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ORIGINAL ARTICLE

Is blood eosinophilia an effective predictor of acute rejection in living donor liver transplantation?*

Yoji Kishi, Yasuhiko Sugawara, Sumihito Tamura, Junichi Kaneko, Nobuhisa Akamatsu, Junichi Togashi and Masatoshi Makuuchi

Artificial Organ and Transplantation Division, Department of Surgery, Graduate School of Medicine, University of Tokyo, Tokyo, Japan

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Correspondence
Yasuhiko Sugawara MD, Artificial Organ and Transplantation Division, Department of Surgery, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. Tel.: 81 3 3815 5411; fax: 81 3 5684 3989; e-mail: yasusuga-tky@umin.ac.jp

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Summary

The association of blood eosinophilia with acute cellular rejection (ACR) after living donor liver transplantation has not been examined yet. The subjects were the 167 recipients who underwent liver biopsy (314 times). The blood eosinophil counts in the preoperative period ($n = 167$), 3 days before ($n = 314$) and on the day of biopsy ($n = 314$) were compared among the groups stratified by severity of ACR. Among 314 biopsy specimens, the 140 biopsy specimens were diagnosed with ACR. In the 140 ACR episodes, eosinophil counts before and after therapy was compared between the episodes that responded to therapy ($n = 80$) and those not ($n = 60$). The sensitivity and specificity of preoperative eosinophilia (eosinophil counts >130 mm^3) to predict ACR was 33% and 65%, respectively. The eosinophil counts >400 mm^3 3 days before and on the day of biopsy was associated with the severity of ACR ($P < 0.0001$). The sensitivity to predict ACR was 26% and 33%, and the specificity, 94% and 93%, respectively. There was no significant difference in changes of eosinophil counts between the steroid-responders versus the nonresponders. The present results suggested the limited role of eosinophilia as a predictor of ACR after living donor liver transplantation.

Introduction

In liver transplantation, acute cellular rejection (ACR) is still a major complication that can lead to mortality. Early diagnosis is necessary for prompt treatment, which must be based on liver biopsy. Several reports indicate a relationship between blood eosinophilia and acute rejection in liver transplantation [1–4]. Infiltration of eosinophils into the graft and peripheral blood eosinophilia might relate to ACR. In most studies, eosinophilia preceded ACR by 2–4 days [1,5]. One report demonstrated a close relationship between pretransplantation peripheral blood eosinophilia and postoperative ACR [6]. All of these reports, however, were based on data from deceased donor liver transplantation. In living donor liver transplantation (LDLT), the relation between eosinophilia and ACR has not been examined.

It is controversial that whether there is a difference in the frequency of ACR rejection between LDLT and deceased donor liver transplantation [7,8]. Some authors

reported lower incidence of steroid resistant [9] or late onset ACR [10] after LDLT. This might be due in part to the length of graft cold ischemic time [7] or the HLA haplotype matching in living-related donor cases [9]. The difference in the frequency and severity of ACR between deceased donor liver transplantation and LDLT led us to examine whether blood eosinophilia can predict ACR after LDLT.

Patients and methods

Patients

Subjects were 305 consecutive patients that underwent LDLT at our hospital. Two patients complicated by chronic rejection and eight patients who underwent emergent transplantation were excluded. Of the remaining 299 patients, biopsies were performed in 167 patients consisting 131 adults [47 ± 1.0 (mean ± SE) in age] and 36 children (6.3 ± 1.0 years old). The indications for LDLT included HCV related cirrhosis ($n = 39$), hepatitis B virus related cirrhosis ($n = 14$), cirrhosis of other etiologies ($n = 7$), biliary atresia ($n = 37$), primary biliary cirrhosis ($n = 33$), primary sclerosing cholangitis ($n = 4$), autoimmune hepatitis ($n = 5$), fulminant hepatic failure ($n = 15$), metabolic diseases ($n = 7$) and others ($n = 6$).

Acute cellular rejection was diagnosed based on biopsy and graded into four classes according to the Banff scheme [11] [Grade 0 (G0): no evidence of rejection, Grade 1 (G1): mild rejection, Grade 2 (G2): moderate rejection, Grade 3 (G3): severe rejection; Fig. 1]. Postoperative immunosuppression was achieved with tacrolimus and methylprednisolone [12]. Tacrolimus was administered to control the trough level at approximately 16–18 ng/ml for the first week, and gradually tapered to 5–8 ng/ml over 6 months. Steroids were also tapered day by day from 3 mg/kg on the first postoperative day to 0.3 mg/kg on the fifteenth postoperative day. The dose was then decreased slowly to 0.06 mg/kg over 6 months. When the diagnosis of ACR was confirmed, 20 mg/kg of methylprednisolone was administered, which was then tapered by reducing the dose by half each day until the same dose as before therapy was achieved.

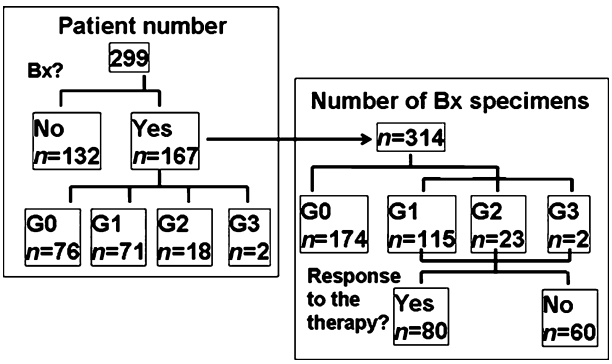

Figure 1 The numbers of the patients and liver specimens studied. Bx, liver biopsy.

Biopsy was performed when levels of all blood liver function tests, including transaminases, bilirubin, gamma-glutamyl transpeptidase and alkaline phosphatase, elevated. No protocol biopsy was performed.

Analysis

The relationship between preoperative eosinophilia and ACR stratified by grade was examined. Preoperative eosinophilia was defined as absolute eosinophil count (AEC) >130 mm^3 [6]. The relationship of eosinophilia 3 days before or on the day of biopsy and ACR grouped by grade was examined. Here, the number of eosinophils was evaluated as AEC or relative eosinophil count (REC: AEC × 100/total leukocyte count). Postoperative eosinophilia was defined as AEC more than 400/mm^3 and/or REC more than 4% [3].

Pre- or post-treatment AEC, REC, and eosinophil count changes were compared between patients that responded to the treatment and those that did not. Treatment was judged successful when transaminase and bilirubin levels improved to normal levels and did not increase again during the following month. If liver dysfunction recurred again within 1 month, followed by biopsy-proven ACR, the treatment was defined as failed.

Statistics

Data were expressed as mean ± SE. Sensitivity and specificity of eosinophilia was calculated for the prediction of ACR or improvement of ACR. AEC and REC were compared between groups using an unpaired *t*-test or one-way ANOVA. A *P*-value of <0.05 was considered statistically significant.

Results

Preoperative eosinophilia

An average of 2.2 biopsies were performed per patient. The interval between transplantation and biopsy was on 32 ± 2.0 days. The degree of ACR included G1 in 71, G2 in 18 and G3 in two patients. Other 76 patients showed only indeterminate evidence of ACR in every biopsy samples and were classified to G0. Preoperative AEC of the patients with and without postoperative ACR was 168 ± 27/mm^3 and 114 ± 16/mm^3, respectively ($P = 0.78$). There was no significant difference in REC (G0,

2.6 ± 0.34%; G1, 2.9 ± 0.52%; G2, 3.7 ± 0.98%; $P = 0.54$) or AEC (G0, 114 ± 18/mm^3; G1, 159 ± 27/mm^3; G2, 217 ± 51/mm^3; $P = 0.10$) among the G0–G2 grades of ACR (Fig. 2a). Two G3 specimens were excluded from the analysis. Preoperative eosinophilia predicted ACR with a sensitivity of 33% and a specificity of 65%, respectively (Table 1).

Eosinophilia 3 days before the biopsy

Eosinophil counts 3 days before the biopsy were available for 314 biopsy samples (Fig. 1), graded as G1 ($n = 115$) and G2 ($n = 25$). The other 174 samples showed indeterminate evidence of ACR and were classified to G0. The major findings the samples included nonspecific hepatitis with or without cholestasis ($n = 122$), congestion ($n = 15$), recurrent hepatitis C ($n = 15$) only mild lymphocyte infiltration or endothelialitis ($n = 5$), cholangitis ($n = 3$) and no abnormal findings ($n = 14$). REC and AEC 3 days before biopsy in patients complicated with ACR were 2.5 ± 0.3% and 234 ± 33/mm^3, respectively. REC and AEC in patients without ACR were 0.8 ± 0.1% and 77 ± 12/mm^3, respectively. When the biopsy samples were grouped according to the severity of ACR, there was a significant difference between the groups both in REC ($P < 0.0001$) and AEC ($P < 0.0001$; Fig. 2b). Eosinophilia (REC > 4%) 3 days before the biopsy predicted ACR with a sensitivity of 26% and a specificity of 94%, respectively (Table 1).

Eosinophilia on the day of biopsy

Eosinophil counts on the day of the biopsy were available for 314 biopsy samples. The REC and AEC on the day of the biopsy with findings of ACR were 3.3 ± 0.3% and 312 ± 35/mm^3, respectively, being significantly higher than those without ACR ($n = 174$, 0.8 ± 0.1%, $P < 0.0001$ and 78 ± 13/mm^3, $P < 0.0001$). When biopsy episodes were grouped according to the severity of ACR, there was a significant difference between groups both in REC ($P < 0.0001$) and AEC ($P < 0.0001$; Fig. 2c). Eosinophilia (REC > 4%) on the day of biopsy predicted ACR with a sensitivity of 33% and a specificity of 93%, respectively (Table 1).

Eosinophil count in response to treatment

Eosinophil count changes (count 1 week after treatment minus that just before treatment) could be calculated in the 140 biopsy episodes. Of these, 80 were responsive to steroid recycling therapy and 60 were resistant. Pretreatment REC and AEC were 2.8 ± 0.4% and 226 ± 35/mm^3 in the responding group and 4.0 ± 0% and 426 ± 65/mm^3 in the nonresponding group, respectively. Post-treatment REC and AEC were 2.3 ± 0.5% and 176 ± 32/mm^3 in the responding group and 2.6 ± 0.6% and 202 ± 55/mm^3 in the nonresponding group, respectively. There was a significant difference between groups in the pretreatment AEC ($P = 0.04$), but not in pretreatment REC ($P = 0.07$), post-treatment REC ($P = 0.49$), or post-treatment AEC ($P = 0.48$).

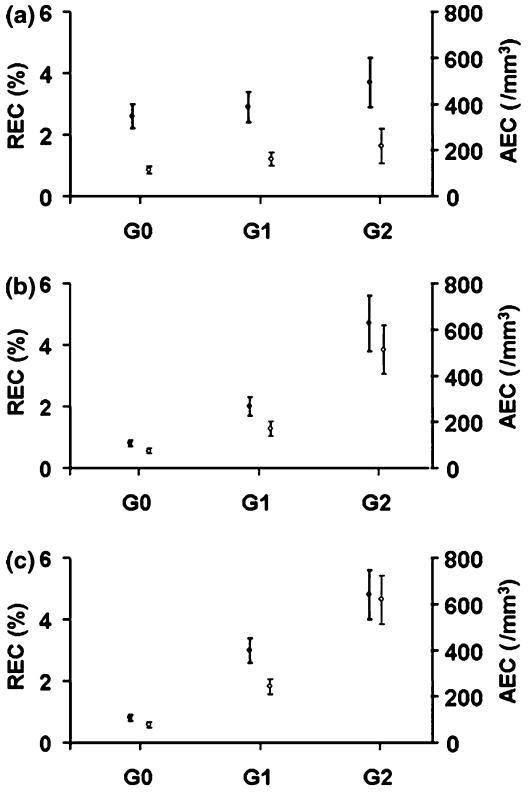

Figure 2 Relative (REC, thick bar and closed circle) and absolute eosinophil counts (AEC, thin bar and open circle) stratified by grade of rejection at preoperative (a) $n = 197$; 3 days before the biopsy (b) $n = 314$; and on the day of biopsy (c) $n = 314$. $P < 0.0001$ after comparison among the groups in the analyses of (b) and (c).

Table 1. Significance of eosinophil counts to predict acute cellular rejection.

Conditions	Events	Results	Sensitivity (%)	Specificity (%)
Pre-Tx	AEC > 130	ACR	33	65
Before Bx	REC > 4	ACR	26	94
	AEC > 400		20	95
On Bx	REC > 4	ACR	33	93
	AEC > 400		28	97
Before and after SRT	Decreased REC	Improvement of ACR	45	50
	Decreased AEC		50	43

Tx, transplantation; Bx, biopsy; SRT, steroid recycle therapy; ACR, acute cellular rejection; AEC, absolute eosinophil count; REC, relative eosinophil count.

Relative eosinophil count decreased in 36 and 30 treatments in the responding and nonresponding groups, respectively, whereas AEC decreased in 40 and 34 treatments. A decrease in REC or AEC predicted successful treatment of ACR with a sensitivity of 45% or 50% and a specificity of 50% or 43% (Table 1).

Discussion

Few studies have evaluated whether preoperative eosinophilia predicts ACR [6]. Nagral *et al.* [2] reviewed 129 biopsy cases. They demonstrated that there was no association between preoperative eosinophil count and the severity of ACR. They also demonstrated that AEC 1 or 2 days before or on the day of biopsy predicted ACR with low sensitivity (30.3–37.5%) and high specificity (83.3–91.8%). In our study also, eosinophilia both 3 days before and on the day of biopsy predicted ACR with low sensitivity and high specificity.

In contrast, Hughes *et al.* [13] emphasized that monitoring blood eosinophil count and serum eosinophil cationic protein was useful for early ACR diagnosis because they increase 2–3 days earlier than serum transaminase or alkaline phosphatase levels. Foster *et al.* [14] reported high sensitivity and specificity of blood eosinophilia in predicting ACR when they combined elevated serum transaminase or alkaline phosphatase levels. The exact reason for the discrepancy remains unclear, but might be due to a different dose of methylprednisolone for basal immunosuppression in our protocol: 3.0 mg/kg on the first postoperative day versus 1.5 mg/kg in Foster's report. The baseline eosinophil numbers might be decreased because of higher doses of steroid [15].

Our results indicated a higher pretreatment AEC in the steroid nonresponding ($426 \pm 65/mm^3$) compared with that of the responding group ($226 \pm 35/mm^3$, $P = 0.04$). They may support the phenomenon that the eosinophil count before or on the day of biopsy correlated well with the grade of ACR. A similar association was also reported by Barnes *et al.* [3] in liver transplantation and Trull *et al.* [15] in cardiac and lung transplantation. However REC was not a predictor of the response to the steroids, indicating the association between eosinophil counts before the treatment and the response to the treatment was not to be firm. Additionally the decrease in REC and AEC was not useful for predicting the effect of steroids on ACR in our series. Our results revealed a significant decrease in REC and AEC after steroid recycle therapy irrespective of the response to therapy. The finding might be explained by the hypothesis that steroids downregulate eosinophilia [16].

In summary, eosinophilia in the preoperative period, 3 days before and on the day of biopsy, predicted consequent ACR with high specificity, but low sensitivity. The present results suggested the limited role of eosinophilia as a predictor of ACR after LDLT.

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