Transfusion associated complications in cardiac surgery : the swan song of the allogeneic leukocytes ?

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Chapter 6

Mannose-Binding Lectin is Involved in Multiple-Organ-Dysfunction-Syndrome after Cardiac Surgery: Effects of Blood Transfusions

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ABSTRACT

Background: Serum levels of mannose-binding lectin (MBL), a recognition molecule of the lectin pathway of complement, are highly variable, based on genetic variation. After cardiac surgery, extracorporeal circulation and ischemia/reperfusion injury initiate a systemic inflammatory response, which can evolve to multi-organ dysfunction syndrome (MODS). Peroperative transfusions of allogeneic leukocytes contribute to infectious and inflammatory complications. The present study investigates the role of MBL in relation to blood transfusions and complications following cardiac surgery.

Methods: In cardiac surgery patients who participated in a randomised trial comparing leukoreduced with buffy-coat-depleted red blood cell transfusions, circulating MBL was measured pre- and post-operatively by ELISA. Data were related to the incidence of complications and to the transfusions the patients received.

Results: Patients with high pre-operative serum MBL levels (>400 ng/ml) show a significant (52 ± 12%) decrease of serum MBL post-operatively, while patients with low serum MBL levels (<400 ng/ml) show a significant increase of serum MBL levels after surgery (140 ± 106%), which was further enhanced by fresh-frozen plasma (FFP) transfusions. MBL levels were not associated with infections, sepsis, or death. Patients with MBL deficiency (MBL <80 ng/ml) were protected against development of MODS (p=0.016), whereas FFP transfusion abolished this protection (p=0.048).

Conclusions: Cardiac surgery is associated with MBL consumption, independent of the transfusion of allogeneic leukocytes. Patients with MBL deficiency develop no MODS, unless they have been transfused with FFP, which is associated with MBL reconstitution. Therefore, sustained MBL deficiency may be a favourable status for patients undergoing cardiac surgery.

INTRODUCTION

Cardiac surgery associated with ischemia/reperfusion injury is often followed by a systemic inflammatory response syndrome (SIRS). Moderate SIRS usually resolves with supportive care, although severe SIRS can evolve to multiple-organ-dysfunction-syndrome (MODS). The outcome of cardiac surgery is closely related with the severity of MODS and development of severe infections [1].

One of the components of the inflammatory response which is activated during cardiac surgery is the complement system [2,3]. The complement system can be activated by three pathways: the classical pathway, the alternative pathway and the lectin pathway. While the classical pathway is activated by antibodies and immune complexes, the lectin pathway can be triggered by binding of carbohydrates exposed on a wide range of micro-organisms to mannose-binding lectin (MBL) [4]. Polymorphisms in the MBL gene result in a wide range of functional MBL levels. Roughly 30% of the Caucasian population has reduced levels of MBL, due to single nucleotide polymorphisms in exon 1 of the *MBL2* gene, and approximately 5-10% has a functional MBL deficiency [5].

MBL deficiency in itself does not lead to clinical problems, but several studies have shown that MBL deficiency confers an increased susceptibility for infections in immune-compromised patients [6-9]. The role of MBL deficiency on the development and outcome of SIRS and sepsis syndrome is controversial. Worse outcome in patients with sepsis is described [10,11]. However, in animal models, inhibition of the lectin pathway was shown to protect against ischemia-reperfusion injury and diminished neutrophil accumulation and cytokine release [12], and MBL-deficient mice were protected against severe ischemia/reperfusion injury in various organ systems [13-16]. These studies suggest that MBL deficiency could have favourable effects on tissue injury following ischemia and reperfusion.

In cardiac surgery, red blood cell transfusions dose-dependently increase the risk of morbidity and mortality. In randomized trials we observed that red blood cells containing leukocytes compared with filtered leukoreduced red blood cells were associated with increased postoperative infections and mortality with MODS [17]. After complicated cardiac surgery, approximately 20-25% of the patients develop MODS and the combination of MODS and infections is associated with increased mortality [18]. However the role of blood component transfusions on activation of the complement system, especially on the lectin pathway, is not known. Cellular interactions of MBL have been described *in vitro* for several cell types. Interaction of polymorphonuclear leukocytes with ligand-bound MBL *in vitro* was shown to induce cell aggregation and superoxide production [19]. Direct

interactions between leukocytes and the complement system may provide an explanation for the beneficial effect of leukocyte depletion in red cell transfusion. We hypothesize that MBL and the lectin pathway may contribute to complications after cardiac surgery, both via activation of the complement cascade and via cellular interactions.

In the present study, we evaluated the effect of cardiac surgery and blood transfusions on pre- and postoperative MBL levels and we assessed whether MBL levels were associated with postoperative complications after cardiac surgery.

MATERIALS AND METHODS

Patients

A randomized, double-blinded controlled trial had been conducted in two hospitals and included patients undergoing valve surgery with or without coronary artery bypass graft (CABG). Patients were randomized to receive (when needed) standard buffy-coat depleted or pre-storage leukoreduced red blood cells. Patient samples were collected after informed consent was obtained. The endpoints of the study were: postoperative infections, MODS and mortality (90-days and in-hospital). The design and clinical outcome of the study has been described elsewhere [17]. In this trial infections were defined according to the criteria of the Center for Disease Control (CDC) [20]. The following infections were taken into account: respiratory tract, urinary tract, sepsis and wound infections. The incidence and duration of organ dysfunction was described as defined by Knaus [21]. MODS was defined as failure of two or more organ systems. The blood products used in this study were prepared and controlled according to the Dutch standards for blood banks.

Blood samples were taken pre-operatively before the start of the surgical procedure and at admission on the ICU, immediately centrifuged, and the sera stored at -80°C.

Measurement of MBL

The concentration of MBL was measured by sandwich enzyme linked immunosorbent assay (ELISA) as described previously [22]. In summary, 96-well ELISA plates were coated with mAb 3E7 (monoclonal anti-MBL antibody kindly provided by Dr. T. Fujita, Fukushima, Japan). After blocking residual binding sites with PBS containing 1% BSA and washing, serum samples were diluted and incubated, followed by detection with digoxygenin (dig-) conjugated 3E7 and HRP-conjugated sheep Fab anti-Dig antibodies (from Roche Applied Science, Mannheim, Germany), respectively. Enzyme activity was developed using ABTS.

The optical density was measured using a microplate reader, and results were calculated on basis of a calibration line using pooled normal human serum with a known concentration of MBL.

To estimate the effect of the surgical procedure on the MBL serum concentration, the ratio of the post- and preoperative MBL serum level was calculated.

Statistics

Categorical characteristics among MBL groups were compared using cross-tables with calculation of p-values. Continuous variables were analyzed using Student t-test or, when appropriate Mann-Whitney test was used. The differences in MBL levels before and after surgery were analyzed by the Wilcoxon's signed rank test. Spearman Rank correlation coefficients were used for correlation. A logistic regression model was used to evaluate the effect of per-operative factors on the postoperative/preoperative MBL ratio. P-values <0.05 were considered as statistically significant. All analyses were performed in SPSS (SPSS Inc, Chicago, IL, USA).

RESULTS

Patients Characteristics

Of the 474 patients included in the trial, serum for MBL determination was available from 400 patients prior to surgery. From these 400 patients, postoperative MBL levels could be assessed in 330 patients. The main characteristics of the patient population are presented in Table 1.

Serum MBL Levels

Pre-operative serum levels of MBL were highly variable as expected in the human population (range 15-3709 ng/ml; Table 1). In Figure 1A the post-/ preoperative MBL ratios are shown. The ratio of post-operative and pre-operative MBL concentration was negatively correlated with the pre-operative MBL concentration ($R = -0.73$, $p<0.0001$). Patients with high serum MBL levels before surgery (>400 ng/ml) showed an average decrease of $52 \pm 12\%$ of the serum MBL level after the procedure (range ratio 0.19-0.93; $p < 0.0001$, Figure 1B). In contrast, patients with low serum MBL levels before operation (< 400 ng/ml) generally showed an increase of serum MBL levels, as represented by a ratio significantly higher than 1 ($p<0.0001$, Figure 1B).

Table 1 | Patient Characteristics

	Total	Randomization*		P value
		Buffy-coat depleted red blood cells	Leuko-reduced red blood cells	
Patient number	400	194	206	
Age (mean ±SD)	65.8 ± 13.9	66.5 ± 12.5	65.1 ± 15	0.36
Type of surgery (N (%))				
Valve	269 (67.3%)	133 (68.6%)	136 (66.0%)	0.60
Valve + CABG	131 (32.8%)	61 (31.4%)	70 (34.0%)	
Female (N (%))	178 (44.5%)	85 (43.8%)	93 (45.1%)	0.84
Parsonnet score	13.8 ± 8.2	13.8 ± 8.6	13.8 ± 7.8	0.98
Blood transfusions during surgery†				
Red blood cells (mean units per patient ±SD)	3.2 ± 3.6	3.4 ± 4.7	3.0 ± 2.1	0.23
FFP (mean units per patient ±SD)	3.2 ± 4.1	3.6 ± 5.4	2.8 ± 1.7	0.22
Patients without FFP transfusions (N (%))	224 (56.0%)	103 (53.1%)	121 (58.7%)	0.27
Patients without red blood cell transf. (N (%))	114 (28.5%)	54 (27.8%)	60 (29.1%)	0.82
Cardiopulmonary bypass time (minutes; mean ±SD)	140 ± 62	145 ± 66	136 ± 57	0.13
Aortic cross clamping time (minutes; mean ±SD)	96± 46	96 ± 47	96 ± 44	0.98
Serum total protein (gram/l) (mean ±SD)				
Pre-operative	74.5 ± 4.3	74.3 ± 4.0	75 ± 4.6	0.55
Post-operative	49.9 ± 6.2	49.6 ± 6.6	50.2 ± 5.8	0.50
Infections (N (%))	113 (28.3%)	66 (34.0%)	47 (22.8%)	0.02
MODS (N (%))	89 (22.3%)	45 (23.2%)	44 (21.4%)	0.72
Sepsis (N (%))	17 (4.3%)	10 (5.2%)	7 (3.4%)	0.46
Hospital mortality (N (%))	34 (8.5%)	23 (11.9%)	11 (5.3%)	0.04
Mean MBL (ng/ml) (mean ±SD)				
Pre-operative (N=400)	837 ± 796	820 ± 802	854 ± 792	0.68
Post-operative (N=330)	453 ± 350	449 ± 356	457 ± 346	0.82
Median MBL (range)				
Pre-operative	571 (15-3709)	534 (21-3619)		
Postoperative	339 (35-1934)	329 (35-1934)		
Pre-operative MBL <80 (ng/ml) (N (%))	38 (9.5%)	21 (10.8%)	17 (8.2%)	0.40
Pre-operative MBL <400 (ng/ml) (N (%))	164 (41.0%)	81 (41.7%)	83 (40.3%)	0.84

* Patients were randomized to receive either buffy-coat-depleted red blood cells or leukocyte depleted red blood cells, when needed. † Units per patients transfused during surgery. Only patients who received red blood cells and FFP transfusions are included for calculation of mean and SD.

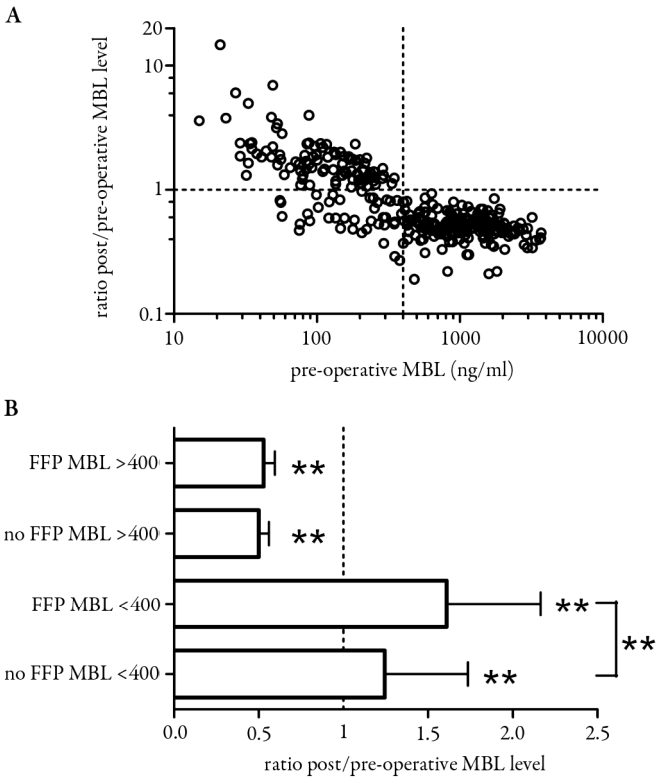

Figure 1 | The effect of plasma transfusion on levels of serum MBL in patients who undergo cardiac surgery. **A.** Ratios of post/pre-surgery MBL levels were plotted against pre-operative serum MBL levels in all patients (N=330). The dashed line indicates a serum MBL level of 400 ng/ml. **B.** Mean ratios post/pre-surgery MBL levels ± SD were calculated for patients above and below 400 ng/ml who did or did not receive FFP during the operation, as indicated. ** indicates statistical significance. Ratios in all groups were significantly different from 1 ($P<0.0001$; Wilcoxon signed rank test). The difference between patients with serum MBL level below 400 ng/ml who did or did not receive plasma was tested with the Mann Whitney test ($P=0.0039$).

Ratios of MBL levels were not different between recipients of leukoreduced or buffy-coat depleted red blood cells. In both groups the MBL level decreased or increased with the same ratios determined by the preoperative MBL level (not shown). In contrast, FFP transfusions were associated with an additional increase of post-operative serum MBL in patients with a pre-operative MBL level below 400 ng/ml (median post/pre MBL ratio 1.25 versus 1.61,

p=0.0039; Figure1B), whereas FFP transfusion did not show an effect in patients with MBL levels above 400 ng/ml.

Following surgery, all patients displayed a dilution effect, as shown by a decreased serum total protein concentration (average ratio total protein post/pre-surgery =0.67 ± 0.08; range 0.46-0.86). However, this dilution factor was not correlated to post/pre-surgery MBL ratios, also not when patients with pre-MBL levels above 400 ng/ml were analysed only ($R<0.1$).

In a logistic regression model we further evaluated the contribution of various per-operative factors on the post/pre-surgery MBL ratio. The type of surgery (p=0.23), duration of cardiopulmonary bypass (p=0.19), duration of aortic crossclamping (p=0.25), number of red blood cell transfusions (p=0.19), randomization arm (p=0.74) and the decrease in total protein concentrations (p=0.43) did not show a significant effect on the post/preoperative MBL ratio.

MBL Serum Levels and Complications of Cardiac Surgery

Following cardiac surgery 113 of 400 patients developed infections (28.3%), 89 patients developed MODS (22.3%), 17 patients had sepsis (4.3%) and 34 patients died (8.5%; Table 1). Infections and mortality were significantly higher in patients receiving leukocyte-containing transfusions than in patients randomized for leuko-reduced red blood cells (p=0.02 and p=0.04, respectively). Patients who developed MODS had a significantly longer duration of cardiopulmonary bypass (median 148 versus 124 minutes; p=0.0002) and aortic crossclamping (median 98 versus 90 minutes; p=0.008).

Since data as presented above show that the surgical procedure and plasma transfusions have an effect on serum MBL levels, the relation between serum MBL and complications of cardiac surgery were further evaluated based on pre-operative MBL levels in patients who did not receive plasma. Using an MBL level of 400 ng/ml as a cut-off value, no significant association could be identified between higher or lower pre-operative MBL levels and infections, sepsis, MODS or mortality in the 224 patients who did not receive FFP transfusions (Table 2). However, patients with an MBL level below 80 ng/ml, indicating overt MBL deficiency, did not develop MODS (Figure 2 and Table 2; p=0.016). The incidence of infections, sepsis and mortality was not significantly different in patients with pre-operative MBL serum levels below or above 80 ng/ml.

In total 176 patients received FFP transfusions. In patients with pre-operative MBL levels below 80 ng/ml, the FFP transfusions increased the risk for development of MODS until the same degree as for patients with preoperative MBL concentrations above 80 ng/ml

(Table 3; p=0.048). In patients with pre-operative serum MBL levels above 80 ng/ml or above 400 ng/ml, FFP transfusion did not show any effect on the development of MODS.

Table 2 | Complications in Relation to Pre-operative MBL Levels in Patients who did not Receive Plasma

MBL (ng/ml)*	All	≤80	>80	p-value§	≤400	>400	p-value§
N†	224	18	206		93	131	
Infection (%)‡	25.0	22.2	25.2	1.0	28.0	22.9	0.43
Sepsis (%)‡	3.6	0	3.9	1.0	5.4	2.3	0.28
MODS (%)‡	21.0	0	22.8	0.016	19.4	22.1	0.74
Mortality (%)†	6.3	0	6.8	0.61	7.5	5.3	0.58

*MBL level before surgery; †Number of patients in each category; ‡Percentage of patients who developed this complication; §P value (Fisher exact test; comparison between MBL-low and MBL-high patients); MODS = multiple-organ-dysfunction-syndrome

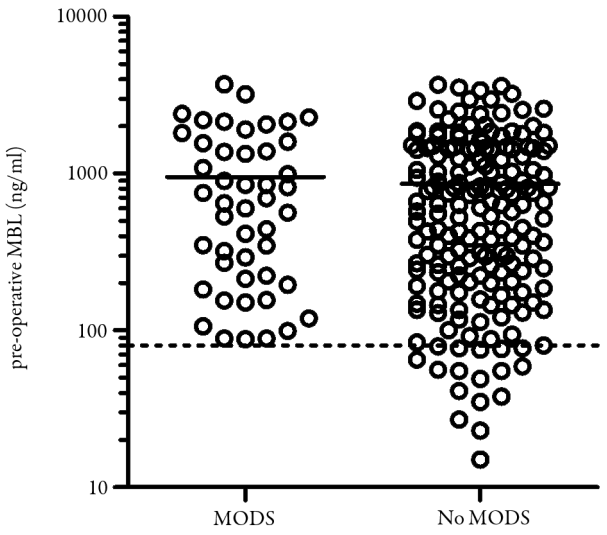

Figure 2 | Low serum MBL levels protect against MODS in patients who did not receive plasma during operation. Pre-operative serum MBL levels are shown in patients who did or did not develop MODS. Only patients who did not receive FFP were plotted.

Table 3 | Plasma Transfusion is a Risk Factor for MODS in Patients with Low Pre-operative MBL Levels

MBL level (ng/ml)*	No plasma (N)†	% MODS‡	Plasma (N)†	% MODS‡	p-value§
All	224	21	176	24	0.55
≤80	18	0	20	25.0	0.048
>80	206	22.8	156	23.7	0.90
≤400	93	19.4	7 1	25.3	0.45
>400	131	22.1	105	22.9	1.00

* MBL level before surgery; †Number of patients who did or did not receive plasma during the procedure; ‡Percentage of patients who developed multi-organ dysfunction syndrome; §P value (Fisher exact test; comparison between patients who did or did not receive plasma)

CONCLUSIONS

In a randomised study in cardiac surgery patients we observed a lower incidence of postoperative infections and mortality associated with MODS after transfusion of filtered leukoreduced red blood cell transfusions compared with standard buffy-coat depleted red blood cells [17]. Pre-operative and postoperative blood samples had been taken for investigation of mechanisms, which could explain differences between both randomization arms, should these occur. Because in previous studies low MBL levels have been associated with infections and high levels with ischemia/reperfusion injury [6-12], both important complications of cardiac surgery, we hypothesized that leukocytes in red blood cell units may play a role by activation of the innate immune system via the lectin pathway.

The results of this study show that absence or presence of allogeneic leukocytes in erythrocyte products had no effect on MBL concentrations after surgery. In both randomisation arms, patients with higher (>400 ng/ml) pre-operative MBL levels show post-surgical decrease by almost 50%. Although patients with preoperative MBL values below 400 ng/ml increased their postoperative MBL levels, especially if FFP had been administered during the procedure. If the preoperative MBL value was above 80 ng/ml, preoperative and postoperative MBL values were not associated with postoperative infections, MODS or mortality. However, none of the 18 patients with MBL deficiency below 80 ng/ml who did not receive per-operative FFP developed MODS. In contrast, the incidence of MODS in 20 MBL-deficient patients who received FFP was comparable with the incidence in patients with higher preoperative MBL concentrations.

This is, to the best of our knowledge, the first study that reports MBL serum levels after cardiac surgery. In a previous study in a small number of 23 patients undergoing abdominal aneurysm aorta surgery, a postoperative decrease of approximately 40% of MBL was observed, whereas in a control group, undergoing biliary and pancreatic surgery, there was no effect on MBL levels after the operation [23]. In patients undergoing abdominal surgery for respectable esophagus carcinoma a clear increase of serum MBL was observed, with a slow kinetics starting on day 5 postoperatively [24]. In gastric surgery patients, no effect on post-surgery MBL levels was observed as assessed on day 3 [25]. These variable results in patient cohorts undergoing different surgical interventions, in studies with relatively small cohort sizes, indicate interplay of different factors modifying the MBL serum level. In our large patient population undergoing cardiac surgery, we observed an increase as well as a decrease of MBL concentrations. The change in MBL concentration after surgery was significantly associated with the patient's MBL status prior to surgery. Because the MBL level in the population shows a wide range, which is genetically based, this relationship is easily missed when small groups of patients are evaluated. The measured decrease of 50% in the MBL concentration was somewhat overestimated because of postoperative hemodilution, reflected by a decrease of the total protein concentration. In reverse, due to this hemodilution, the increase of MBL after cardiac surgery is somewhat underestimated. When calculation of MBL post/pre ratios was corrected for this hemodilution effect, we confirmed a median 24% decrease of serum MBL levels ($P<0.0001$, not shown) in patients with pre-operative MBL levels above 400 ng/ml, suggesting an independent cause for the decrease of serum MBL.

The reduction in serum MBL in patients with higher preoperative MBL levels is most likely the result of consumption by activation of the lectin pathway during surgery, presumably mainly by interaction of MBL with ischemic and injured tissue. Accordingly, MBL consumption was previously observed in patients undergoing aorta aneurism operation [23], a procedure also associated with extended ischemia, but not in patients undergoing other surgical interventions [23-25]. The finding that this decrease was not compensated in our cardiac surgery patients, neither by endogenous production nor by FFP transfusions, suggests a stronger MBL consumption in these patients than in patients with lower preoperative MBL levels. In patients with preoperative MBL concentrations below 400 ng/ml we do not know whether (compensated) per-operative MBL consumption may occur, but after surgery endogenous production is increased and the level further increases after FFP transfusions. The latter observation, i.e. MBL reconstitution of MBL-deficient patients upon FFP transfusion, has also been reported for patients undergoing aortic aneurysm repair [3]. It is well conceivable that MBL consumption is more efficient in patients with a high MBL

level before transplantation, since levels of MBL above 400 ng/ml are strongly associated with the MBL wildtype genotype and normal lectin pathway function, whereas MBL from low-MBL patients may show impaired interaction with ligand [26]. Further studies with sampling during the various phases in cardiac surgery are needed revealing the kinetics of MBL during surgery to explain the mechanisms of this dichotic MBL behavior.

In the present study no association was observed between pre- or post-surgery MBL levels and postoperative infections after cardiac surgery, in contrast to studies performed in immunocompromised patients showing an association of MBL deficiency with increased risk of infections [6-9]. However, patients with an obvious MBL deficiency before surgery were significantly protected against the development of MODS. Only in these patients, FFP transfusions, resulting into (partial) reconstitution of the MBL status, reversed this protection against MODS. This is in agreement with observations in experimental renal and myocardial ischemia/reperfusion injury and septicaemia in rats and mice, which suggest that low MBL ameliorates ischemia/reperfusion injury and sepsis-induced organ damage [12,13,27,28]. Lower MBL levels have also been associated with less graft loss in kidney transplantation in humans [22]. In the setting of aortic aneurism repair, one patient has been presented who was MBL deficient and who did not respond to the surgical procedure with production of soluble complement activation products and cytokines, in contrast to all MBL-sufficient patients who developed an evident inflammatory reaction [3]. MBL may mediate an inflammatory response via activation of the complement cascade and probably also by direct interaction with and activation of leukocytes [19]. However we observed a beneficial effects of transfusion with leukoreduced red blood cells in cardiac surgery [17], no effects of allogeneic leukocytes could be observed in this study in postoperative MBL levels. Taken together, results now presented in our study support a role for MBL in the activation of the complement system and induction of a systemic inflammatory response upon ischemia and reperfusion injury, which may evolve into multiple organ dysfunction. MBL deficiency have probably a protective role in development of MODS, which disappears with transfusions of FFP and is not directly related with allogeneic leukocytes.

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