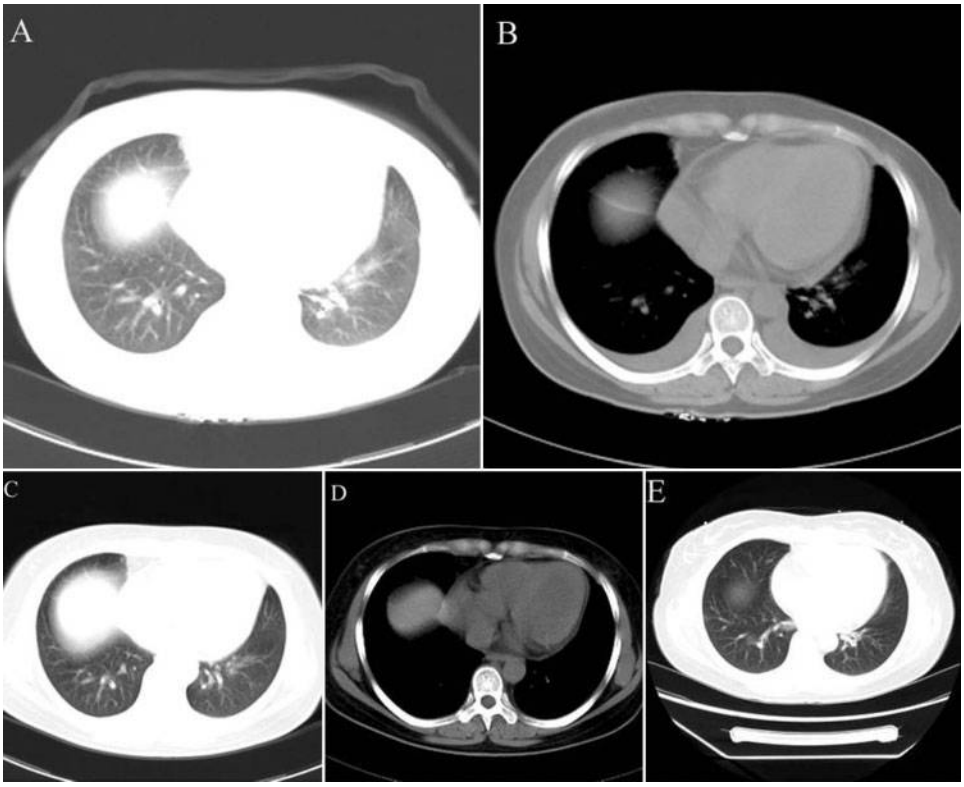

Figure 2 **This patient had only respiratory symptoms and abnormal chest HRCT without skin, liver and gut involvement on +122 day post-transplantation. Chest HRCT showed diffused interstitial infiltrate (A), bilateral pleural effusion and hydropericardium (B) at the onset. Chest HRCT showed that interstitial infiltrate was obviously improved (C), pleural effusion disappeared and hydropericardium obviously improved (D) after treatment for GVHD for 11 days. Chest HRCT returned to normal after anti-GVHD for 88 days (E)**

Characteristics of chest HRCT

Chest HRCT scans were performed in 47 patients and 20 patients had imaging abnormalities. Seventeen cases were suspected of acute GVHD-induced lung injury and three cases were diagnosed with IFI relapse, invasive *Aspergillus* pneumonia and bacterial pneumonia, respectively. In 17 cases suspected of acute GVHD-induced lung injury, HRCT revealed diffused interstitial infiltrate in five cases, diffused interstitial and alveolar infiltrate in seven cases, and diffused interstitial and segmental lobar infiltrate in five cases accompanied by bilateral pleural effusion and hydropericardium in nine patients (Fig. 3). HRCT showed that 14 cases had the whole lung involved and three cases had the median and inferior lobe involved.

Levels of serum IFNγ and TNFα and analysis of hydrothorax

The levels of serum IFNγ were 6·901 $\pm$ 1·751 ng/ml in the cases with lung injury, 6·280 $\pm$ 1·150 ng/ml in the cases without lung injury and 3·343 $\pm$ 0·737 ng/ml in the healthy group. The levels of serum TNFα were 399·514 $\pm$ 101·598 pg/ml in the cases with lung

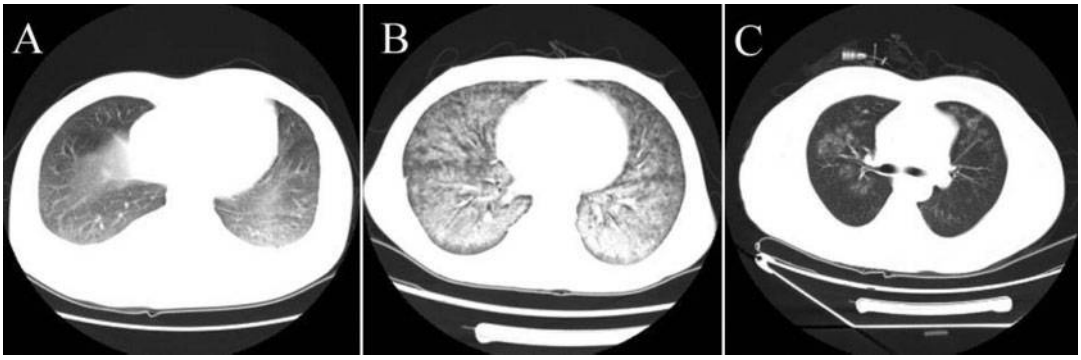

Figure 3 **(A) Diffused interstitial infiltrate; (B) diffused interstitial and alveolar infiltrate; (C) diffused interstitial and segmental lobar infiltrate**

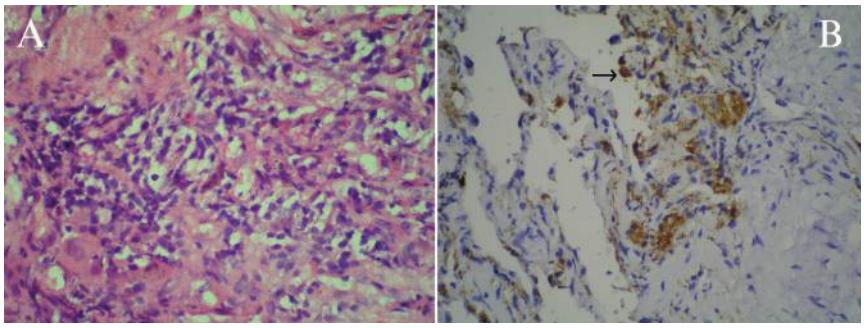

Figure 4 (A) H&E-stained histological section of the transbronchial lung biopsy. There is lymphocytic infiltrate which consists mostly of T lymphocytes (HE, ×200). (B) Immunohistochemistry. There is macrophage infiltrates (CD68+, ×200)

injury, 427·871 ± 83·287 pg/ml in the cases without lung injury and 79·298 ± 14·555 pg/ml in the healthy group. No statistical significance was shown in the levels of serum IFNγ and TNFα between cases with and without lung injury (IFNγ: p=0·202, TNFα: p=0·306), but the levels of serum IFNγ and TNFα in patients were higher than in the healthy group (IFNγ: p=0·000, TNFα: p=0·000). Four of nine patients with bilateral pleural effusion had thoracentesis. Examination of hydrothorax showed that white blood cell count 1200 ± 517·204/μl (800–2200/μl) and the quantity of protein 38·00 ± 13·06 g/l (25·00–62·00 g/l) increased. Bacterium or fungus cultures of hydrothorax were negative.

Therapeutic effect

Forty-seven cases all attained the treatment for acute GVHD and 26 of them received CR: nine were PR and 12 were NR. The total effective rate was 74·47% and the CR rate was 55·32% in the treatment for acute GVHD. The total effective rates of treatment for acute GVHD were 88·24 and 66·67% in patients with (CR: 10 cases, PR: five cases and NR: two cases) and without lung injury (CR: 16 cases, PR: four cases and NR: 10 cases), respectively. There was no statistical significance in the effective rate of treatment for acute GVHD between patients with and without lung injury (p=0·200). Seventeen patients suspected of acute GVHD-induced lung injury rechecked chest HRCT during 7–14 days after anti-GVHD. Rechecked HRCT scans showed that 10 patients received CR: six patients were PR and one patient aggravated. The total effective rate of treatment for acute GVHD-induced lung injury was 94·12% and the CR rate was 58·82%. Comparison between the effective rates of treatment for acute GVHD and acute GVHD-induced lung injury showed no statistical significance (p=0·169). The effective rate of treatment for acute GVHD-induced lung injury positively correlated with that for acute GVHD (r=0·771, p=0·001).

Histopathology and immunohistochemistry

Transbronchial biopsy was performed in four patients whose HRCT showed PR during 26–52 days after the onset of acute GVHD. The histopathology of the lung tissue was characteristic by disorganization, epithelial cell damage, interstitial fibroplasias and cells infiltrate. Immunohistochemistry showed that three cases had predominately T lymphocyte infiltrate and one case had macrophage infiltrate (Fig, 4).

Pulmonary function

Seven patients had abnormal pulmonary function in nine patients with acute GVHD-induced lung injury who survived more than 6 months, including six patients with obstructive defect and one patient with restrictive defect. Four patients had abnormal pulmonary function in 15 patients without acute GVHD-induced lung injury who survived more than 6 months, including two patients with obstructive defect, one patient with restrictive defect and one patient with both. There was significance in the incidence of abnormal pulmonary function between patients with or without acute GVHD-induced lung injury (p=0·033, cross-tabs).

The relationship between acute GVHD-induced lung injury and late-onset non-infectious lung injury

Three patients developed late-onset non-infectious lung injury in nine patients with acute GVHD-induced lung injury and three had late-onset non-infectious lung injury in 15 patients without acute GVHD-induced lung injury who survived more than 6 months, according to diagnostic criteria of clinical manifestation, imaging characteristics and pulmonary function in references of late-onset non-infectious lung injury.[9,10] The incidence of late non-infectious lung injury between these two groups had no significant difference (p=0·635).

Survival status

Until now, 18 patients still survived and disease free survival rate for 3 years was 38·30%. Six patients died of acute GVHD, three secondary infections and one late-onset non-infectious lung injury, in 17 patients with acute GVHD-induced lung injury. Ten cases died of acute GVHD, five secondary infection, one late-onset non-infectious lung injury, one renal inadequacy, one leukaemia relapse and one lymphoproliferative diseases in 30 patients without lung injury. Disease free survival rate for 3 years was 41·18 $\pm$ 11·94 and 26·48 $\pm$ 12·36% in cases with and without lung injury, respectively. There was no statistical significance of disease free survival rate between patients with and without lung injury (p=0·707).

Discussion

Lung injury is a major complication of allo-HSCT that occurs in 25–50% of recipients and can account for approximately 50% of transplant-related mortality.[2,3] It is a major prognostic factor following allo-HSCT. Lung injury after allo-HSCT can be divided into infectious and non-infectious lung injury according to the pathogeneses and can be either early lung injury which occurs within 100 days or late-onset lung injury that occurs over 100 days after allo-HSCT. Historically, about half of all lung injury after allo-HSCT has been secondary to infection, but the judicious use of broad-spectrum antimicrobial prophylaxis in recent years has altered the balance of lung injury from infectious to non-infectious causes. A large body of literature demonstrated that late-onset non-infectious lung injury after allo-HSCT was immunologically mediated disease and shared similar pathogenesis with chronic GVHD.[1–3,11] On the issue about the relationship of acute GVHD and early lung injury, in 1978, Beschorner *et al.* reported a group of patients who developed lymphocytic bronchitis associated with acute GVHD after allo-HSCT.[4] Many studies suggested that the lung was the target organ of acute GVHD and some early lung injury was related to acute GVHD confirmed by animal experiment and clinical cases, but whether the lung is one of the target organs of acute GVHD remains controversial.[1,2,11] Some reports suspected the association between early lung injury and acute GVHD based on the absence of specific epithelial apoptosis in the lung tissue which is considered as histological feature for acute GVHD that existed in the skin, liver and gut, and there is no consistency of the occurrence of early lung injury and acute GVHD. However, the thymus is a known target of GVHD and displays extensive cytolytic damage in the course of GVHD, but epithelial cell apoptosis is not a prominent histological feature.[12] Recently, Cooke *et al.* reported that lung damage was also detectable in mice after T lymphocyte depleted allo-HSCT when signs of clinical and histological acute GVHD were absent.[2] Bolanos-Meade *et al.* and Knox *et al.* reported patients who presented with pneumonia as the early manifestation of acute GVHD without skin, liver and gut involvement after allogeneic peripheral haematopoietic stem cell transplantation and liver transplantation, respectively.[1,13] In this report, 20 of 47 patients with grades II–IV acute GVHD after allo-HSCT had abnormal chest HRCT. Three cases were diagnosed of infectious lung injury and 17 cases were suspected of acute GVHD-induced lung injury. In total, 94·12% of patients' lung injury was improved in17 cases suspected of acute GVHD-induced lung injury after anti-GVHD treatment. The efficacy of treatment for acute GVHD-induced lung injury paralleled with that for GVHD. Additionally, two of these 17 patients had no any stigmata of acute GVHD in skin, liver and gut early at the onset of acute GVHD-induced lung injury. Clinical condition and chest HRCT improved after anti-GVHD treatment. These two patients presented with manifestations of acute GVHD (grade II) on skin and liver 9 and 11 days after the onset of lung injury, respectively, and evolved to limited chronic GVHD. These confirmed that lung injury correlated to acute GVHD and lung injury might be the initial manifestation of acute GVHD.

There are few reports about chest imaging characteristics of acute GVHD-induced lung injury. Early idiopathic pneumonia syndrome after allo-HSCT, which is reported to be related to acute GVHD, has multilobar infiltrate as one of the chest imaging diagnosis criteria.[14–17] Recently, Bolanos-Meade *et al.* reported a case of acute GVHD-induced lung injury after allo-HSCT. Chest imaging characteristics of the reported case included diffuse centrilobular nodules, lung parenchymal and interstitial infiltrate.[1] In this study, 17 cases suspected of acute GVHD-induced lung injury had 14 cases with the whole lung involved and three cases with the median and inferior lobe involved. HRCT characteristics including diffused interstitial and alveolar infiltrate were similar to the previous reports. Furthermore, nine cases suspected of acute GVHD-induced lung injury in this study had bilateral pleural effusion and hydropericardium.

The pathogenesis of acute GVHD-induced lung injury is not clear. Yanik and Cooke[3] proposed the mechanisms of T-cell axis and gut–liver–lung

inflammatory cytokine axis. The lymphocyte activation pathway depends on interactions between donor T cells and host antigen-presenting cells. Donor-derived T cells are activated by host antigen presenting cells and secrete inflammatory cytokines like IFNγ, IL-2 and IL-1 which subsequently recruit to the lung by specific chemokine receptor, and finally contribute to the early pro-inflammatory events associated with lung injury. Donor macrophages, primed by cytokines produced by gut–liver–lung inflammatory cytokine axis, are recruited to the lung where they are triggered by lipopolysaccharide to secrete inflammatory cytokines like TNFα, resulting in enhanced chemokine expression, the recruitment of neutrophils to the lung and increased tissue damage.[2,3,5,6,14] In our four patients, immunohistochemistry of lung tissue showed T lymphocyte infiltrate predominately in three cases and macrophage infiltrate in one case. It suggested that T lymphocyte and macrophage might play a role in acute GVHD-induced lung injury. In this study without non-GVHD transplant controls, the difference of the levels of serum IFNγ and TNFα in patients with and without lung injury showed no statistical significance, but the levels of serum IFNγ and TNFα in patients were significantly higher than that in healthy group. The results suggested that IFNγ and TNFα might be involved in lung injury. The comparison of levels of serum IFNγ and TNFα in GVHD and non-GVHD patients might further demonstrate the participation of IFNγ and TNFα in acute GVHD-induced lung injury.

Reports about the association between early non-infectious lung injury and late-onset non-infectious lung injury after allo-HSCT are rare. It was reported that the incidence of late-onset non-infectious lung injury was higher in patients with acute GVHD than in those without acute GVHD, which suggested that acute GVHD might be related to late-onset non-infectious lung injury.[10,17–19] Twenty-four of 47 patients with grades II–IV of acute GVHD who survived more than 6 months were followed up. Three cases each developed late-onset non-infectious lung injury in cases with and without lung injury. Although the difference of the incidence of late non-infectious lung injury was not significant, there was significance in the incidence of abnormal pulmonary function between cases with and without lung injury. These suggested that early acute GVHD-induced lung injury could evolve to late-onset non-infectious lung injury.

These results suggested that lung might be one of the target organs of acute GVHD and participation of T lymphocyte, macrophage and cytokines like IFNγ and TNFα might play a role in the pathogenesis of acute GVHD-induced lung injury. GVHD-induced early lung injury might progress to late-onset non-infectious lung injury.

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