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Predicting severity and clinical course of acute rejection after liver transplantation using blood eosinophil count

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Summary

Acute cellular rejection remains an important source of morbidity after liver transplantation, particularly if rejection is moderate or severe, as this usually is treated. Currently liver biopsies are seldom performed, so diagnostic noninvasive markers would be useful. We evaluated 690 consecutive first liver transplant patients to assess whether peripheral eosinophilia could predict moderate-severe rejection and its course. A protocol biopsy was performed 6 ± 2.5 days after transplant. A second biopsy was taken 6.1 ± 2 days after the first in 487 patients to assess histological improvement. Liver function tests, peripheral eosinophil count and changes between first and second biopsy, were evaluated using logistic regression. Histological rejection was present in 532 patients (77.1%), with moderate (30.6%) and severe rejection (3.9%). Peripheral eosinophil count was strongly associated with moderate-severe rejection (OR = 2.15; $P = 0.007$), although the area under ROC curve (AUROC) was 0.58. On second biopsy, rejection improved in 119 (24.4%) patients. The delta in eosinophil count between the first and second biopsies was the only independent predictor of histological improvement (OR = 3.12; $P = 0.001$), irrespective of whether bolus steroids were used (OR = 2.77; $P = 0.004$); AUROC was 0.72. Peripheral eosinophilia is not sufficiently predictive of moderate-severe histological rejection. However the changes in eosinophil count over time can accurately predict the histological resolution of rejection.

Introduction

Acute cellular rejection (ACR) is a frequent complication following liver transplantation [1,2] with varying prevalence according to the diagnostic methods used. Although it may be a cause of graft dysfunction, patients are usually asymptomatic and conventional liver function tests are not diagnostically specific [3], and are only late diagnostic markers [4]. Thus, liver biopsy is the current gold standard for assessment and grading of ACR. The clinical significance of ACR and its interpretation have changed over the decades. ACR had a prominent role, such that some centres implemented routine protocol biopsies to detect and treat rejection promptly [5]. Currently many centres only perform liver biopsies if there is significant derangement of liver function tests, and will treat possible rejection empirically also considering that complications can occur, even with a transjugular biopsy [6]. Avoiding biopsy is a strategy that leads to misdiagnose ACR in many patients [2,7,8]. As yet a consensus definition for 'clinical' rejection (i.e. without biopsy) does not exist. In 1992 a definition based on liver function tests was compared with histological rejection [8]: the correlation was not good and 40% of patients biopsied had histological rejection, not encompassed by the clinical definition. Therefore identifying noninvasive markers able to predict

the risk of moderate or severe ACR would lead to a more rational decision to biopsy and/or to treat empirically.

Eosinophils are typically involved in ACR, first reported as an association with ACR in kidney transplantation [9] and subsequently in lung and heart transplants [10]. In the liver graft, a portal tract eosinophilic infiltrate is a typical finding of ACR which contributes diagnostically, adding to the Banff criteria [11]. As graft eosinophils come from blood, a high peripheral eosinophil count might predict histological ACR after liver transplantation. Absolute eosinophil count (AEC) increases in blood 2–3 days earlier than liver function tests and 3–4 days before ACR is proven histologically [4], and there is a positive correlation with eosinophilia in the liver graft [12].

However the diagnostic utility of blood eosinophilia for ACR has varied. The first report [13] found AEC (threshold $>0.5 \times 10^9$/l) to have a negative predictive value (NPV) of 99% and a positive predictive value (PPV) of 44% for ACR. The only prospective study included only 20 patients [14]. Other studies [4,15,16] showed that AEC was a specific predictor of ACR with a high negative predictive power, but with inadequate sensitivity and low positive predictive power. In addition the predictive ability of a reduction in AEC following treatment of ACR is less studied [13–16].

Previous studies have important limitations. Firstly, the major endpoint was prediction of any degree of ACR. However mild rejection is usually not treated and maintenance immunosuppression is not modified [17]. Secondly the sample size (20–167 patients) was insufficient to perform multivariate analyses, and inadequate to address whether combining liver function tests and eosinophil count could predict ACR more accurately. Finally several biopsies were evaluated per patient without differentiating the interval from transplantation, thus introducing systematic errors, and making results less clinically relevant.

The aims of the present study were (i) to assess whether peripheral blood eosinophil count is predictive of histologically proven moderate and severe ACR, together with or without clinical parameters and liver function tests and (ii) to evaluate the relationship between changes in blood eosinophil count and clinical course of ACR in both treated and untreated patients.

Materials and methods

We identified 690 patients in our prospectively collected liver transplant database between October 1988 and February 2008 during which interval protocol biopsies were obtained 5–7 days after first liver transplantation to establish the presence and severity of ACR. There were another 75 patients in whom graft biopsy was not available in the first 2 weeks after transplant because of early death ($n = 20$), retransplant ($n = 13$) or other complications and were not analyzed. Routine laboratory tests including liver function profile were evaluated on the day of the biopsy. AEC (normal range $0–0.46 \times 10^9$/l) was recorded the day before and on the day of the biopsy. Relative eosinophil count (REC) was calculated with the following formula: AEC × 100/total white cell count (threshold 3.5%). AEC on the day of the second biopsy and ΔAEC between the first and second biopsy were evaluated as potential predictors of clinical course of ACR and response to treatment. The threshold chosen for ΔAEC was the null value (0×10^9/l) which meant no change in eosinophil count between the first and second biopsy.

Liver biopsies were examined to assess and grade ACR according to the Royal Free system [11] which predates the Banff schema [18]. The Royal Free ACR system applies the same histopathological diagnostic criteria (mixed mainly portal inflammation, endothelitis and bile duct damage) as the Banff schema, except that the Royal Free system evaluation of eosinophils in the inflammatory infiltrate is included as a separate additional axis of assessment. The immunosuppression protocol started immediately after transplant with intravenous methylprednisolone (1 mg/kg/day until July 1997 or 16 mg daily thereafter, followed in both cases by 20 mg oral prednisolone daily once gut function was restored) and azathioprine (1 mg/kg/day) in addition to either tacrolimus (initially 0.1 mg/kg/day in two divided doses) or cyclosporin (initially 10 mg/kg/day in two divided doses). Calcineurin inhibitor doses were run on the lower side of the therapeutic range and adjusted according to serum levels, the presence of infection or toxicity. Between October 1996 and January 1997 a clinical trial was conducted [19] during which patients were randomized to receive monotherapy with tacrolimus versus cyclosporine. From May 1997 to April 1999 patients were randomized to triple therapy based on either tacrolimus or cyclosporine [20]. Thereafter a cohort of patients received tacrolimus monotherapy [21]. At all times standard treatment for ACR consisted of 1 g of intravenous methylprednisolone given on three consecutive days. However 28 patients (9.5%) received two boluses and 20 patients (6.8%) received just one bolus because of individual clinical circumstances.

In 487 patients, a second biopsy was obtained after 6.1 ± 2 days from the first one to assess the course and response to treatment. We evaluated the whole group for the presence of rejection, and then patients who had or who had not received boluses of steroids in relation to the change in eosinophil count. According to the grade of rejection in the second biopsy, patients were classified in three groups: (i) Improvement: when rejection grade improved from moderate or severe to mild or no

rejection; (ii) Deterioration: opposite of the previous; (iii) No change: when no significant histological change was found.

Statistical analysis

Statistical analysis was performed using SPSS 18.0 (SPSS Inc., Chicago, USA). Variables are displayed in frequency tables or expressed as means and standard deviations, except those with an asymmetric distribution, which are described with medians and interquartile ranges (IQR). Testing for differences between groups were performed using Chi square test for frequencies, student's *t* test or ANOVA tests for quantitative variables and Mann–Whitney's *U* test or Kruskal-Wallis for variables with an asymmetric distribution. The optimal threshold value for peripheral eosinophil count with respect to moderate/severe ACR was established by receiver operating characteristic (ROC) curves. We used multiple logistic regression to control for possible confounding factors and to evaluate the combination of eosinophil count and other routine laboratory tests, which have also been used in previous papers, (AST, ALT, AST/ALT ratio, ALP, GGT, bilirubin, albumin, urea and creatinine) in predicting moderate/severe ACR. The same method was used to identify those variables independently related with histological improvement of ACR. Every hypothesis tested was two tailed and considered statistically significant if $P < 0.05$.

Results

Descriptive evaluation

There were 690 patients of whom 425 (61.6%) were men. Major aetiologies were alcoholic liver disease (17.1%), hepatitis C (13.3%), or their combination (5.1%), hepatocarcinoma (11.4%), primary biliary cirrhosis (13%), acute liver failure (8%), primary sclerosing cholangitis (7%), hepatitis B (5.8%) and cryptogenetic cirrhosis (5.1%). A protocol liver biopsy was obtained 6 ± 2.5 days after liver transplantation. ACR was found in 532 patients (77.1%) which was mild in 294 (42.6%), moderate in 211 (30.6%) and severe in 27 (3.9%) biopsies respectively and 158 patients (22.9%) had no histological rejection. In 294 cases (42.6%) boluses of corticosteroids were given after the first biopsy, with 90 (30.6% treated) patients having mild rejection, 178 (84.4% treated) moderate rejection and 26 (96.3% treated) severe rejection.

A second biopsy was taken 6.5 ± 2 days after the first one in 487 patients (70.6%). The group who had a second biopsy had more severe ACR on the first biopsy (moderate-severe rate 42.3% vs. 15.8%; $P < 0.001$) and subsequently received more corticosteroid boluses and azathioprine (Table 1). In the group that had received bolus steroids, an improvement was seen in 102 (40.6%) patients, 23 (9.2%) showed deterioration and 126 (50.2%) remained unchanged. With regard to the patients who did not receive bolus of steroids initially, only 17 (7.2%) improved while 56 (23.7%) showed deterioration and 163 (69.1%) remained unchanged. Considering subgroups according to the grade of ACR on the first biopsy, of 99 patients initially classified as 'no rejection' 37 (37.4%) remained unchanged while 62 patients worsened (45.5% to mild rejection and 13.1% to moderate-severe rejection). From 182 patients with mild rejection on the first biopsy, improvement to no rejection was seen in 30 patients (16.5%) and deterioration to moderate-severe rejection occurred in 66 patients (34%). Finally from 206 patients with moderate-severe rejection at baseline, improvement was detected in 119 cases [97 (47.1%) passed to mild rejection, and 22 (10.7%) to no rejection].

Laboratory variables as predictors of moderate or severe rejection in the first biopsy

The univariate analysis showed that both AEC and REC were higher in ACR patients especially when moderate-severe ACR occurred (Fig. 1). In the ROC analysis, the area under curve was 0.58, 0.59 and 0.57 for AEC, AEC (day-1) and REC respectively. Values of sensitivity, specificity, PPV and NPV tested for several cut-off points related to moderate or severe rejection are shown in Table 2. It is noteworthy that, although sensitivity and specificity vary (occasionally exceeding 90%) depending on the cut-off point chosen, predictive values for moderate-severe rejection are relatively constant and lower than 70% for most scenarios.

The initial immunosuppression regimen used immediately after transplantation, and the indication for liver

Table 1. Baseline characteristics in patients who had a protocol first liver biopsy after transplantation (days 6 ± 2.5) who then had or did not have a second biopsy.

	Group with 2nd biopsy (n = 487)	Group without 2nd biopsy (n = 203)	P
Grade of ACR in first biopsy (moderate-severe)	206 (42.3%)	32 (15.8%)	<0.001
Tacrolimus	343 (70.4%)	155 (76.4%)	0.11
Ciclosporine	113 (23.2%)	38 (18.7%)	0.19
Azathioprine	214 (43.9%)	69 (34%)	0.015
Mycophenolate	50 (10.3%)	19 (9.4%)	0.71
Prednisone	226 (46.4%)	93 (45.8%)	0.88
Steroid boluses	251 (51.5%)	43 (21.2%)	<0.001

ACR, acute cellular rejection.

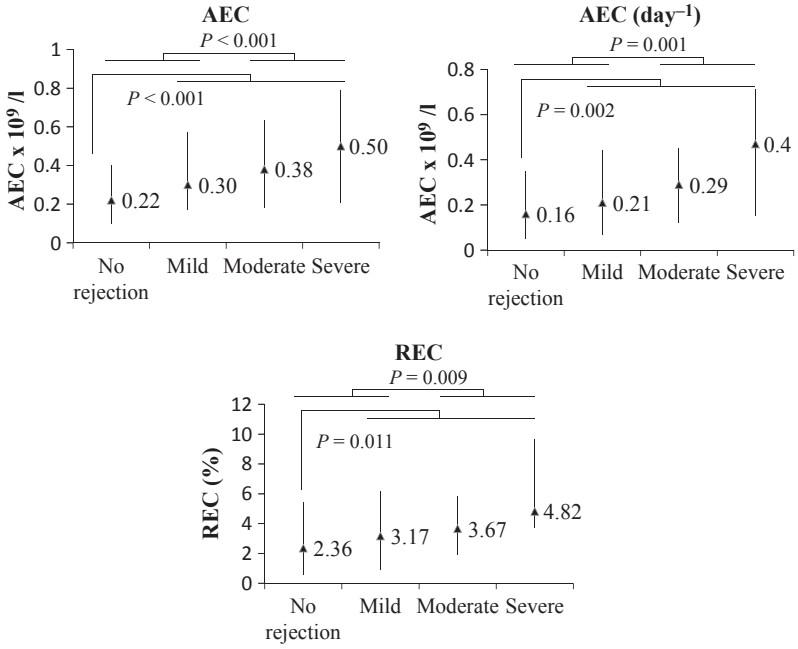

Figure 1 Absolute eosinophil counts on the day of the biopsy (AEC) and on the day before (AEC day-1) and relative eosinophil count (REC) according to histological grade of rejection in the first protocol biopsy performed 6 ± 2.5 days after liver transplantation. Medians and IQR are shown.

Table 2. Accuracy of AEC and REC to predict histological moderate or severe rejection in the first (protocol) biopsy after liver transplantation (days 6 ± 2.5).

	Cut-off point	Sensitivity	Specificity	PPV	NPV
AEC ($\times 10^9$)	1	8.2%	93.6%	44.4%	62.7%
	0.46	43.8%	73%	49.7%	68.1%
	0.2	70.6%	39.5%	41.5%	68.9%
	0.1	86.6%	21%	40%	72%
AEC (day-1)	1	2.7%	96.7%	33.3%	61.9%
($\times 10^9$)	0.46	27.3%	79.4%	44.7%	64.1%
	0.2	62.6%	52.6%	44.7%	69.7%
	0.1	78.1%	37.9%	43.5%	70.9%
REC (%)	6	25.7%	76.2%	40%	62.4%
	3.5	57.2%	55.4%	44.2%	66.7%
	2.5	70.1%	45.5%	44.3%	71.1%
	1.2	81.8%	29.4%	41.7%	72.4%

Normal range of AEC: 0–0.46 × 10^9/l where 0.46 × 10^9/l is the upper limit of the normal range.
AEC, absolute eosinophil count; NPV, negative predictive value; PPV, positive predictive value; REC, relative eosinophil count.

transplant did not influence either AEC or grade of rejection (data not shown). Nevertheless those patients with primary sclerosing cholangitis showed higher levels of AEC (0.6 × 10^9/l; IQR 0.14–1.3) at first biopsy than other indications (0.3 × 10^9/l; IQR 0.16–0.52) ($P = 0.01$).

Patients with moderate to severe rejection were also characterized by higher bilirubin and cholestasis parameters with lower AST, AST/ALT ratio, albumin, urea and creatinine (Table 3) than those with mild or no ACR. Serum bilirubin, GGT, albumin, urea and AEC on the day of biopsy were independently related with the degree of rejection in the multivariate analysis (Table 3). The combination of these serum parameters in the logistic regression analysis had 73% sensitivity and 52.9% specificity which was only a marginal improvement compared with AEC alone (global precision improved from 0.62 to 0.65). ALP and creatinine were tested within the model instead of GGT and urea respectively but they did not reach statistical significance. It is noteworthy that the ALT value, which is widely used as a marker of rejection in clinical practice, was not related to the presence or grading of ACR (Fig. 2). The ALP was related to rejection but because of the wide overlap it cannot be used as a marker of rejection nor its severity (Fig. 2). The immunosuppression regimen and the indication for liver transplant (primary sclerosing cholangitis) were both included and then excluded as possible confounding factors for the association between AEC and grade of rejection (Table 3).

Peripheral eosinophil count as a surveillance tool for rejection

In the second biopsy, 89 cases (18.3%) showed no rejection, 232 (47.6%) had mild rejection, 135 (27.7%) moderate rejection and 31 (6.4%) severe rejection. Compared with the first biopsy, there was an improvement for 119 patients (24.4%) and a deterioration for 79 (16.2%) while 289 (59.3%) remained unchanged. The AEC on the day of the second biopsy and the change in AEC between the first and the second biopsy were closely related with the

Table 3. Relationship between laboratory variables and histological grade of rejection in the first protocol liver biopsy after liver transplantation (days 6 ± 2.5). Univariate analysis and multiple logistic regression (n = 690 patients).

	Univariate analysis Histological rejection			Multivariate analysis Moderate-severe rejection		
	None-mild	Moderate-severe	*P*	OR	95% CI	*P*
AEC (×10^9/l)	0.28 (IQR 0.13–0.50)	0.40 (IQR 0.18–0.64)	<0.001	2.15	1.2–3.8	0.007
Bilirubin (μmol/l)	92 ± 82	97.5 ± 76.2	0.39	1.003	1.001–1.006	0.043
AST (IU/l)	88 (IQR 50–206)	74 (IQR 48–129)	0.01			
ALT (IU/l)	279 (IQR 136–565)	250 (IQR 132–531)	0.65			
AST/ALT	0.53 (IQR 0.32–0.80)	0.43 (IQR 0.26–0.68)	<0.001			
ALP (IU/l)	106 (IQR 70–161)	133 (IQR 87–213)	<0.001			
GGT(IU/l)	189 (IQR 105–328)	284 (IQR 167–412)	<0.001	1.002	1.001–1.003	0.003
Albumin (g/l)	30 ± 8.2	27.4 ± 7.7	<0.001	0.96	0.93–0.99	0.019
Urea (mg/dl)	13.5 ± 8.8	10.6 ± 7.4	<0.001	0.96	0.93–0.99	0.041
Creatinine (μmol/l)	133 ± 73	120.3 ± 72.2	0.034			
Confounding factors controlled for						
Aetiology (Primary sclerosing cholangitis)				1.41	0.69–2.86	0.33
Immunosuppression (cyclosporine versus tacrolimus)				1.14	0.66–1.99	0.62
Immunosuppression (maintenance prednisone)				0.94	0.65–1.35	0.74
Immunosuppression (maintenance azathioprine)				0.83	0.57–1.22	0.35

Multiple logistic regression data: R^2 = 0.12, partial F = 16.96, Freedom Degrees = 6, P = 0.18.
AEC, absolute eosinophil count; IQR, interquartile ranges.

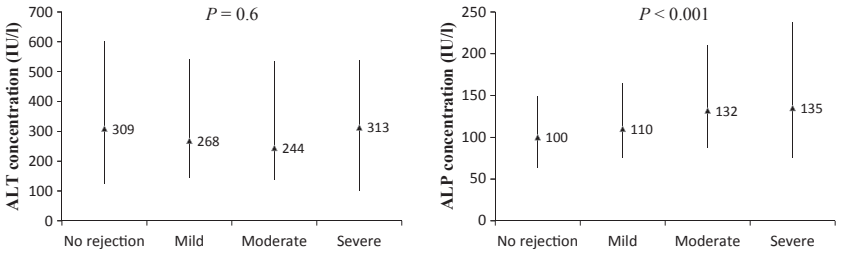

Figure 2 ALT and ALP concentration and histological level of rejection in the first protocol liver biopsy performed 6 ± 2.5 days after liver transplantation.

histological course of ACR (see Fig. 3). A decrease in AEC was associated with an improvement of the histological grade of rejection. In the ROC curve the AUC for ΔAEC was 0.72 (95% CI 0.66–0.78) while for AEC on the day of the second biopsy it was 0.34 (95% CI 0.27–0.40). The best threshold for ΔAEC was no increase i.e. 0×10^9/l (Sensitivity = 75%; Specificity = 64%). In the subgroup of patients with moderate-severe ACR on the first biopsy, AEC decreased in those patients who achieved histological improvement (ΔAEC = 0.19×10^9/l; IQR 0.007–0.46) while a trend to rise in AEC was seen in patients who remained unchanged (ΔAEC = -0.06×10^9/l; IQR −0.23–0.27), with statistically significant differences between them ($P < 0.001$). With regard to liver function tests, there was a trend to a rise in parameters of cholestasis (GGT and ALP) between the first and the second biopsy,

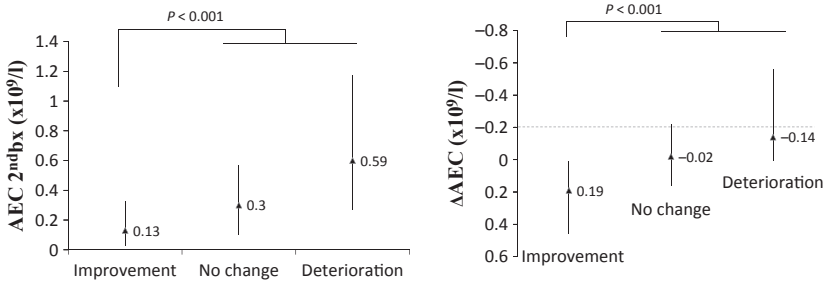

Figure 3 Delta of absolute eosinophil count (ΔAEC) between the first protocol biopsy and the second biopsy and absolute eosinophil count on the day of the second biopsy (AEC 2ndbx) after liver transplantation and their relationship with histological change of rejection in the whole cohort (n = 690). Medians and IQR are shown.

which was significantly greater for ALP in those cases without improvement in the second biopsy (Table 4). Nevertheless in the multiple logistic regression, the only independent predictors of good histological course were ΔAEC and treatment with boluses of steroids (Table 4).

In the present cohort, 108 patients at the first biopsy had ALT levels lower than 100 IU, and among these 39 patients (36.1%) showed moderate or severe ACR. In this subgroup of patients, the ΔAEC was more accurate in predicting clinical course of ACR. In the ROC curve, the area under the curve was 0.81 and, with a threshold of no increase i.e. 0×10^9/l, the sensitivity was 82% and specificity was 69%.

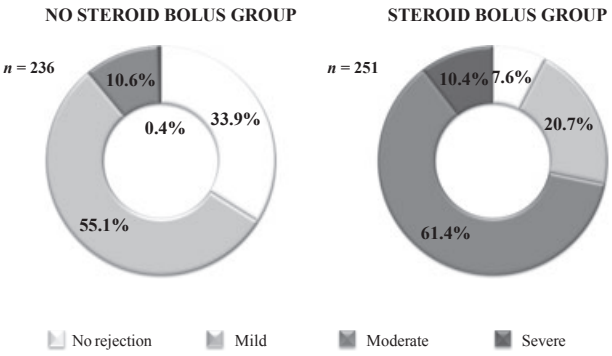

Figure 4 Grade of acute cellular rejection in the first protocol biopsy after liver transplantation depending on whether boluses of steroids were subsequently given. The proportion of moderate-severe rejection was higher at baseline in steroid group ($P < 0.001$).

Blood eosinophils and histological improvement of ACR. Evaluation in the group who had received boluses of corticosteroids and the group who had not

AEC on the day of the first biopsy was comparable between patients who had a second biopsy and patients without a second biopsy (0.33×10^9 IQR 0.17–0.58 vs. 0.30×10^9 IQR 0.14–0.5; $P = 0.44$). Among the patients with a second biopsy, the subgroup who received boluses with steroids achieved improvement in the biopsy grade of ACR (102/251; 40.6%), more frequently than the subgroup who did not receive bolus steroids (17/236; 7.2%) ($P < 0.001$). This may be explained in part because of differences in the grade of ACR in the first biopsy (Fig. 4).

In the subgroup of 251 patients who were given corticosteroids boluses, AEC on the day of the second biopsy and ΔAEC between the first and the second biopsy were related to the likelihood of treatment response ($P = 0.001$ and $P < 0.001$ respectively) (Fig. 5). In the ROC curve, the area under curve for ΔAEC was higher (0.66) than for AEC on the day of the second biopsy (0.35). An AEC rising higher than 0.3×10^9/l between the first and second biopsy was associated with a high likelihood of no response to bolus steroids (78.3%), with a sensitivity for this threshold of 94.8%. Among patients with moderate-severe ACR on the first biopsy who received bolus steroids, the AEC decrease was greater in those cases with improvement ($\Delta AEC = 0.19 \times 10^9$/l; IQR 0–0.48) compared with those who did not ($\Delta AEC = -0.04 \times 10^9$/l; IQR −0.23–0.38) ($P = 0.001$). Improvement in grade of ACR was also more frequent in patients who received 3 boluses of steroids (97/212; 45.8%) than in those who received a lower dose (5/39; 12.8%) ($P < 0.001$); nevertheless the number of boluses did not influence AEC on

Table 4. Variables related to histological improvement of acute cellular rejection in the second biopsy performed to assess course of rejection (6.1 ± 2 days after first biopsy). Univariate analysis and multiple logistic regression ($n = 487$ patients).

	Univariate analysis			Multivariate analysis (Improvement)		
	Improvement	No change/deterioration	*P*	OR	95% CI	*P*
Bolus steroids (%)	102/119 (85.7%)	149/368 (40.5%)	<0.001	10.09	4.7–21.4	<0.001
ΔAEC (×10^9/l)	0.25 (IQR 0.05–0.5)	−0.04 [IQR (−0.27)–0.19]	<0.001	3.12	1.5–6.2	0.001
ΔBilirubin (μmol/l)	10.5 [IQR (−25)–38]	5 [IQR (−40)–28]	0.16			
ΔAST (IU/l)	15.5 [IQR (−26)–54]	18 [IQR (−28)–73]	0.41			
ΔALT (IU/l)	98.5 (IQR 11–382)	143 (IQR 18–362)	0.97			
ΔALP (IU/l)	−80 [IQR (−241)–(−7.5)]	−130 [IQR (−269)–(−17)]	0.036			
ΔGGT (IU/l)	−38 [IQR (−318)–74]	−138 [IQR (−342)–0]	0.095			
Confounding factors controlled for						
Aetiology (primary sclerosing cholangitis)				0.86	0.30	0.78
Immunosuppression (cyclosporine versus tacrolimus)				1.63	0.63	0.30
Immunosuppression (maintenance prednisone)				1.37	0.77	0.28
Immunosuppression (maintenance azathioprine)				0.70	0.38	0.24

Multiple logistic regression data: $R^2 = 0.33$, partial F = 94.9, Freedom Degrees = 9, $P = 0.08$.
AEC, absolute eosinophil count; IQR, interquartile ranges.

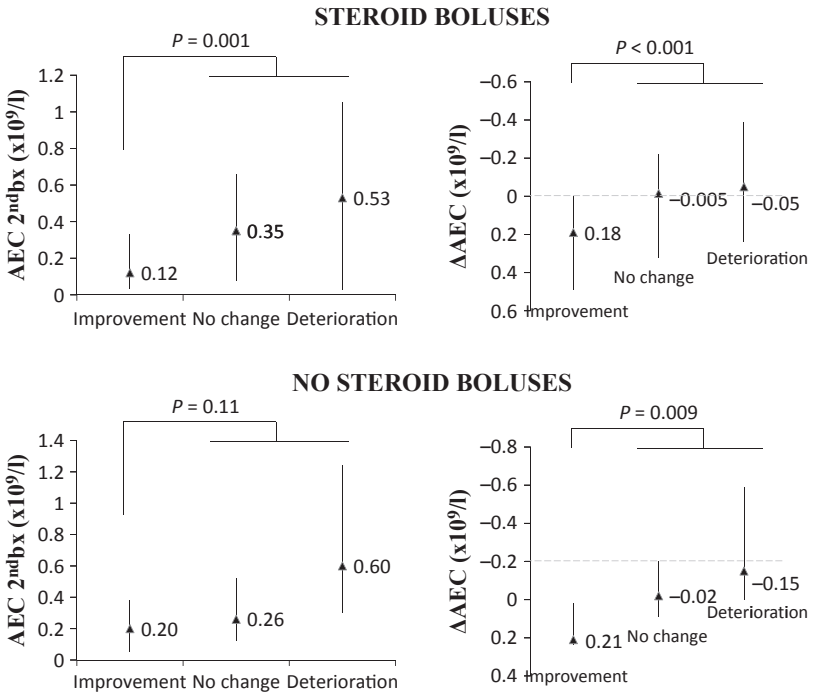

Figure 5 Delta of absolute eosinophil count (ΔAEC) between the first protocol biopsy and second biopsy after liver transplantation and absolute eosinophil count on the day of the second biopsy (AEC 2ndbx) related to histological course of rejection whether boluses of steroids were used (n = 251) or not (n = 236). Medians and IQR are shown.

the day of the second biopsy (P = 0.63) and neither ΔAEC between the first and second biopsies (P = 0.21). The delta value of liver function tests (bilirubin, AST, ALT, AST/ALT ratio, ALP and GGT) did not correlate with the likelihood of treatment response (P = 0.22, P = 0.89, P = 0.31, P = 0.34 and P = 0.11 respectively). The multivariate analysis (which included ΔAEC, steroid dose, immunosuppression protocol and delta of liver function tests) identified ΔAEC as the only independent variable able to predict the histological response of ACR after treatment with boluses of steroids (OR = 2.77; 95%CI = 1.4–5.5; P = 0.004) although the association was marginally less than in the group overall (OR decreased from 3.12 to 2.77).

With regard to the subgroup of 236 patients who had not received steroids, the ΔAEC, but not the AEC, on the day of the second biopsy was related to the likelihood of ACR improvement (P = 0.009 and P = 0.11 respectively) (Fig. 5). Delta values of bilirubin, AST, ALT, and GGT between the first and the second biopsies were not related to the likelihood of ACR improvement in the second biopsy (P = 0.19, P = 0.91, P = 0.23 and P = 0.08 respectively). As described previously in the whole cohort, a larger difference in ΔALP was seen in the group who did not improve (106 vs. 23 IU/l; P = 0.015). Among patients in this group with moderate-severe ACR on the first biopsy, the AEC decreased in those patients who improved (ΔAEC = 0.21×10^9/l; IQR 0.02–0.24) while it increased in those who did not (ΔAEC = -0.11×10^9/l; IQR −0.37–0) (P = 0.008). Multivariate analysis could not be performed in this subgroup because of the small number of patients that improved the grade of ACR in the second biopsy (n = 17).

Discussion

This study evaluated the clinical usefulness and accuracy of peripheral blood eosinophil count for predicting moderate and severe ACR, as well as its clinical course and response to treatment with steroids in a large cohort of liver transplant patients. Since there is no consensus on the definition of ACR based on liver function tests, which are also poorly correlated with its grade, histological ACR was used as the gold standard to evaluate the accuracy of eosinophils.

Absolute eosinophil count measured on the day before or on the day of the biopsy was higher in patients with ACR, which is in agreement with previous reports [12–16], and was related to the histological grade of rejection confirming our previous observations in 275 liver biopsies from 101 patients [15]; this correlation is more likely as eosinophils in the histological infiltrate are an independent marker of ACR [11]. However using the upper limit of normal range (0.46×10^9/l) the PPV and NPV were only 66.5% and 51.9% respectively. Even when thresholds with a higher sensitivity and specificity were tested, the predictive values did not exceed 70% in any situation, so this parameter is not itself sufficiently predictive to guide

therapeutic decisions. Our results do not confirm the high NPV in 51 patients [4], and in 60 patients [13], nor high specificity in 167 patients [16] of a raised AEC, found in previous studies.

Other studies have shown that several routine biochemical laboratory tests are related to the presence of ACR [22–24]. When we assessed these parameters in association with the AEC in predicting moderate-severe ACR, the AEC was the strongest related parameter (OR = 2.15) and a higher bilirubin and GGT with a lower albumin and urea, independently predicted moderate-severe ACR. However, the combination of these tests only marginally improved the global precision of AEC (0.62 to 0.65). Thus the benefit of combining AEC with routine biochemical laboratory tests was limited.

Previous studies [13,15] did not evaluate relationships between peripheral eosinophil counts and histological changes. A higher AEC or REC before treatment predicted biochemical response to bolus corticosteroids with a sensitivity and specificity ranging from 45% to 50% in one study of 140 paired biopsies [16]. In our study, only the ΔAEC between the first and second biopsy and treatment with bolus steroids were independent predictive factors for histological improvement in the multivariate analysis. In the group overall the sensitivity and specificity of ΔAEC for predicting improvement was 75% and 64% respectively (threshold 0×10^9 which meant no difference in ΔAEC between the first and second biopsies). These results were consistent among the group of patients with moderate-severe rejection on the first biopsy. Importantly when transaminases were low (ALT < 100 IU/l), the accuracy of ΔAEC was improved (sensitivity 82% and specificity 69%). Thus ΔAEC is a simple noninvasive parameter that helps to assess the course of ACR, whereas differences in standard liver function tests were not helpful.

In patients treated with boluses of corticosteroids, it is reasonable to expect a lower predictive power for ΔAEC because steroids lower blood eosinophil counts. However this was not a major issue: OR for ΔAEC in the multivariate analysis decreased from 3.12 in the group overall to 2.77 in the bolus corticosteroid group. The ΔAEC was not affected by type of maintenance immunosuppression including steroids which were used at much lower doses compared with bolus doses.

In conclusion, we found that, although the AEC is independently related to moderate and severe ACR, its predictive power is not accurate enough to make therapeutic decisions using this parameter alone. The combination of AEC with other ACR independently associated variables (i.e. bilirubin, GGT, albumin and urea) only had a marginal benefit in terms of diagnostic precision. However, the changes in AEC used as a monitoring test can provide valuable information about the histological course and the likelihood of response to boluses of steroids for treatment of moderate-severe rejection. This finding is particularly useful if biopsies are not routinely performed to diagnose acute cellular rejection and assess response to therapy. Nevertheless it would be useful to have a consensus definition for clinical rejection, which might include peripheral eosinophil count and would need a correlation with protocol biopsies.

Authorship

MRP: analyzed data and wrote manuscript. GG and ET: collected and analyzed data. NR: Collected data. TVL and APD: analyzed tissue samples. DT, JO and DP: contributed important concepts. AKB: designed research and wrote manuscript.

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