A significant association of viral loads with corneal endothelial cell damage in cytomegalovirus anterior uveitis

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Outcome of unrelated umbilical cord blood transplantation in 88 patients with primary immunodeficiency in Japan

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Summary

We report the results of umbilical cord blood transplantation (UCBT) performed in 88 patients with primary immunodeficiency (PID) between 1998 and 2008 in Japan; severe combined immunodeficiency (SCID, $n = 40$), Wiskott–Aldrich syndrome (WAS, $n = 23$), chronic granulomatous disease ($n = 7$), severe congenital neutropaenia (SCN, $n = 5$) and other immunodeficiencies ($n = 13$). Five-year overall survival (5-year OS) for all patients was 69% [95% confidence interval (CI), 57–78%], and was 71% and 82% for SCID and WAS, respectively. The main cause of death before day 100 was infection (17/19), while that after day 100 was graft-versus-host disease (GVHD) (5/7). Using multivariate analyses, pre-transplant infection, no conditioning, ≥2 human leucocyte antigen (HLA) mismatches or diagnosis other than SCID, SCN or WAS were all associated with poor prognosis. Reduced-intensity conditioning was associated with decreased overall mortality compared with myeloablative therapy. The cumulative incidence of grade 2–4 acute GVHD at day 100 was 28% (95% CI, 19–38%), and that of chronic GVHD at day 180 was 13% (95% CI, 7–23%). We conclude that UCBT should be considered for PID patients without an HLA-matched sibling. The control of pre-transplant infection and selection of HLA-matched donors will lead to a better outcome.

Keywords: primary immunodeficiency, severe combined immunodeficiency, Wiskott-Aldrich syndrome, cord blood transplantation, reduced-intensity conditioning.

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Allogeneic haematopoietic stem cell transplantation (HSCT) has been successfully used as a curative therapy for most severe forms of primary immunodeficiency (PID) (Zeidler *et al*, 2000; Antoine *et al*, 2003; Sakata *et al*, 2004; Rao *et al*, 2005; Kobayashi *et al*, 2006; Mazzolari *et al*, 2007; Dvorak & Cowan, 2008; Griffith *et al*, 2008; Cuvelier *et al*, 2009). Stem cell transplantation from a human leucocyte antigen (HLA)-identical family donor provides better prognosis than bone marrow transplantation from an unrelated donor (Antoine *et al*, 2003). Survival with this type of transplantation from a matched unrelated donor has improved significantly over the years in patients with severe combined immunodeficiency (SCID), whereas no improvement in survival has been observed with this transplantation in non-SCID patients (Antoine *et al*, 2003). The optimal stem cell source for PID patients with no HLA-identical sibling remains to be determined (Dvorak & Cowan, 2008; Griffith *et al*, 2008; Cuvelier *et al*, 2009).

Umbilical cord blood grafts from unrelated donors have been successfully used, primarily in children and subsequently in adults (Kurtzberg *et al*, 1996; Wagner *et al*, 1996; Gluckman *et al*, 1997; Rubinstein *et al*, 1998; Rocha *et al*, 2000, 2004; Laughlin *et al*, 2004). Theoretically, unrelated cord blood transplantation (UCBT) has the following distinct advantages in PID patients: (i) the cord blood product is rapidly accessible in most cases; (ii) the incidence and severity of graft-versus-host disease (GVHD) is not excessive, even in mismatched transplantation and (iii) the risk of latent viral transmission is low. The disadvantages of UCBT include slower haematopoietic/immunological reconstitution and graft failure, which have been observed with UCBT for malignant disorders, and naivety of lymphocytes to pathogens (Brown *et al*, 2008; Griffith *et al*, 2008; Szabolcs *et al*, 2008). Rapid immune reconstitution is particularly important in PID patients with ongoing infection who undergo UCBT.

The limited data available show that UCBT can be a curative measure in patients with SCID, Wiskott–Aldrich syndrome (WAS), chronic granulomatous disease (CGD) and severe congenital neutropaenia (SCN) (Knutsen & Wall, 2000; Bhattacharya *et al*, 2003, 2005; Fagioli *et al*, 2003; Knutsen *et al*, 2003; Kobayashi *et al*, 2006). Most of the available data have come from a single centre, and thus, detailed information on the outcome and problems associated with UCBT in PID patients is still lacking. In this study, we report the results of UCBT performed in 88 PID patients between 1998 and 2008 in Japan.

Methods

Collection of data

All UCBTs carried out for PIDs through the Japan Cord Blood Bank Network (JCBBN) between August 1998 and January 2008 was enrolled in this study. Eighty-eight patients with PID underwent UCBT during this period. All data were provided by JCBBN, which collects recipients' clinical information at day 100 after transplantation. Recipients' data on survival, disease status and long-term complications are renewed annually by administering follow-up questionnaires. Latest data acquisition was performed in November 2009. The present study was approved by the institutional ethical and data management committees of JCBBN.

Patients

A summary of patients enrolled in this study is shown in Table I. Forty patients had SCID (45%) and 48 (55%) had non-SCID. Patients with familial haemophagocytic syndrome were not included in this study. The median age at the time of transplantation was 10 months (range, 0–248 months).

Procedures

Cryopreserved, unrelated cord blood cells were used as the source of haematopoietic stem cells. The type of conditioning used and median cell dose infused are shown in Table I.

In most cases, HLA matching was performed by both serological and DNA typing for HLA-A, HLA-B and HLA-DRB1. In this study, HLA mismatch was defined according to serological or low-resolution molecular typing for HLA-A and HLA-B and high-resolution molecular typing for HLA-DRB1. Of the UCB donors, 29 (33%) were HLA fully compatible. Of the mismatched donors, 40 (45%) were 1-antigen mismatched, 15 (17%) were 2-antigen mismatched and four (5%) were 3-antigen mismatched (Table I). In 48 patients in whom high-resolution genotypical typing was performed for HLA-A, HLA-B and HLA-DRB1, 11 were fully matched, 13 were 1-antigen mismatched, 16 were 2-antigen mismatched, five were 3-antigen mismatched and three were 4-antigen mismatched.

Immunosuppressive prophylaxis against GVHD after UCBT consisted of ciclosporin A (CyA)- and tacrolimus-based regimens in 48 and 35 patients, respectively. Five patients were not administered any immunosuppressive drug after UCBT.

Various techniques including karyotyping, HLA typing and fluorescence *in situ* hybridization for the XY chromosome and variable number of tandem repeats were used to confirm the engraftment of donor cells.

Definitions

Neutrophil recovery was defined by an absolute neutrophil count of at least $0{\cdot}5 \times 10^9$/l for three consecutive days. Platelet recovery was defined by a count of at least 20×10^9/l, unsupported by transfusion for 7 d. Reticulocyte recovery was defined by a count of at least 20‰.

Patients without conditioning or with only anti-thymocyte globulin (ATG) were categorized as receiving no conditioning. Patients administered busulfan (BU)/cyclophosphamide (CY) ± total body irradiation (TBI) or total lymphoid irradiation

Table I. Age at the time of transplantation, type of conditioning and HLA disparity.

	Patients (*N*)	Median age at transplantation (months) (range)	Median cell dose ($\times 10^7$/kg) (range)	Second or third transplantation (*N*)	Conditioning			HLA disparity			
					No (*N*)	RIC (*N*)	MAT (*N*)	0 (*N*)	1 (*N*)	2 (*N*)	3 (*N*)
Total	88	9 (0–248)	8·60 (1·89–31·1)	8	14	31	43	29	40	15	4
SCID	40	6·5 (0–27)	11·4 (4·55–31·1)	1	12	18	10	17	15	5	3
WAS	23	14 (4–84)	6·49 (2·89–13·6)	1	0	2	21	7	10	6	0
CGD	7	63 (31–248)	6·00 (1·89–12·3)	5	1	4	2	2	4	1	0
SCN	5	10 (4–124)	5·99 (4·16–9·19)	0	0	1	4	1	4	0	0
Others	13	37 (6–194)	8·11 (3·01–19·8)	1	1	6	6	2	7	3	1

RIC, reduced-intensity conditioning; MAT, myeloablative therapy. Definition of conditioning regimens are described in *Methods* section. 'Others' include four CD40L deficiency, two common variable immunodeficiency and one of each of the following disorders: Major histocompatibility complex (MHC) class II deficiency, DiGeorge syndrome, X-linked lymphoproliferative disorder, NEMO (NF-κ-B essential modulator) deficiency, IPEX (immunodysregulation polyendocrinopathy enteropathy X-linked) syndrome, Idiopathic CD4 lymphopenia and Blau syndrome.

(TLI), BU/CY + ATG ± TLI, BU/CY + fludarabine (Flu) or CY/etoposide/high-dose cytarabine were categorized as receiving myeloablative therapies (MATs). CY dose ranged from 120 to 240 mg/kg (median, 200 mg/kg) in patients receiving MAT.

TBI < 4 Gy was classified as 'low-dose TBI'. Patients administered Flu/melphalan (L-PAM) ± low-dose TBI or TLI, Flu/BU ± TLI or Flu/CY (50–60 mg/kg) ± low-dose TBI/TLI, Flu + low-dose TBI or Flu + ATG were categorized as receiving reduced-intensity conditioning (RIC). L-PAM dose was ≤140 mg/m^2 in patients receiving RIC.

GVHD was graded according to the standard criteria (Przepiorka *et al*, 1995).

Statistical analyses

The probability of survival was estimated by the product-limit method, and the log-rank test was used for group comparisons. Cumulative incidence curves were used in a competing-risks setting to calculate the probability of neutrophil, platelet and reticulocyte recovery and that of acute and chronic GVHD. Death before recovery was the competing event for haematological recovery, and death without GVHD was the competing event for GVHD. Gray's test was used for group comparisons of cumulative incidence (Gray, 1988; Gooley *et al*, 1999). The Cox regression model was used to analyse data for the identification of prognostic factors. Factors found to be significant ($P < 0{\cdot}05$) or marginally significant ($P < 0{\cdot}1$) in univariate analysis were included in multivariate analysis. The variables considered were patient age at the time of transplantation, diagnosis, duration from diagnosis to transplantation, second or third transplantation, HLA disparity, presence of infection at the time of transplantation, conditioning regimen and cell dose infused. Variables with >2 categories were included in the final model using dichotomized dummy variables when at least one of the categories showed significant effect on survival. Continuous variables were dichotomized for the prognostic factor analyses. Variables were dichotomized as follows; patient age greater or <12 months at transplantation, dichotomized at a median nucleic cell dose of $<8{\cdot}2 \times 10^7$/kg vs. $\geq 8{\cdot}2 \times 10^7$/kg and CD34 cell dose of $<2{\cdot}1 \times 10^5$/kg and $\geq 2{\cdot}1 \times 10^5$/kg, shorter than or equal to or longer than 180 d for time to transplant. All *P*-values were two-sided.

Results

Engraftment

Sixty-seven patients (76%) achieved stable engraftment. The cumulative incidence of neutrophil, platelet and reticulocyte recovery at day 100 after transplantation was 77% [95% confidence interval (CI), 66–85%], 56% (95% CI, 45–65%) and 64% (95% CI, 53–73%) respectively (Fig 1A, B; data not shown). The median time for neutrophil, platelet and reticulocyte recovery was 19 d (range, 9–104 d), 40 d (range, 10–122 d) and 27 d (range, 12–98 d), respectively. The cumulative incidences of neutrophil recovery were not statistically different among the disease groups (SCID, 74%; WAS, 91% and others, 68% at day 100 after transplantation) (Fig 1C), although incidence was low in CGD patients ($N = 7$, 43%).

The time required for neutrophil recovery was similar in all disease groups, while that required for platelet recovery varied to some extent among the different disease groups. Platelet engraftment was slightly delayed in WAS patients, but the time required for engraftment in these patients was not significantly different from that required in other patients (Fig 1D).

Forty-three, 31 and 14 patients received MAT, RIC and no conditioning, respectively. No difference was observed in the incidence of neutrophil recovery between the MAT and RIC groups (84% vs. 87% at day 100). Similarly, no difference was observed in platelet recovery between these two groups (data not shown).

The cell dose infused ranged from 1·89 to $31{\cdot}1 \times 10^7$/kg, with a median of $8{\cdot}60 \times 10^7$/kg. No correlation was observed between the cell dose infused and engraftment.

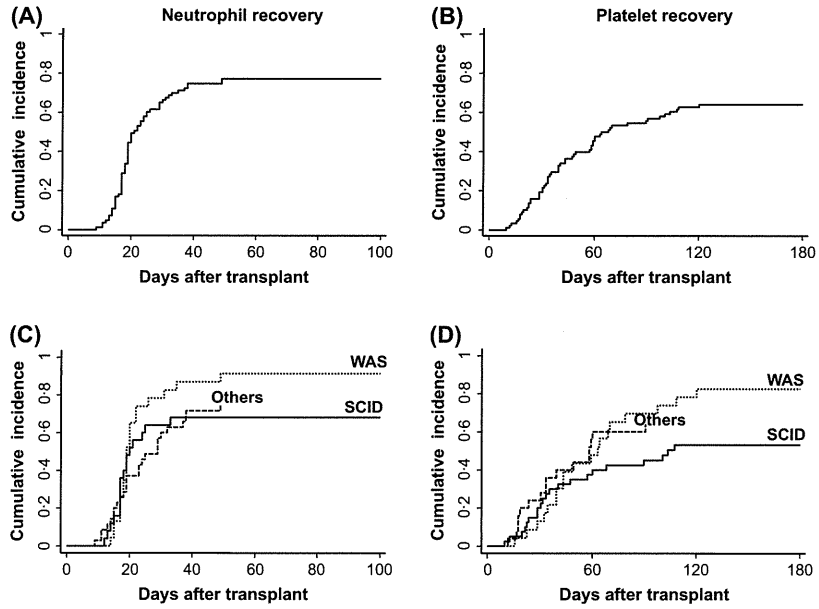

Fig 1. Cumulative incidence of neutrophil and platelet recovery after UCBT. (A) The cumulative incidence of neutrophil recovery 77% (95% CI, 66–85%). (B) The cumulative incidence of platelet recovery 56% (95% CI, 45–65%). The cumulative incidence of neutrophil (C) and platelet (D) recovery according to disease category is shown.

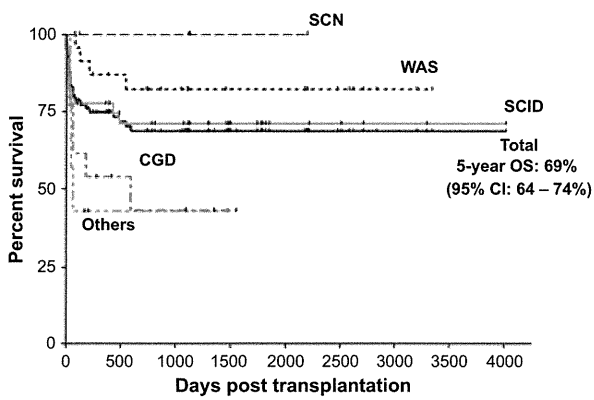

Fig 2. Kaplan–Meier estimates of overall survival after umbilical cord transplantation.

Five of 21 patients with engraftment failure received a second transplantation. Two WAS patients achieved successful engraftment in the second transplantation, while one SCID and two CGD patients did not survive the second transplantation. Only two of the remaining 16 patients who rejected the UCB graft remained alive at the latest data analysis.

Survival and causes of death

Of the 88 PID patients who underwent UCBT, 62 remained alive at the latest follow-up. Five-year OS for all patients was 69% (95% CI, 57–78%) (Fig 2), while that for SCID and WAS patients was 71% and 82%, respectively. All five SCN patients remained alive, although one patient had rejected the graft on day 79 after UCBT. Three of seven CGD patients survived UCBT; this low survival rate may be due to the fact that UCBT was selected in five patients after the first or second failed bone marrow transplantation (BMT). Seven of 14 patients categorized as 'other diseases' remained alive at the latest follow-up.

Table II summarizes the survival and causes of death after UCBT. Of the 26 patients who died, 19 had died within day 100 (17 from infection) and seven (SCID, six and congenital CD4 lymphopenia, one) had died within day 28 after UCBT.

Causes of early death (≤28 d) were cytomegalovirus (CMV) disease (three patients), *Pneumocystis* pneumonia (one patient), interstitial pneumonia (one patient), bacterial infection (one patient) and veno-occlusive disease (VOD) (one patient). All those who died of CMV disease had CMV pneumonia before transplantation.

The cause of death between days 28 and 100 in the remaining 12 patients was bacterial infection (seven had concomitant fungal infection, one also had VOD and one had CMV disease), CMV disease (two patients), fungal infection (one patient), multiple organ failure (one patient) and VOD (one patient). Four of seven CGD patients died of bacterial or fungal infection without engraftment. Although detailed data on bacterial/fungal infections at the time of transplantation were not collected, all the CGD patients were administered both antimicrobial and antifungal agents at the time of transplantation.

The causes of death after day 100 were GVHD (five patients), Epstein–Barr virus (EBV)-associated post-transplant lymphoproliferative disorder (EBV-PTLD, one patient) and

Table II. Survival and causes of death.

			Death (day)				Cause of death (<day 100)			Cause of death (≥day 100)	
	Cases (*N*)	Alive (*N*)	<28 (*N*)	<100 (*N*)	≥100 (*N*)	Infection at CBT (*N*)	Bac/Fung infection (*N*)	Viral infection (*N*)	Others (*N*)	GVHD (*N*)	Others (*N*)
Total	88	62	7	19	7	18	10	7	VOD 3 MOF1	5	PTLD 1 AI 1
SCID	40	29	6	9	2	11	2	6	1 (VOD)	1	1 (AI)
WAS	23	19	0	1	3	1	1	0	0	3	0
CGD	7	3	0	4	0	5	4	0	1 (VOD)	0	0
SCN	5	5	0	0	0	0	0	0	0	0	0
Others	13	6	1	5	2	1	3	1	1 (VOD) 1 (MOF)	1	1 (PTLD)

Bac/Fung infection, bacterial and/or fungal infection. VOD, veno-occlusive disease; MOF, multiple organ failure; AI, adrenal insufficiency; PTLD, post-transplant lymphoproliferative disorder. Cause of death total does not equal the number of deceased patients because one patient died of VOD and bacterial infection.

adrenal insufficiency (one patient). None of the other patients died of infection after day 100.

GVHD

All but five patients in the present study received either CyA- or tacrolimus-based immunosuppressant prophylaxis for GVHD. The cumulative incidence of grade 2–4 acute GVHD at day 100 was 28% (95% CI, 19–38%), and that of grade 3–4 GVHD was 8% (95% CI, 4–15%) (Fig 3A, D).

The incidence of grade 2–4 GVHD was higher in patients who underwent 2- or 3-antigen-mismatched UCBT compared with those who underwent HLA-matched or HLA-1-antigen–mismatched UCBT, but it was not statistically significant ($P = 0{\cdot}071$) (Fig 3B). On the other hand, no difference was observed in the incidence of grade 3–4 GVHD between <2-antigen-mismatched and >2-antigen-mismatched transplants (Fig 3E), although grade 3–4 GVHD was not observed by high-resolution DNA typing in patients who underwent genotypically HLA-matched transplantation.

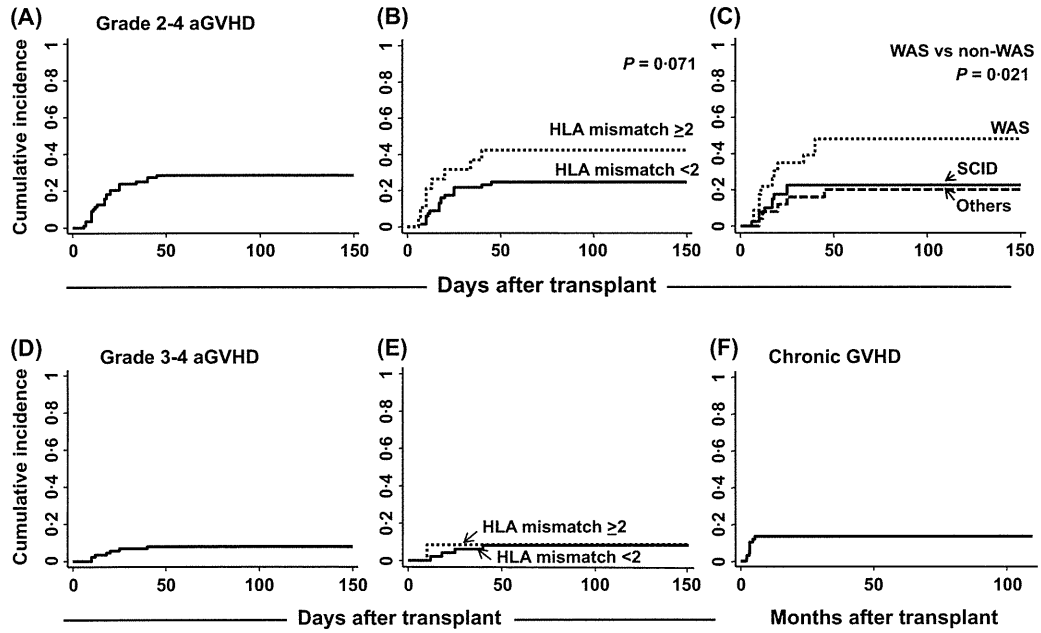

Fig 3. Cumulative probability of acute and chronic GVHD after UCBT. The cumulative incidence of grade 2–4 acute GVHD (aGVHD) at day 100 was 28% (95% CI, 19–38%) (A). The incidence was higher in transplantation mismatched for ≤2 antigens (B) and in that for WAS patients (C). The cumulative incidence of grade 3–4 acute GVHD at day 100 was 8% (95% CI, 4–15%) (D) and the incidence was not different between patients undergoing transplantation for ≥2-antigen mismatched transplant and those undergoing <2-antigen mismatched transplant (E). The cumulative incidence of chronic GVHD at day 180 was 13% (95% CI, 7–23%) (F).

When focusing on differences among the disease groups (Fig 3C), a significantly higher incidence of grade 2–4 GVHD was observed in WAS patients than in non-WAS patients, $P = 0{\cdot}021$. In addition, three of five WAS patients who developed grade 3–4 GVHD died of either GVHD (two patients) or VOD (one patient).

Chronic GVHD was observed in nine patients, and its cumulative incidence at day 180 was 13% (95% CI, 7–23%) (Fig 3F).

Infections

Twenty-eight patients (SCID, 11; WAS, eight; CGD, three and other diseases, six) developed bacterial infection after UCBT. Sixteen of the 28 patients remained alive at the time of data collection.

Fungal infection mainly caused by *Aspergillus* species was observed in eight patients (CGD, three; SCID, two; WAS, two and X-linked hyperIgM syndrome, one). Three of the eight patients died of bacterial infection, bacterial/fungal infection or GVHD.

Twenty patients (SCID, eight; WAS, four; CGD, two; SCN, two and others, four) developed CMV disease after UCBT. CMV was detected before conditioning in all eight SCID patients of which four patients died of CMV disease after transplantation. Twelve of the 20 patients remained alive at the time of analysis.

Other notable virus-related complications were respiratory syncytial virus bronchiolitis accompanied by chronic GVHD in one SCID patient and EBV-PTLD in one patient with Blau syndrome; both infections led to a fatal outcome. One WAS patient had severe haemorrhagic colitis caused by Coxsackie virus B infection, which was treated successfully by infusion of expanded CD4 T cells prepared from the infusion residual of donor cord blood (Tomizawa *et al*, 2005). Another WAS patient had persistent norovirus infection. Interstitial pneumonia not due to CMV or *Pneumocystis* was noted in three patients of which one patient had parainfluenza/rhinovirus infection, while the causative agent for infection in the remaining two patients was not identifiable.

Risk factors for overall mortality

Lastly, we analysed the factors contributing to overall survival. Using univariate analyses, the following were found to be significant contributory factors to a poor prognosis: HLA mismatch of ≥2 antigens, time to transplant >180 d, second or third transplantation, ongoing infection at the time of transplantation, no conditioning for UCBT and diagnosis other than SCID, SCN or WAS (Table III). The dose of transfused nucleated cells or CD34-positive cells did not affect the 5-year OS.

Using multivariate regression analyses, the following were found to be significant contributory factors to patient death: infection at the time of transplantation, no conditioning, HLA mismatch of >2 antigens and diagnosis other than SCID, SCN or WAS (Table IV). RIC was determined to be the favourable factor for patient survival ($P = 0{\cdot}01$) (Fig 4 and Table IV).

Table III. Univariate analyses of factors that contributed to 5-year OS.

Factors	Hazard ratio	95% CI	*P*-value
Age: ≥12 months	1·73	(0·78–3·83)	0·175
Diagnosis			
WAS and SCN	1·00		
SCID	2·34	(0·75–7·36)	0·145
Other diseases	5·39	(1·70–17·0)	0·004*
Nucleic cell dose: ≥8·2 × 10^7/kg	1·51	(0·69–3·29)	0·299
CD34 cell dose: ≥2·1 × 10^5/kg	0·86	(0·36–2·08)	0·744
HLA disparity			
6/6 matched	1·00		
5/6 matched	1·68	(0·58–4·83)	0·337
4/6 matched	3·78	(1·23–11·60)	0·020*
3/6 matched	3·24	(0·63–16·74)	0·160
4/6 or 3/6 matched	2·64	(1·20–5·83)	0·016*
Time to transplant: ≥180 d	1·89	(0·85–4·17)	0·117
Infection at transplant	6·24	(2·61–14·9)	<0·0001*
Second or third transplantation	3·37	(1·26–9·02)	0·016*
Conditioning			
MAT	1·00		
RIC	0·41	(0·13–1·23)	0·111
No conditioning	2·89	(1·21–6·93)	0·017*

*Significant contributory factors to the poor prognosis.

Discussion

This paper reports the outcome of UCBT for 88 PID patients, the largest cohort of PIDs to receive UCBT to date. The overall survival rate for PID patients undergoing UCBT was comparable to that previously reported for 46 Japanese PID patients undergoing BMT from either HLA-identical siblings or unrelated donors (Sakata *et al*, 2004), and also to that reported by the European Society of Immunodeficiency and other stem cell transplantation centres for PID patients receiving BMT from HLA-matched related donors, HLA-mismatched related donors or unrelated donors (Antoine *et al*, 2003; Rao *et al*, 2005; Dvorak & Cowan, 2008). The time for haematopoietic recovery was comparable to or better than the median recovery time observed in a large cohort of UCBT in children with haematopoietic disorders (Thomson *et al*, 2000; Michel *et al*, 2003) and in adults with leukaemia (Laughlin *et al*, 2004; Atsuta *et al*, 2009). The incidence of grade 2–4 GVHD (28%) in UCBT was lower compared with that reported in unrelated donor BMT in PID patients in Japan (47%) (Sakata *et al*, 2004), with that reported in BMT in 90 SCID patients (34%) (Neven *et al*, 2009) and with that observed in the studies of UCBT for childhood haematological malignancies (Thomson *et al*, 2000; Michel *et al*, 2003; Sawczyn *et al*, 2005). The incidence of chronic GVHD (13%) after UCBT was slightly

Table IV. Multivariate analyses of factors that contributed to 5-year OS.

Factors	HR	95% CI	*P*-value
Diagnosis			
WAS and SCN	1·00		
SCID	1·71	(0·39–7·38)	0·475
Other diseases	7·50	(2·06–27·19)	0·002*
HLA disparity			
6/6 matched	1·00		
5/6 matched	1·53	(0·50–4·66)	0·454
4/6 matched	5·64	(1·66–19·14)	0·006*
3/6 matched	1·04	(0·68–23·96)	0·124
4/6 or 3/6 matched	3·87	(1·63–9·19)	0·002*
Infection at transplant	4·61	(1·74–12·16)	0·002*
Conditioning			
MAT	1·00		
RIC	0·20	(0·06–0·69)	0·011†
No conditioning	4·87	(1·79–13·3)	0·002*

*Significant contributory factors to an unfavourable prognosis.
†Significant contributory factors to a favourable prognosis.

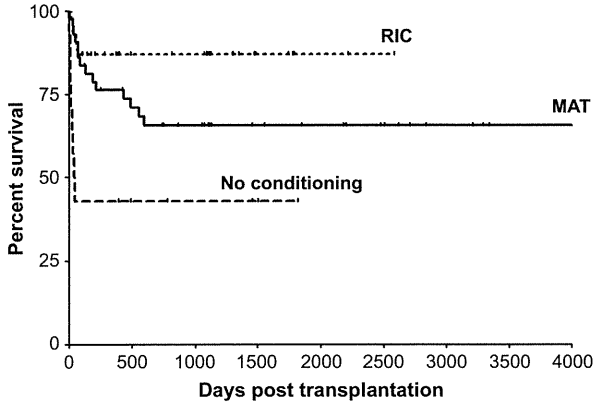

Fig 4. Kaplan–Meier estimates of overall survival after umbilical cord transplantation. Comparison of overall survival between reduced intensity conditioning (RIC), myeloablative therapy (MAT), and no conditioning is shown. For 5-year OS, MAT *versus* RIC, $P = 0{\cdot}111$, MAT *versus* no conditioning, $P = 0{\cdot}017$ in univariate analysis.

lower than that after URBMT in PID patients in Japan (20%) (Sakata *et al*, 2004), and was lower compared to that in UCBT studies for childhood leukaemia (Michel *et al*, 2003; Sawczyn *et al*, 2005). Thus, UCBT in PID patients in the present study was associated with a good survival rate, good engraftment rate, rapid haematological recovery and a lower incidence of acute and chronic GVHD.

Given that the 5-year OS for SCID patients (71%) was better than that for SCID patients receiving bone marrow from HLA-mismatched related donors in both Japan (5-year OS, 36%, Imai, Morio, Kamachi, Kumaki, Ariga, Nonoyama, Miyawaki, and Hara, unpublished observations) and Europe (5-year OS, 52%, Antoine *et al*, 2003), UCBT would be particularly beneficial for patients requiring rapid access to donor units yet lacking a matched related donor.

The present study found that several key risk factors were associated with overall mortality. First, infection was the major cause of mortality during the first 100 d after UCBT in PID patients, and the frequency was much higher than that observed in other disorders following UCBT (Rocha & Gluckman, 2006; Kurtzberg *et al*, 2008, Szabolcs *et al*, 2008). As predicted and reported in previous studies (Antoine *et al*, 2003; Cuvelier *et al*, 2009), infection at the time of transplantation was associated with poor survival ($P < 0{\cdot}0001$), suggesting that the control of pre-existing infection at the time of UCBT is critically important.

Eight of 11 SCID patients who had active infection, mainly CMV pneumonia, died before day 50, while 26 of 28 patients without infection at the time of UCBT remained alive at the time of data collection. UCBT without conditioning was selected for 12 patients, of which seven had CMV infection and one had *Pneumocystis* pneumonia at the time of transplantation. Six out of the seven patients died of CMV infection; and one patient with *Pneumocystis* pneumonia did not survive UCBT.

UCBT in WAS patients achieved a good 5-year OS, as reported in a previous study of 15 cases (Kobayashi *et al*, 2006). One of the key factors would have been the time from diagnosis to transplantation. In our WAS patients, UCBT was performed at a median age of 14 months (range, 4–84 months), when most patients were thrombocytopenic, but did not yet have uncontrolled infection or autoimmunity.

Four CGD patients died of bacterial or fungal infection without engraftment. Although these patients were not categorized as those with active infection at the time of transplantation, they required intravenous administration of antimicrobial and antifungal agents before and after transplantation.

Second, HLA disparity was a risk factor associated with overall mortality. Lower survival was observed in UCB recipients transplanted with a ≥2 antigen-mismatched graft compared with those transplanted with a <2 antigen-mismatched graft [Hazard Ratio (HR) = 3·87, $P = 0{\cdot}002$]. Although no difference was observed in 5-year OS between recipients of HLA-matched and those of HLA 1-antigen mismatched UCBT in the present study, we would need data from a larger number of patients with information on more extensive and sensitive HLA typing to discuss the impact of fully matched HLA on transplant outcome.

Finally, non-SCID/SCN/WAS patients showed a significantly lower survival rate (HR = 5·40, $P < 0{\cdot}0001$ by multivariate analyses). Although a previous large-scale study showed that results of HSCT according to disease did not show obvious disease-specific findings (Antoine *et al*, 2003), it is not yet known if UCBT is suitable for all types of PIDs. This may indicate donor source other than UCB is preferable for certain types of PID. Although the success of UCBT noted for X-linked hyperIgM syndrome, bare lymphocyte syndrome and

X-linked recessive anhidrotic ectodermal dysplasia with immunodeficiency (Tono *et al*, 2007) is encouraging, optimization of transplantation procedures and determination of suitable timing for UCBT may be necessary for this group of patients. Alternatively, this may simply indicate an expansion of transplantation to less favourable clinical conditions or to less favourable transplantation conditions. Studies on a larger cohort are necessary for drawing any conclusion on whether diagnosis is significant overall.

Recent studies suggest improved survival after BMT for PID with the RIC regimen; however, to date, comparison of CBT using RIC *versus* MAT has not been made. In our study, 87% of patients on the RIC regimen and 66% on the MAT regimen remained alive at the latest follow-up. Multivariate analyses revealed that the RIC regimen is associated with a higher 5-year OS than the MAT regimen (HR = 0·20, $P = 0·011$). Although it is premature to conclude that RIC provides an equal or superior outcome to MAT for all PID patients, non-myeloablative treatment may be beneficial at least for certain types of PID. RIC was selected preferentially in SCID and CGD patients, with good survival rates: 17 of 18 SCID patients and three of four CGD patients remain alive. As a result of this, we are in the process of initiating a clinical trial of UCBT with RIC in SCID patients. On the other hand, only two of 23 WAS patients received RIC. Our previous data showed that a conditioning regimen other than BU/CY or BU/CY/ATG was the only independent factor associated with failure in HSCT for WAS patients (Kobayashi *et al*, 2006). However, whether this holds true for UCBT in younger WAS patients should be determined.

Notably, although the outcome of UCBT for WAS in this cohort was excellent compared with that from previously reported HSCT results using different donor sources (Kobayashi *et al*, 2006; Friedrich *et al*, 2009), UCBT in WAS patients was associated with a high rate of grade 2–4 acute GVHD (11 of 23 patients) and a post-transplant infectious episode (13 of 23 patients). Eight patients experienced bacteraemia/sepsis and six suffered a viral infection (CMV pneumonia, four; Coxsackie virus enterocolitis, one and persistent norovirus infection, one). The high rate of serious infections and GVHD in WAS patients after transplantation warrants further study in search of preventive measures that might include RIC for severe, transplantation-related toxicities.

Long-term follow-up of the clinical and immunological status is necessary when considering the lifespan of PID patients. Recent studies on the long-term outcome after HSCT for SCID revealed the presence of relatively late complications, such as chronic GVHD, autoimmune events, severe or recurrent infections, chronic human papilloma virus infection, nutritional problems and late rejection in 50% of patients (Mazzolari *et al*, 2007; Neven *et al*, 2009). Similarly, long-term follow-up of HSCT in WAS patients revealed that 20% of patients developed chronic GVHD-independent autoimmunity (Ozsahin *et al*, 2008). One possible measure that might be taken to avoid the chronic problems associated with CBT would be to select a HLA-matched UCB unit, as HLA disparity was a risk factor for both overall survival and the development of GVHD in our study. The advantage of RIC over MAT in preventing late complications needs careful assessment, together with data on mortality, engraftment and early post-transplant complications.

Finally, the issue of SCID patients who died before or without receiving SCT, most likely due to uncontrolled infection, still remains unresolved. This suggests that the early diagnosis of SCID and prevention of opportunistic infection within a protected environment and the administration of appropriate prophylactic drugs is critically important for the improvement of survival in SCID patients in general. To that end, neonatal screening with the employment of T cell receptor excision circles should be beneficial for an improved outcome in SCID patients (McGhee *et al*, 2005; Morinishi *et al*, 2009).

We report the results of UCBT for 88 PID patients in Japan. Despite the limitations of a retrospective, non-randomized study, our study suggests that unrelated umbilical cord blood can be considered as a promising stem cell source for children with congenital immunodeficiency when a HLA-matched related donor is not available.

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References

Antoine, C., Muller, S., Cant, A., Cavazzana-Calvo, M., Veys, P., Vossen, J., Fasth, A., Heilmann, C., Wulffraat, N., Seger, R., Blanche, S., Friedrich, W., Abinun, M., Davies, G., Bredius, R., Schulz, A., Landais, P. & Fischer, A.; European Group for Blood and Marrow Transplanation; European Society for Immunodeficiency (2003) Long-term survival and transplantation of haemopoietic stem cells for immunodeficiencies: report of the European experience 1968–99. [see comment]. *Lancet*, **361**, 553–560.

Atsuta, Y., Suzuki, R., Nagamura-Inoue, T., Taniguchi, S., Takahashi, S., Kai, S., Sakamaki, H., Kouzai, Y., Kasai, M., Fukuda, T., Azuma, H., Takanashi, M., Okamoto, S., Tsuchida, M., Kawa, K., Morishima, Y., Kodera, Y. & Kato, S.; Japan Cord Blood Bank Network (2009) Disease-specific analyses of unrelated cord blood transplan-

tation compared with unrelated bone marrow transplantation in adult patients with acute leukemia. *Blood*, **113,** 1631–1638.

Bhattacharya, A., Slatter, M., Curtis, A., Chapman, C.E., Barge, D., Jackson, A., Flood, T.J., Abinun, M., Cant, A.J. & Gennery, A.R. (2003) Successful umbilical cord blood stem cell transplantation for chronic granulomatous disease. *Bone Marrow Transplantation*, **31,** 403–405.

Bhattacharya, A., Slatter, M.A., Chapman, C.E., Barge, D., Jackson, A., Flood, T.J., Abinun, M., Cant, A.J. & Gennery, A.R. (2005) Single centre experience of umbilical cord stem cell transplantation for primary immunodeficiency. *Bone Marrow Transplantation*, **36,** 295–299.

Brown, J.A. & Boussiotis, V.A. (2008) Umbilical cord blood transplantation: basic biology and clinical challenges to immune reconstitution. *Clinical Immunology*, **127,** 286–297.

Cuvelier, G.D.E., Schultz, K.R., Davis, J., Hirschfeld, A.F., Junker, A.K., Tan, R. & Turvey, S.E. (2009) Optimizing outcomes of hematopoietic stem cell transplantation for severe combined immunodeficiency. *Clinical Immunology*, **131,** 179–188.

Dvorak, C.C. & Cowan, M.J. (2008) Hematopoietic stem cell transplantation for primary immunodeficiency disease. *Bone Marrow Transplantation*, **41,** 119–126.

Fagioli, F., Biasin, E., Berger, M., Nesi, F., Saroglia, E.H., Miniero, R., Martino, S. & Tovo, P.A. (2003) Successful unrelated cord blood transplantation in two children with severe combined immunodeficiency syndrome. *Bone Marrow Transplantation*, **31,** 133–136.

Friedrich, W., Schutz, C., Schulz, A., Benninghoff, U. & Honig, M. (2009) Results and long-term outcome in 39 patients with Wiskott-Aldrich syndrome transplanted from HLA-matched and -mismatched donors. *Immunologic Research*, **44,** 18–24.

Gluckman, E., Rocha, V., Boyer-Chammard, A., Locatelli, F., Arcese, W., Pasquini, R., Ortega, J., Souillet, G., Ferreira, E., Laporte, J.P., Fernandez, M. & Chastang, C. (1997) Outcome of cord-blood transplantation from related and unrelated donors. Eurocord Transplant Group and the European Blood and Marrow Transplantation Group. [see comment]. *New England Journal of Medicine*, **337,** 373–381.

Gooley, T.A., Leisenring, W., Crowley, J. & Storer, B.E. (1999) Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Statistics in Medicine*, **18,** 695–706.

Gray, R. (1988) A class of k-sample tests for comparing the cumulative incidence of a competing risk. *Annals of Statistics*, **16,** 1141–1154.

Griffith, L.M., Cowan, M.J., Kohn, D.B., Notarangelo, L.D., Puck, J.M., Schultz, K.R., Buckley, R.H., Eapen, M., Kamani, N.R., O'Reilly, R.J., Parkman, R., Roifman, C.M., Sullivan, K.E., Filipovich, A.H., Fleisher, T.A. & Shearer, W.T. (2008) Allogeneic hematopoietic cell transplantation for primary immune deficiency diseases: current status and critical needs. *Journal of Allergy & Clinical Immunology*, **122,** 1087–1096.

Knutsen, A.P. & Wall, D.A. (2000) Umbilical cord blood transplantation in severe T-cell immunodeficiency disorders: two-year experience. *Journal of Clinical Immunology*, **20,** 466–476.

Knutsen, A.P., Steffen, M., Wassmer, K. & Wall, D.A. (2003) Umbilical cord blood transplantation in Wiskott Aldrich syndrome. *Journal of Pediatrics*, **142,** 519–523.

Kobayashi, R., Ariga, T., Nonoyama, S., Kanegane, H., Tsuchiya, S., Morio, T., Yabe, H., Nagatoshi, Y., Kawa, K., Tabuchi, K., Tsuchida, M., Miyawaki, T. & Kato, S. (2006) Outcome in patients with Wiskott-Aldrich syndrome following stem cell transplantation: an analysis of 57 patients in Japan. *British Journal of Haematology*, **135,** 362–366.

Kurtzberg, J., Laughlin, M., Graham, M.L., Smith, C., Olson, J.F., Halperin, E.C., Ciocci, G., Carrier, C., Stevens, C.E. & Rubinstein, P. (1996) Placental blood as a source of hematopoietic stem cells for transplantation into unrelated recipients. [see comment]. *New England Journal of Medicine*, **335,** 157–166.

Kurtzberg, J., Prasad, V.K., Carter, S.L., Wagner, J.E., Baxter-Lowe, L.A., Wall, D., Kapoor, N., Guinan, E.C., Feig, S.A., Wagner, E.L. & Kernan, N.A. (2008) Results of the Cord Blood Transplantation Study (COBLT): clinical outcomes of unrelated donor umbilical cord blood transplantation in pediatric patients with hematologic malignancies. *Blood*, **112,** 4318–4327.

Laughlin, M.J., Eapen, M., Rubinstein, P., Wagner, J.E., Zhang, M.-J., Champlin, R.E., Stevens, C., Barker, J.N., Gale, R.P., Lazarus, H.M., Marks, D.I., van Rood, J.J., Scaradavou, A. & Horowitz, M.M. (2004) Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. [see comment]. *New England Journal of Medicine*, **351,** 2265–2275.

Mazzolari, E., Forino, C., Guerci, S., Imberti, L., Lanfranchi, A., Porta, F. & Notarangelo, L.D. (2007) Long-term immune reconstitution and clinical outcome after stem cell transplantation for severe T-cell immunodeficiency. *Journal of Allergy & Clinical Immunology*, **120,** 892–899.

McGhee, S.A., Stiehm, E.R., Cowan, M., Krogstad, P. & McCabe, E.R.B. (2005) Two-tiered universal newborn screening strategy for severe combined immunodeficiency. *Molecular Genetics & Metabolism*, **86,** 427–430.

Michel, G., Rocha, V., Chevret, S., Arcese, W., Chan, K.W., Filipovich, A., Takahashi, T.A., Vowels, M., Ortega, J., Bordigoni, P., Shaw, P.J., Yaniv, I., Machado, A., Pimentel, P., Fagioli, F., Verdeguer, A., Jouet, J.P., Diez, B., Ferreira, E., Pasquini, R., Rosenthal, J., Sievers, E., Messina, C., Iori, A.P., Garnier, F., Ionescu, I., Locatelli, F., Gluckman, E. & Eurocord Group (2003) Unrelated cord blood transplantation for childhood acute myeloid leukemia: a Eurocord Group analysis. *Blood*, **102,** 4290–4297.

Morinishi, Y., Imai, K., Nakagawa, N., Sato, H., Horiuchi, K., Ohtsuka, Y., Kaneda, Y., Taga, T., Hisakawa, H., Miyaji, R., Endo, M., Oh-Ishi, T., Kamachi, Y., Akahane, K., Kobayashi, C., Tsuchida, M., Morio, T., Sasahara, Y., Kumaki, S., Ishigaki, K., Yoshida, M., Urabe, T., Kobayashi, N., Okimoto, Y., Reichenbach, J., Hashii, Y., Tsuji, Y., Kogawa, K., Yamaguchi, S., Kanegane, H., Miyawaki, T., Yamada, M., Ariga, T. & Nonoyama, S. (2009) Identification of severe combined immunodeficiency by T-cell receptor excision circles quantification using neonatal guthrie cards. *Journal of Pediatrics*, **155,** 829–833.

Neven, B., Leroy, S., Decaluwe, H., Le Deist, F., Picard, C., Moshous, D., Mahlaoui, N., Debre, M., Casanova, J.-L., Dal Cortivo, L., Madec, Y., Hacein-Bey-Abina, S., de Saint Basile, G., de Villartay, J.-P., Blanche, S., Cavazzana-Calvo, M. & Fischer, A. (2009) Long-term outcome after hematopoietic stem cell transplantation of a single-center cohort of 90 patients with severe combined immunodeficiency. *Blood*, **113,** 4114–4124.

Ozsahin, H., Cavazzana-Calvo, M., Notarangelo, L.D., Schulz, A., Thrasher, A.J., Mazzolari, E., Slatter, M.A., Le Deist, F., Blanche, S., Veys, P., Fasth, A., Bredius, R., Sedlacek, P., Wulffraat, N., Ortega, J., Heilmann, C., O'Meara, A., Wachowiak, J., Kalwak, K., Matthes-Martin, S., Gungor, T., Ikinciogullari, A., Landais, P., Cant, A.J., Friedrich, W. & Fischer, A. (2008) Long-term outcome following hematopoietic stem-cell transplantation in Wiskott-Aldrich syndrome: collaborative study of the European Society for Immunodeficiencies and European Group for Blood and Marrow Transplantation. *Blood*, **111,** 439–445.

Przepiorka, D., Weisdorf, D., Martin, P., Klingemann, H.G., Beatty, P., Hows, J. & Thomas, E.D. (1995) 1994 consensus conference on acute GVHD grading. *Bone Marrow Transplantation*, **15,** 825–828.

Rao, K., Amrolia, P.J., Jones, A., Cale, C.M., Naik, P., King, D., Davies, G.E., Gaspar, H.B. & Veys, P.A. (2005) Improved survival after unrelated donor bone marrow transplantation in children with primary immunodeficiency using a reduced-intensity conditioning regimen. *Blood*, **105,** 879–885.

Rocha, V. & Gluckman, E.; Eurocord and European Blood and Marrow Transplant Group. (2006) Clinical use of umbilical cord blood hematopoietic stem cells. *Biology of Blood and Marrow Transplantation*, **12,** 34–41.

Rocha, V., Wagner, Jr, J.E., Sobocinski, K.A., Klein, J.P., Zhang, M.J., Horowitz, M.M. & Gluckman, E. (2000) Graft-versus-host disease in children who have received a cord-blood or bone marrow transplant from an HLA-identical sibling. Eurocord and International Bone Marrow Transplant Registry Working Committee on Alternative Donor and Stem Cell Sources. *New England Journal of Medicine*, **342,** 1846–1854.

Rocha, V., Labopin, M., Sanz, G., Arcese, W., Schwerdtfeger, R., Bosi, A., Jacobsen, N., Ruutu,

T., de Lima, M., Finke, J., Frassoni, F. & Gluckman, E.; Acute Leukemia Working Party of European Blood Marrow Transplant, Group; Eurocord-Netcord Registry (2004) Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. [see comment]. *New England Journal of Medicine*, **351,** 2276–2285.

Rubinstein, P., Carrier, C., Scaradavou, A., Kurtzberg, J., Adamson, J., Migliaccio, A.R., Berkowitz, R.L., Cabbad, M., Dobrila, N.L., Taylor, P.E., Rosenfield, R.E. & Stevens, C.E. (1998) Outcomes among 562 recipients of placental-blood transplants from unrelated donors. [see comment]. *New England Journal of Medicine*, **339,** 1565–1577.

Sakata, N., Kawa, K., Kato, K., Yabe, H., Yabe, M., Nagasawa, M., Mugishima, H., Kigasawa, H., Tsuchida, M., Akiyama, Y., Morisima, Y., Kodera, Y. & Kato, S. (2004) Unrelated donor marrow transplantation for congenital immunodeficiency and metabolic disease: an update of the experience of the Japan Marrow Donor Program. *International Journal of Hematology*, **80,** 174–182.

Sawczyn, K.K., Quinones, R., Malcolm, J., Foreman, N., Garrington, T., Gore, L., Gao, D. & Giller, R. (2005) Cord blood transplant in childhood ALL. [see comment]. *Pediatric Blood & Cancer*, **45,** 964–970.

Szabolcs, P. & Niedzwiecki, D. (2008) Immune reconstitution in children after unrelated cord blood transplantation. *Biology of Blood and Marrow Transplantation*, **14,** 66–72.

Thomson, B.G., Robertson, K.A., Gowan, D., Heilman, D., Broxmeyer, H.E., Emanuel, D., Kotylo, P., Brahmi, Z. & Smith, F.O. (2000) Analysis of engraftment, graft-versus-host disease, and immune recovery following unrelated donor cord blood transplantation. *Blood*, **96,** 2703–2711.

Tomizawa, D., Yuki, A., Nagasawa, M., Morio, T., Kajiwara, M., Sekine, T., Shimizu, N., Kato, M., Yachie, A. & Mizutani, S. (2005) Novel adopted immunotherapy for mixed chimerism after unrelated cord blood transplantation in Omenn syndrome. *European Journal of Haematology*, **75,** 441–444.

Tono, C., Takahashi, Y., Terui, K., Sasaki, S., Kamio, T., Tandai, S., Sato, T., Kudo, K., Toki, T., Tachibana, N., Yoshioka, T., Nakahata, T., Morio, T., Nishikomori, R. & Ito, E. (2007) Correction of immunodeficiency associated with NEMO mutation by umbilical cord blood transplantation using a reduced-intensity conditioning regimen. *Bone Marrow Transplantation*, **39,** 801–804.

Wagner, J.E., Rosenthal, J., Sweetman, R., Shu, X.O., Davies, S.M., Ramsay, N.K., McGlave, P.B., Sender, L. & Cairo, M.S. (1996) Successful transplantation of HLA-matched and HLA-mismatched umbilical cord blood from unrelated donors: analysis of engraftment and acute graft-versus-host disease. *Blood*, **88,** 795–802.

Zeidler, C., Welte, K., Barak, Y., Barriga, F., Bolyard, A.A., Boxer, L., Cornu, G., Cowan, M.J., Dale, D.C., Flood, T., Freedman, M., Gadner, H., Mandel, H., O'Reilly, R.J., Ramenghi, U., Reiter, A., Skinner, R., Vermylen, C. & Levine, J.E. (2000) Stem cell transplantation in patients with severe congenital neutropenia without evidence of leukemic transformation. *Blood*, **95,** 1195–1198.

Acute Cerebellitis and Concurrent Encephalitis Associated with Parvovirus B19 Infection

To the Editors:

Central nervous system (CNS) infections caused by parvovirus B19 (PVB19) have been rarely documented, especially the involvement of the cerebellum.[1] We describe an immunocompetent girl with acute cerebellitis associated with PVB19 infection.

A 5-year-old girl was hospitalized because of seizures of the upper extremities after a 3-day history of fever. On arrival, consciousness was disturbed (Glasgow coma scale: E2V1M4). Laboratory findings on admission included white blood cells 10.6 $\times 10^9$/L, blood glucose 74 mg/dL and sodium 130 mmol/L. Cerebrospinal fluid (CSF) examination showed 229 cells/μL with predominance of polymorphonuclear cells, 144 mg/dL protein and 56 mg/dL glucose. A brain computed tomography image was normal.

The patient began treatment with intravenous ceftriaxone, panipenem/betamipron, dexamethasone and acyclovir. Owing to persistent consciousness disturbance, methylprednisolone therapy was added on the fourth day in a dose of 30 mg/kg for 3 days. Electroencephalography on the sixth day showed high-voltage delta activity in the bilateral occipital regions, leading to the diagnosis of encephalopathy. On the sixth day, brain diffusion-weighted magnetic resonance images (see Fig. A, Supplemental Digital Content 1, http://links.lww.com/INF/B90) showed marked hyperintensity in the bilateral dentate nuclei in the cerebellum, suggesting a diagnosis of acute cerebellitis. These dentate nuclear lesions disappeared on the 10th day, and instead hyperintensity in the cerebellar hemisphere became prominent (see Fig. B, Supplemental Digital Content 1, http://links.lww.com/INF/B90). Thereafter, the patient could gradually follow simple verbal instructions, could sit alone on the 16th day, and could walk with a wide stance on the 26th day. Mutism had remained until the 20th day. Three months later, slurred speech and intention tremor persisted. A follow-up magnetic resonance study 6 months later showed cerebellar atrophy (see Fig. C, Supplemental Digital Content 1, http://links.lww.com/INF/B90).

On the 10th day, maculopapular rash appeared, extending to the face and extremities, suggesting erythema infectiosum. This was confirmed by elevation of serum PVB19 IgM and IgG antibodies (11.63 and 7.79 titers, respectively) using enzyme immunosorbent assay. Polymerase chain reaction analyses detected PVB19 DNA in the CSF (4.5×10^4 copies/mL) and in the plasma (2.8×10^5 copies/mL) samples stored from the time of admission. Polymerase chain reaction was also applied for herpes simplex virus 1 and 2, human herpes virus (HHV) 6, 7 and 8, varicella-zoster virus, cytomegalovirus, Epstein-Barr virus, JC virus and BK virus, showing negative results for all viruses examined except for HHV6 (5.5×10^2 and 3.5×10^3 copies/mL in the CSF and plasma, respectively). Serologically, HHV6-IgG antibody was positive and HHV6-IgM antibody was negative, findings consistent with history of exanthema subitum. Collectively, cerebellitis and concurrent encephalitis were likely caused by CNS PVB19 infection with reactivation of latent HHV6.

It is important to note that clinical and radiologic features in our patient were consistent with those of rotavirus-associated cerebellitis.[2] Takanashi et al[2] documented 11 such cases and proposed it as a novel clinicoradiologic entity because of the homogeneous characteristics. Although rotavirus predominated as causative pathogens,[2] adenovirus type 3,[3] HHV-6[4] and influenza virus[5] have also been reported. Among 31 childhood PVB19 CNS infections,[1] only 2 developed ataxia, but their cerebellar involvement was not radiologically demonstrated.

Thus, this is the first describing PVB19 as a cause for this novel type of cerebellitis and suggests a wider entity and a common mechanism.

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REFERENCES

1. Douvoyiannis M, Litman N, Goldman DL. Neurologic manifestations associated with parvovirus B19 infection. *Clin Infect Dis*. 2009;48:1713–1723.
2. Takanashi J, Miyamoto T, Ando N, et al. Clinical and radiological features of rotavirus cerebellitis. *AJNR Am J Neuroradiol*. 2010;31:1591–1595.
3. Kato Z, Manabe T, Teramoto T, et al. Adenovirus infection mimics the cerebellitis caused by rotavirus infection. *Eur J Pediatr*. 2011;170:405–406.
4. Kato Z, Kozawa R, Teramoto T, et al. Acute cerebellitis in primary human herpesvirus-6 infection. *Eur J Pediatr*. 2003;162:801–803.
5. Fluss J, Ferey S, Menache-Starobinski C, et al. Mild influenza-associated encephalopathy/encephalitis with a reversible splenial lesion in a Caucasian child with additional cerebellar features. *Eur J Paediatr Neurol*. 2010;14:97–100.

Primary Cutaneous Aspergillosis in Two Pediatric Trauma Patients

To the Editors:

Aspergillosis is the most common mold infection in immune compromised patients,[1] and can be nosocomially acquired. We report 2 cases of primary cutaneous aspergillosis (PCA) in previously healthy pediatric patients with multiple traumatic injuries after motor vehicle accidents.

CASE 1

A previously healthy 6-year-old boy with a history of prematurity and mild intermittent asthma was admitted to the pediatric intensive care unit after he suffered pulmonary contusions and a mediastinal hematoma in a motor vehicle accident. He developed acute respiratory distress syndrome requiring venoarterial extracorporeal membranous oxygenation. He received systemic corticosteroids for his lung injury and vancomycin for *Staphylococcus aureus* bacteremia. He developed erythematous bullous lesions on his left proximal forearm, which underwent necrosis with central ulceration (see Fig. 1). Tissue culture grew *Aspergillus fumigatus* and he was treated with intravenous voriconazole for 21 days, followed by 10 days of oral therapy.

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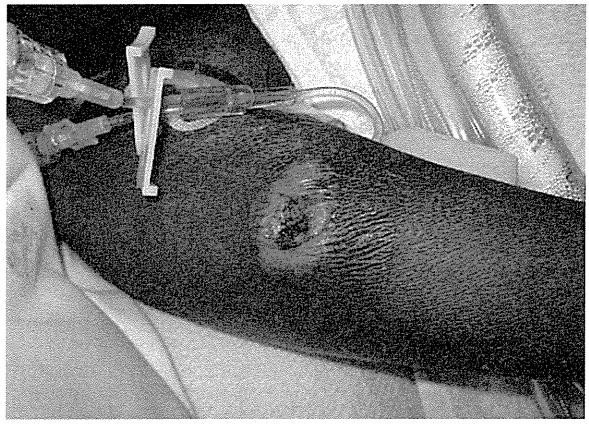

FIGURE 1. Bullous lesion of the left forearm after undergoing necrosis and ulceration.

CASE 2

A previously healthy 2-year-old girl suffered pulmonary contusions and thoracic spondylolisthesis in a motor vehicle accident. She developed acute respiratory distress syndrome requiring high-frequency oscillatory ventilation and completed a 2-week course of methylprednisolone. Sputum cultures grew *Acinetobacter baumanii* and *Moraxella catarrhalis* for which she was treated with vancomycin and cefotaxime. She developed multiple indurated erythematous papules with central eschar on her extremities. Wound culture grew *A. fumigatus*. She completed a 6-week course of voriconazole, with resolution of the lesions.

DISCUSSION

PCA is typically associated with alterations in skin integrity and immune compromise. The morphology of lesions varies. It may first manifest as erythema and induration, but can progress to papules, nodules, macules, plaques, pustules, vesicles, bullae or ulcers.[1] Diagnosis is typically made by skin biopsy for histopathology and culture. Amphotericin B has historically been first-line therapy; however, voriconazole has been shown to be superior in adults, has a good safety profile in children and offers oral administration.[2]

Our patients were previously healthy, without underlying immune suppression, but other factors likely caused transient alteration in their defenses. They suffered multiple traumatic injuries, and may have had unrecognized alterations in skin integrity. Trauma is known to alter cellular and humoral immunity, with an initial proinflammatory response to the injury characterized by activation of nuclear factor-κB with resultant increases in acute phase reactants such as cytokines, specifically interleukin (IL)-1b, IL-6, IL-8 and tumor necrosis factor-α. After this proinflammatory state, a compensatory antiinflammatory response syndrome or immune paralysis can occur. Patients develop impaired monocyte function with decreased expression of human leukocyte antigen DR and decreased production of tumor necrosis factor-α, resulting in predominance of the Th2 lymphocyte pattern.[3] Posttraumatic impairment of monocyte activity has been shown to correlate with severity of injury, and is predictive of the development of systemic inflammatory response syndrome.[4] Corticosteroids have been associated with PCA, with impairment of glycemic control, phagocytic function and strength of the skin barrier.[5] Broad spectrum antibiotics can also increase susceptibility to fungal infections in immune compromised patients.

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REFERENCES

1. Katta R, Bogle MA, Levy ML. Primary cutaneous opportunistic mold infections in a pediatric population. *J Am Acad Dermatol.* 2005;53:213–219.
2. Walsh TJ, Lutsar I, Driscoll T, et al. Voriconazole in the treatment of aspergillosis, scedosporiosis and other invasive fungal infections in children. *Pediatr Infect Dis J.* 2002;21:240–248.
3. Tschoeke SK, Ertel W. Immunoparalysis after multiple trauma. *Injury.* 2007;38:1346–1357.
4. Wutzler S, Maier M, Lehnert M, et al. Suppression and recovery of LPS-stimulated monocyte activity after trauma is correlated with increasing injury severity: a prospective clinical study. *J Trauma.* 2009;66:1273–1280.
5. Walsh TJ. Primary cutaneous aspergillosis—an emerging infection among immunocompromised patients. *Clin Infect Dis.* 1998;27:453–457.

Letter to the Editor

Common variable immunodeficiency classification by quantifying T-cell receptor and immunoglobulin κ-deleting recombination excision circles

To the Editor:

Common variable immunodeficiency (CVID) is the most frequent primary immunodeficiency associated with hypogammaglobulinemia and other various clinical manifestations. CVID was originally reported to be a disease primarily caused by defective B-cell function, with defective terminal B-cell differentiation rendering B cells unable to produce immunoglobulin. However, combined immunodeficiency (CID) involving both defective B and T cells is often misdiagnosed as CVID.[1] Indeed, one study reported that $CD4^+$ T-cell numbers were decreased in 29% of 473 patients with CVID[2]; similarly, another study found that naive T-cell numbers were markedly reduced in 44% (11/25) of patients with CVID.[3] These observations indicated that a subgroup of patients with clinically diagnosed CVID is T-cell deficient. Consistently, some patients with CVID have complications that might be related to T-cell deficiency, including opportunistic infections, autoimmune diseases, and malignancies, which is similar to that observed in patients with CID.[1,4] Therefore identifying novel markers to better classify CVID and distinguish CID from CVID will be required to best manage medical treatment for CVID.

We recently performed real-time PCR-based quantification of T-cell receptor excision circles (TREC) and signal joint immunoglobulin κ-deleting recombination excision circles (KREC) for mass screening of severe combined immunodeficiency (SCID)[5] and B-lymphocyte deficiency[6] in neonates. TREC and KREC are associated with T-cell and B-cell neogenesis, respectively.[7] Here we retrospectively report that TREC and KREC are useful for classifying patients with clinically diagnosed CVID.

Hypogammaglobulinemic patients (n = 113) were referred to our hospital for immunodeficiency from 2005-2011, and the following patients were excluded from the CVID pool by estimating their SCID genes based on clinical manifestations and lymphocyte subset analysis: 18 patients with SCID diagnoses; 14 patients less than 2 years of age (transient infantile hypogammaglobulinemia); 10 patients with IgM levels of greater than 100 mg/dL (hyper-IgM syndrome); 26 patients with diseases other than CVID caused by known gene alterations (10 with X-linked agammaglobulinemia and 11 with hyper-IgM syndrome [*CD40L* or *AICDA* mutated]), (2 with DiGeorge syndrome, and 3 with *FOXP3*, *IKBKG*, or *6p* deletions); and 5 patients with drug-induced hypogammaglobulinemia. The remaining 40 patients with decreased IgG (≥2 SDs below the mean for age), IgM, and/or IgA levels, as well as absent isohemagglutinins, poor response to vaccines, or both were included in this study as patients with CVID and analyzed for TREC/KREC levels, retrospectively.

Ages of patients with CVID ranged from 2 to 52 years (median age, 15.5 years). The sex ratio of the patients was 21 male/19 female patients. Serum IgG, IgA, and IgM levels were 370 ± 33 mg/dL (0-716 mg/dL), 30 ± 7 mg/dL (1-196 mg/dL), and 40 ± 6 mg/dL (2-213 mg/dL), respectively. TREC and KREC quantification was performed by using DNA samples extracted from peripheral blood, as reported previously.[5,6] Clinical symptoms were then assessed retrospectively. The study protocol was approved by the National Defense Medical College Institutional Review Board, and written informed consent was obtained from adult patients or parents of minor patients in accordance with the Declaration of Helsinki.

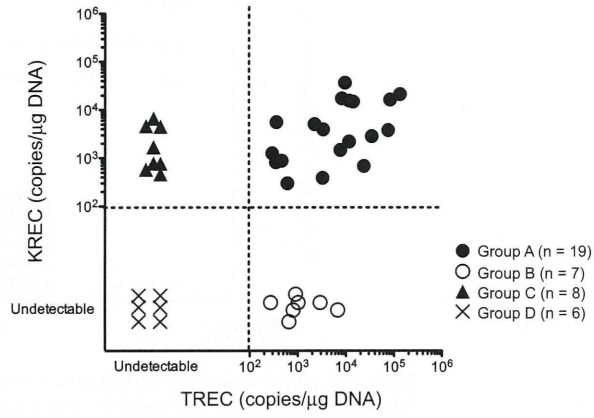

FIG 1. Quantifying TREC and KREC classifies patients with CVID into 4 groups. Patients with CVID were classified as follows: TREC(+)/KREC(+), group A (19 patients); TREC(+)/KREC(−), group B (7 patients); TREC(−)/KREC(+), group C (8 patients); and TREC(−)/KREC(−), group D (6 patients). *Undetectable*, Less than 100 copies/μg DNA.

Based on TREC and KREC copy numbers, the 40 patients with CVID were classified into 4 groups (groups A, B, C, and D; Fig 1). Comparing lymphocyte subsets, $CD3^+$ T-cell numbers were similar among groups A, B, and D but were significantly lower in group C ($P < .05$; group A, 1806 ± 204 cells/μL; group B, 1665 ± 430 cells/μL; group C, 517 ± 124 cells/μL; and group D, 1425 ± 724 cells/μL; $P = .0019$, Tukey multiple comparison test based on 1-way ANOVA). $CD3^+CD4^+CD45RO^+$ memory T-lymphocyte percentages in groups B, C, and D were significantly higher than those in group A ($P < .0001$; group A, 37% ± 16%; group B, 67% ± 13% [$P = .0006$]; group C, 92% ± 8.2% [$P < .0001$]; and group D: 83% ± 14% [$P < .0001$]; see Fig E1 in this article's Online Repository at www.jacionline.org); additionally, the percentages of these cells in groups C and D were higher than in group B ($P = .0115$). These results indicate that group C and D patients have markedly decreased $CD4^+CD45RA^+$ naive T-cell counts than group A patients and that counts in group B are also significantly decreased, although less so than in groups C or D, which is consistent with a report showing lower TREC copy numbers in $CD4^+CD45RO^+$ cells. Some patients in groups B, C, and D exhibited normal $CD4^+CD45RO^+$ percentages, although TREC levels, KREC levels, or both decreased. This discrepancy indicates that TREC/KREC levels could be independent markers to determine the patient's immunologic status in addition to $CD4^+CD45RA^+$; the reasons underlying the discrepancy between $CD4^+CD45RA^+$ and TREC/KREC levels remain unsolved.

$CD19^+$ B-cell numbers in group A were significantly higher ($P < .05$) than those in groups B and D (group A, 269 ± 65 cells/μL; group B, 35 ± 16 cells/μL; group C, 60 ± 11 cells/μL; and group D, 29 ± 16 cells/μL; $P = .0001$). However, B-cell subpopulations, including $CD27^-$, IgD^+CD27^+, and

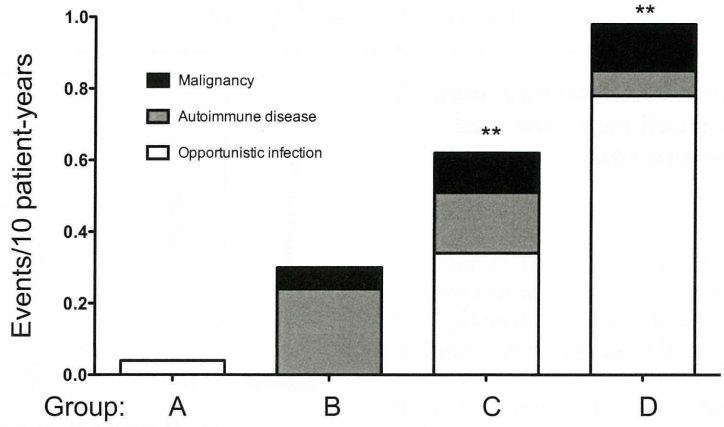

FIG 2. Cumulative incidence of complication events per 10 patient-years differs among groups. Opportunistic infections, autoimmune diseases, and malignancies were evaluated for each patient group. Complication incidences in group D (0.98 events/10 patient-years), group C (0.63 events/10 patient-years), and group B (0.30 events/10 patient-years) were higher than in group A (0.04 events/10 patient-years). Group A versus group D: $^{**}P = .0022$; group A versus C: $^{**}P = .0092$; group A vs group B: $P = .0692$.

IgD^-CD27^+ cells, were not significantly different among the groups. Standardizing KREC copy numbers for each patient by dividing their $CD19^+$ by their $CD27^+$ percentages revealed the same patient classification as that shown in Fig 1 (data not shown), indicating that the original classification was independent of $CD19^+$ B-cell or $CD27^+$ memory B-cell percentages.

Because TREC and KREC levels decrease with age (see Fig E2 in this article's Online Repository at www.jacionline.org)[5,6] and age distribution was wide in this study, we compared patients' ages among groups at the time of analysis to determine whether classification was associated with age. TREC/KREC-based classification was independent of both age and sex because age distribution was not significantly different among groups ($P > .05$; group A, 12.7 ± 2.3 years [2-30 years]; group B, 23.4 ± 4.2 years [6-39 years]; group C, 21.5 ± 6.1 years [4-52 years]; and group D, 25.5 ± 4.4 years [15-46 years]; data not shown) nor was male/female sex ratio (overall, 21/19; group A, 10/9; group B, 2/5; group C, 5/3; and group D, 4/2; $P = .4916$, χ^2 test; data not shown).

We next evaluated whether any correlation existed between TREC/KREC-based classification and clinical symptoms in each patient group. All patients in the study had been treated with intravenous immunoglobulin (IVIG) substitution at the time of analysis. We found that the cumulative events of complications (opportunistic infections, autoimmune diseases, and malignancies) per 10 patient-years were highest in group D (0.98 events/10 patient-years), followed by group C (0.63 events/10 patient-years), group B (0.30 events/10 patient-years), and group A (0.04 events/10 patient-years), where events in groups D and C were significantly higher than group A (group A vs group D, $P = .0022$; group A vs group C, $P = .0092$; group A vs group B, $P = .0692$; Fig 2). Furthermore, we found similar results when evaluating only patients 19 years old or older for group D (1.01 events/10 patient-years), group C (0.56 events/10 patient-years), group B (0.32 events/10 patient-years), and group A (0.06 events/10 patient-years; group A vs group D, $P = .0074$; group A vs group C, $P = .0407$; group A vs group B, $P = .1492$; data not shown). Categorizing patients by using several different previously reported CVID classifications (focused primarily on separating patients based on levels of circulating B-cell subsets), we found that no classification scheme showed any significant event increases in any particular group (see Fig E3 in this article's Online Repository at www.jacionline.org). Assessing longitudinal cumulative opportunistic infection incidence among the groups, group D and C values were significantly higher than in group A (see Fig E4, A, in this article's Online Repository at www.jacionline.org; $P = .0059$). Autoimmune and malignant diseases ($P = .5168$ and $P = .6900$, respectively) were observed in groups B and D but not in group A (see Fig E4, *B* and *C*). Cumulative events were significantly different between groups ($P = .0313$, log-rank test; group A, 5.3% and 5.3%; group B, 14.3% and 57.1%; group C, 27.1% and 63.5%; and group D, 33.3% and 83.3% at 10 and 30 years of age, respectively; see Fig E4, *D*). One patient in group D died of *Pneumocystis jirovecii* pneumonia, and 2 other patients in the same group received hematopoietic stem cell transplantation after complications caused by EBV-related lymphoproliferative disorder.

Assessing these data, TREC/KREC-based classification matches clinical outcomes. Because group D patients exhibited the most frequent complications (opportunistic infections, autoimmune diseases, and malignancies), they could receive a diagnosis of CID based on these symptoms. If they are indeed determined to have CID, then TREC/KREC analysis is helpful to distinguish between CID and CVID. Their TREC(−)/KREC(−) phenotype might relate to defective V(D)J recombination in T- and B-cell development[8] because patients with B-negative SCID (*RAG1*, *RAG2*, *Artemis*, and *LIG4*), as well as patients with ataxia-telangiectasia (AT) and Nijmegen breakage syndrome (NBS; see Fig E5 in this article's Online Repository at www.jacionline.org),[5,6] were also negative for both TREC and KREC; it is intriguing to speculate that an unknown V(D)J recombination gene or genes is responsible. As for treatment, hematopoietic stem cell transplantation should be considered the preferred treatment to "cure" group D patients, as reported in patients with severe CVID/CID, because event-free survival is poor.[9]

In contrast to group D patients, TREC(+)/KREC(+) group A patients treated with IVIG substitution therapy remained healthy. One possible explanation is that these patients harbor

defects only in terminal B-cell differentiation, but not in T cells, and represent typical patients with CVID, as originally reported.

Group C patients had a high frequency of both opportunistic infections and malignancies, suggesting that these TREC(−) patients have T-cell defects. Although group C patients had a similar TREC/KREC pattern to patients with SCID with B cells (*IL2RG* and *JAK3*; see Fig E5, *A*), they do not fulfill the European Society for Immunodeficiencies criteria for SCID, and no mutation was identified in the SCID genes estimated from clinical manifestation and lymphocyte subset analysis. However, from our data, they would likely benefit from undergoing similar treatment to patients with SCID or CID to prevent these complications.

Although opportunistic infections were rare in group B patients, autoimmune diseases were often observed. This is consistent with this group being TREC(+)/KREC(−) and the idea that balance between T and B cells is important to prevent autoimmune diseases in patients with CVID.[1] Intriguingly, a group of patients with AT and NBS were also TREC(+)/KREC(−) (see Fig E4, *B*), which is similar to group B patients. Additionally, $CD45RA^{+}CD4^{+}$ naive T-cell numbers were reduced in most group B patients, which is similar to the phenotype exhibited by patients with AT and NBS. This finding raises the possibility that although some group B patients are also T-cell deficient, as well as B-cell deficient, and should be treated similarly to patients with CID, other patients have only B-cell deficiency and are effectively treated with IVIG substitution therapy.

By analyzing a large CVID patient cohort, the overall survival rate of patients with more than 1 complication was worse than that for patients without other complications.[4] Our findings indicate that low TREC levels, KREC levels, or both are useful markers that correlate well with the overall survival rate in patients with CVID. Therefore we conclude that TREC and KREC are useful markers to assess the clinical severity and pathogenesis of each patient with CVID and to distinguish CID from CVID. Thus patient classification based on TREC/KREC levels would provide a helpful tool for deciding on an effective treatment plan for each patient with CVID.

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REFERENCES

1. Yong PFK, Thaventhiran JED, Grimbacher B. "A rose is a rose is a rose," but CVID is not CVID. common variable immune deficiency (CVID), what do we know in 2011? Adv Immunol 2011;111:47-107.
2. Resnick ES, Moshier EL, Godbold JH, Cunningham-Rundles C. Morbidity and mortality in common variable immune deficiency over 4 decades. Blood 2012;119:1650-7.
3. Moratto D, Gulino AV, Fontana S, Mori L, Pirovano S, Soresina A, et al. Combined decrease of defined B and T cell subsets in a group of common variable immunodeficiency patients. Clin Immunol 2006;121:203-14.
4. Chapel H, Lucas M, Lee M, Bjorkander J, Webster D, Grimbacher B, et al. Common variable immunodeficiency disorders: division into distinct clinical phenotypes. Blood 2008;112:277-86.
5. Morinishi Y, Imai K, Nakagawa N, Sato H, Horiuchi K, Ohtsuka Y, et al. Identification of severe combined immunodeficiency by T-cell receptor excision circles quantification using neonatal Guthrie cards. J Pediatr 2009;155:829-33.
6. Nakagawa N, Imai K, Kanegane H, Sato H, Yamada M, Kondoh K, et al. Quantification of κ-deleting recombination excision circles in Guthrie cards for the identification of early B-cell maturation defects. J Allergy Clin Immunol 2011;128:223-5.e2.
7. van Zelm MC, Szczepanski T, Van Der Burg M, Van Dongen JJM. Replication history of B lymphocytes reveals homeostatic proliferation and extensive antigen-induced B cell expansion. J Exp Med 2007;204:645-55.
8. Verbsky JW, Baker MW, Grossman WJ, Hintermeyer M, Dasu T, Bonacci B, et al. Newborn screening for severe combined immunodeficiency; the Wisconsin experience (2008-2011). J Clin Immunol 2012;32:82-8.
9. Rizzi M, Neumann C, Fielding AK, Marks R, Goldacker S, Thaventhiran J, et al. Outcome of allogeneic stem cell transplantation in adults with common variable immunodeficiency. J Allergy Clin Immunol 2011;128:1371-2.

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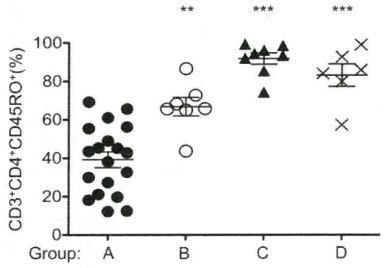
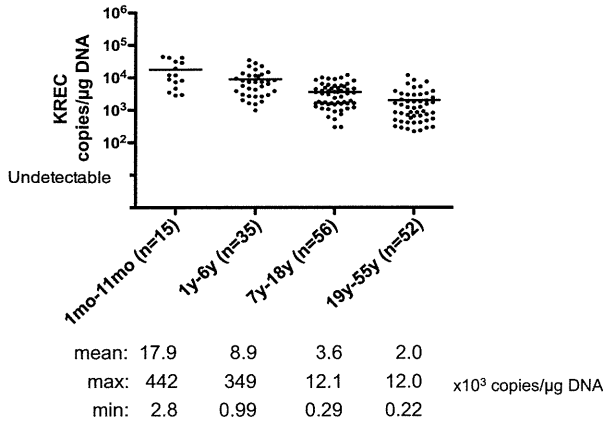


FIG E1. $CD45RO^+CD3^+CD4^+$ T-cell frequency within $CD4^+CD3^+$ lymphocytes was analyzed among groups. $CD45RO^+CD3^+CD4^+$ lymphocyte counts were significantly higher in groups B, C, and D compared with those in group A ($P < .0001$). Group A: 37% ± 16%; group B: 67% ± 13% (**$P < .01$); group C: 92% ± 8.2% (***$P < .001$); and group D: 83% ± 14% (***$P < .001$).



mean:	17.9	8.9	3.6	2.0
max:	442	349	12.1	12.0
min:	2.8	0.99	0.29	0.22

x10^3 copies/µg DNA

FIG E2. KREC levels were analyzed in genomic DNA samples extracted from peripheral blood of control subjects at different age groups (n = 158; age range, 1 month to 55 years). KREC levels were significantly higher in infants (17.9 ± 3.9 × 10^3 copies/µg DNA) compared with other children's age groups (8.9 ± 1.3 × 10^3 copies/µg DNA in the 1- to 6-year-old group and 3.6 ± 3.8 × 10^3 copies/µg DNA in the 7- to 18-year-old group) and adults (2.0 ± 3.3 × 10^3 copies/µg DNA; $P < .0001$).

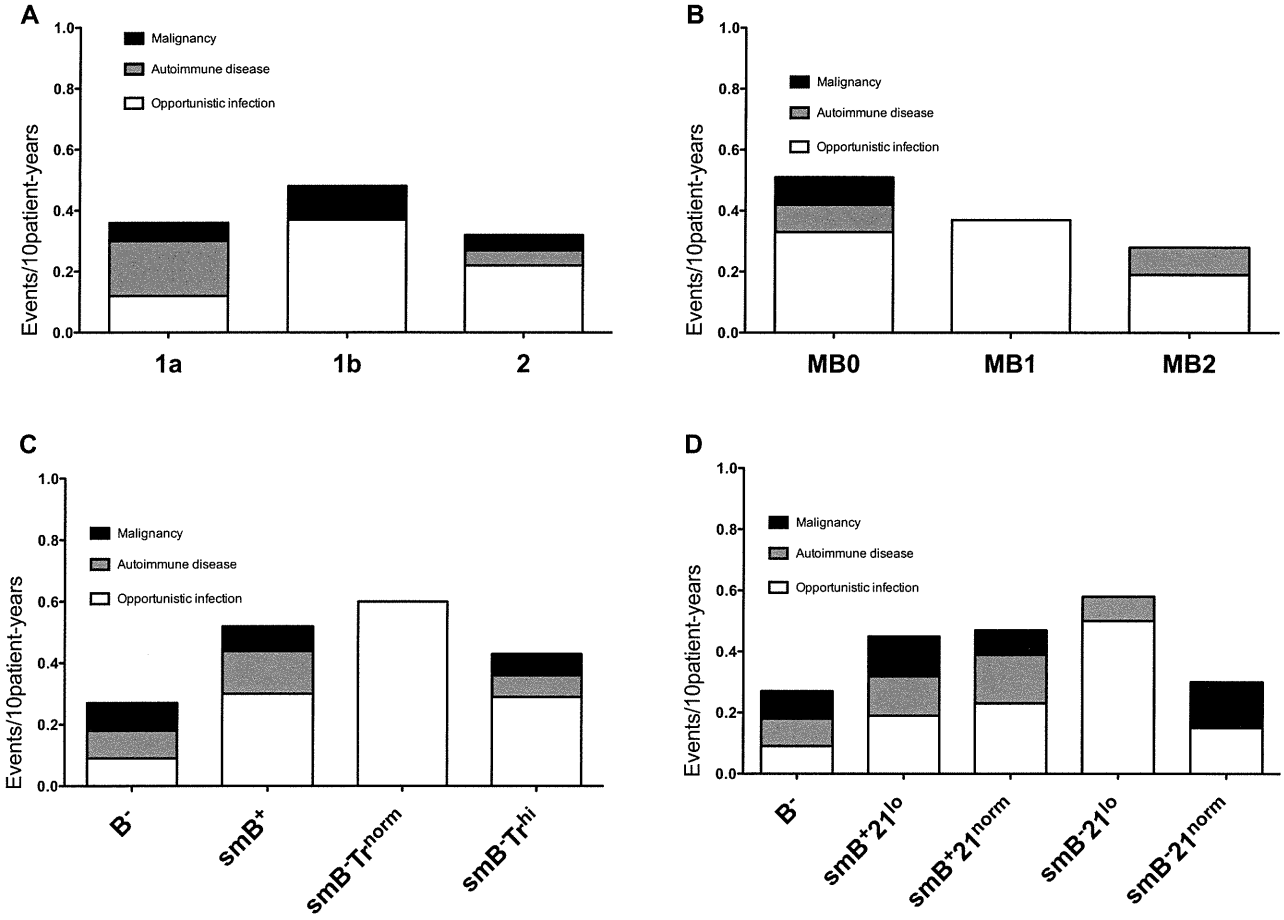


FIG E3. Patients were classified in the following way and analyzed for cumulative incidence of complications: **A,** Freiburg; **B,** Paris; and **C,** EUROclass classifications, according to $CD38^{hi}IgM^{hi}$ transitional B cells (Fig E3, *A-C*) or $CD21^{low}$ B cells **(D)**. Five patients were excluded from the Freiburg and Paris classifications because of decreased B-cell numbers (<1%). Additionally, we excluded 4 patients in the Freiburg classification, 1 patient in the Paris classification, and 4 patients in the EUROclass classification for transitional B cells and 8 in the EUROclass classification for $CD21^{low}$ B cells because of lack of data. The following cumulative events/10 patient-years were found. Freiburg classification: 1a, 0.36; 1b, 0.48; 2, 0.32. Paris classification: MB0, 0.50; MB1, 0.37; MB2, 0.28. EUROclass classification according to transitional B cells: B^-, 0.27; smB^+, 0.52; smB^-Tr^{norm}, 0.60; smB^-Tr^{high}, 0.43. EUROclass classification according to $CD21^{lo}$ B cells: B^-, 0.27; smB^+21^{lo}, 0.45; smB^+21^{norm}, 0.47; smB^-21^{lo}, 0.58; smB^-21^{norm}, 0.30. No classification showed any significantly increased events in any particular group according to calculated *P* values, as follows—Freiburg classification: 1a vs 2 = .898, 1b vs 2 = .479, 1a vs 1b = .838; Paris classification: MB0 vs MB2 = .179, MB1 vs MB2 = .654, MB0 vs MB1 = .764; EUROclass classification according to transitional B cells: B^- vs smB^+ = .298, smB^-Tr^{norm} vs smB^+ = .809, smB^-Tr^{hi} vs smB^+ = .702, smB^-Tr^{hi} vs smB^-Tr^{norm} = .641, smB^-Tr^{norm} vs B^- = .329, smB^-Tr^{hi} vs B^- = .508; EUROclass classification according to $CD21^{lo}$ B cells: B^- vs smB^+21^{norm} = .443, smB^+21^{lo} vs smB^+21^{norm} = .930, smB^-21^{lo} vs smB^+21^{norm} = .695, smB^-21^{norm} vs smB^+21^{norm} = .575, B^- vs smB^-21^{norm} = .926, smB^+21^{lo} vs smB^-21^{norm} = .609, smB^-21^{lo} vs smB^-21^{norm} = .399, B^- vs smB^+21^{lo} = 0.474, B^- vs smB^-21^{lo} = 0.270, smB^+21^{lo} vs smB^-21^{lo} = 0.618.

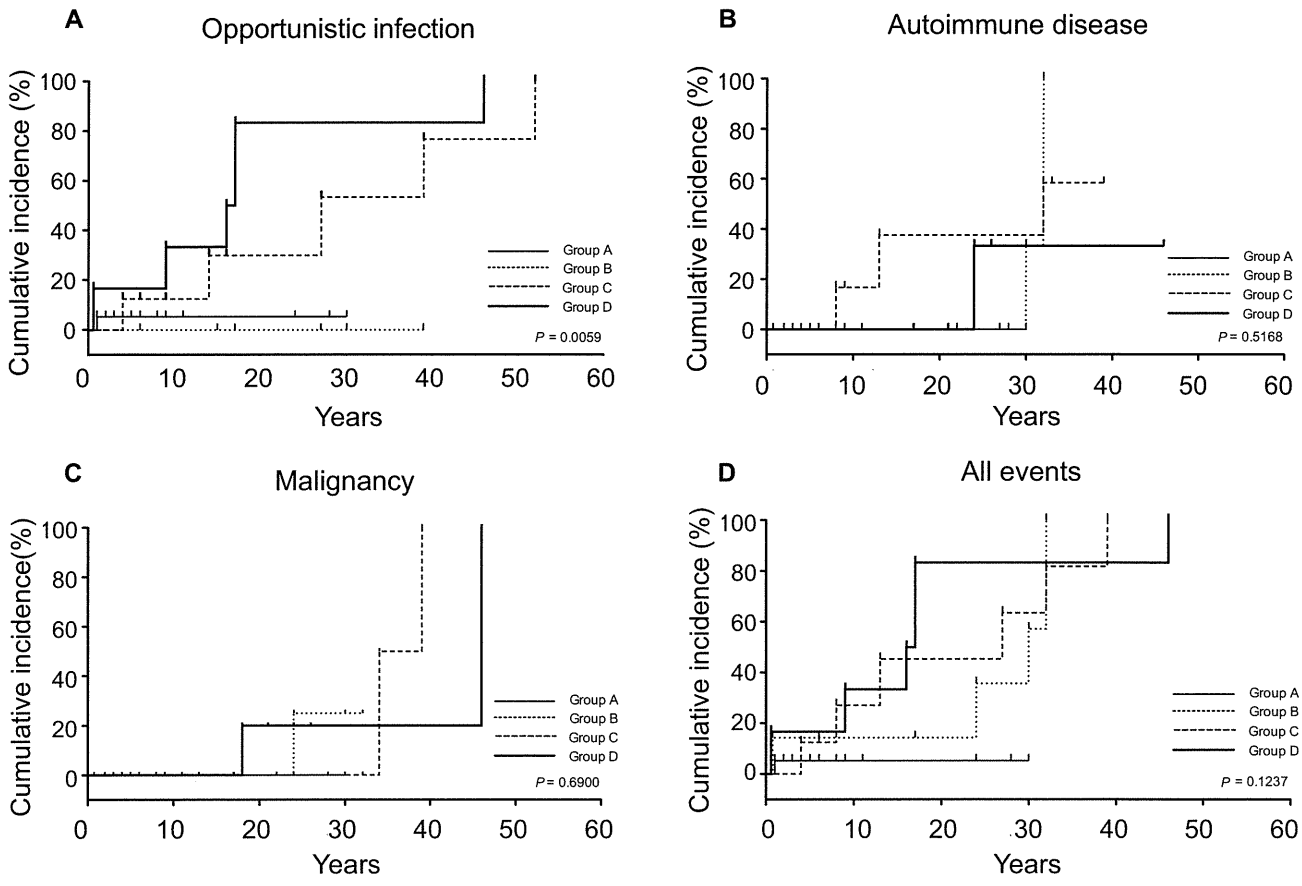


FIG E4. Comparing longitudinal cumulative incidence of complication events among groups. Cumulative incidence was estimated separately and longitudinally by using the Kaplan-Meier method and statistically compared between groups by using the log-rank test. The cumulative incidence of opportunistic infections **(A)**, autoimmune diseases **(B)**, malignancies **(C)**, and all events **(D)** is shown.