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Evaluation of Neutrophil to Lymphocyte Ratio and Platelets Count after Liver Transplantation

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Abstract

Background: The neutrophil-to-lymphocyte ratio (NLR) is the index that indicates systemic inflammatory changes after transplantation. Aim: The present study aimed to determine the role of NLR and PLT count in early allograft dysfunction (EAD) development after liver transplantation (LT). Patients and Methods: One hundred twenty patients with living donor liver transplantation in Gastrointestinal Surgery Center Mansoura University were determined throughout the present study. Patients divided into two main groups: Eighty-two non-early allograft dysfunction group (NEAD) and thirty-eight early allograft dysfunction group (EAD). Blood levels of neutrophil, lymphocyte and platelets were estimated at preoperative day and postoperative days from day one to day seven using complete blood count (CBC) data. The NLR was calculated as the ratio of neutrophil count to lymphocyte count. Results: The NLR ratio showed significant difference between the two groups ($p<0.05$) on post- operative days from day 2 to day 7. The area under curves (AUC) ranged from 0.630 to 0.676 with sensitivity from 68% - 81%, specificity from 52% - 59%. However, PLT in EAD group showed significant difference ($p<0.05$) on preoperative day and highly significance on postoperative days from day1 to day 7 compared to NEAD group and exhibited area under curves (AUC) ranged from 0.618 to 0.682 with sensitivity from 65% - 81%, specificity from 52% - 59%. Conclusion: Determination of NLR ratio and PLT count were associated with EAD development from post-operative day 1 to post-operative7 after liver transplantation and maybe as useful biomarkers for EAD prediction.

Keywords: Early allograft dysfunction – Living donor liver transplantation – Platelets– neutrophil-to-lymphocyte ratio

Introduction

Liver transplantation (LT) is the gold standard treatment for patients with different liver diseases such as acute liver failure, hepatocellular carcinoma **(Rajakumar et al., 2023)**.

Early allograft dysfunction (EAD) is a complication that occurs post liver transplant that associated with graft failure and risk of poor prognosis **(Masior and Grat 2022, Zhang, Han et al. 2022)**. Prognosis of EAD is an important target to decrease the mortality and morbidity in patients undergoing LT **(Liu et al. 2022)**. In the early phase of post-transplantation, the function of the new graft liver shows intense and adverse reactions through laboratory blood tests, reflecting common poor sensitivity and specificity of EAD **(Vos et al. 2014).**

The neutrophil-to-lymphocyte ratio (NLR) considered as systemic indicator of inflammation and calculated by the ratio of neutrophil count to lymphocyte count **(Niu et al., 2024)**. Although preoperative NLR is a well-established prognostic indicator for a number of cancers, it is unknown how postoperative NLR affects recipients of living donor liver transplantation (LDLT) **(Kouki et al., 2024).** NLR outperformed other indicators, indicating that preoperative NLR could be a useful indicator for forecasting graft performance after LDLT **(Kwon et al., 2019).** Through inflammatory alterations, ischemia reperfusion injury manifests as an increase in NLR. Furthermore, NLR has been shown to predict the prognosis of the recipient following liver transplantation for hepatocellular carcinoma (**Harimoto et al., 2013).** A recent study highlights the importance of systemic inflammation in trans-jugular intrahepatic portosystemic shunt (TIPS) patients by indicating that NLR is a useful prognostic factor for long-term progression **(Zhang Q et al., 2025)**.

Persistent thrombocytopenia can affect graft function since platelets are thought to play a major role in hepatocyte regeneration. Although hematological changes are very common in pre-transplant patients, thrombocytopenia is the most prevalent abnormality. Additionally, some medications used intraoperatively and surgical variables may be involved in persistent post-operative thrombocytopenia **(Pathik, 2024)**. The number of platelets in the acute phase of LT has been reported as a prognostic factor for early post-transplant survival or graft function **(Lesurtel et al., 2014; Li et al., 2015)**. In addition to their involvement in physiological hemostasis, platelets are essential for liver damage, ischemia-reperfusion injury, tissue healing, and liver regeneration. Furthermore, the patient's prognosis may impacted by problems like as infection, spontaneous bleeding, and other issues brought on by a decline in platelet count **(Ma et al., 2024)**. In order to determine their role in the prognosis of early graft malfunction, the current study aimed to assess the NLR and platelet count in patients both before and after liver transplantation.

Patients and Methods:

The Research and Ethical Committees of Al-Azhar Faculty of Medicine, Damietta, Egypt, ethically approved the present study. One hundred twenty patients under went living donor liver transplantation in Gastrointestinal Surgery Center Mansoura University were subjected to the present study. Patients divided into two groups: non-early allograft dysfunction group (NEAD, 82 patients) and early allograft dysfunction group (EAD, 38 patients). EAD defined according to the following criteria: total serum levels of bilirubin ≥ 10mg/dL or INR ≥ 1.6 on postoperative day-7; and ALT or AST level > 2000 U/L within the first 7 postoperative days. Two milliliters of peripheral blood were collected into a tube containing anticoagulant from each patient on preoperative day and postoperative days from day one to day 7. Neutrophil, lymphocyte and platelets were determined from complete blood count (CBC) data count using haematology analyser device Cell Tac MEK – 6510 -6500. Japan. Neutrophil-lymphocyte ratio (NLR) was calculated as the ratio of neutrophil count to lymphocyte count.

Statistical analysis:

Continuous data are expressed as the mean ± standard deviation or median and interquartile range (IQR) and were compared by the student *t* test, or the Mann–Whitney *U* test when appropriate. Categorical data were presented as the number and proportion and evaluated using the χ^2 test or Fisher's exact test, as appropriate. Changes in hematological levels between the preoperative samples and those taken on postoperative from day one to day seven were analyzed using the Wilcoxon signed rank test. The accuracy of the predictive markers for the development of EAD was analyzed using the area under the receiver operating characteristics curve (AUC). The

association between independent variables and EAD development were investigated using simple logistic regression analyses as appropriate. All of the tests were two sided, and a *P*-value<0.05 was considered statistically significant. Statistical analyses were conducted using SPSS version 26.0 for Windows (SPSS Inc., Chicago, IL).

Results:

Demographic study:

Males represent the higher percentage of cases than females without significant difference in two groups (p=0.159) as shown in figure 1

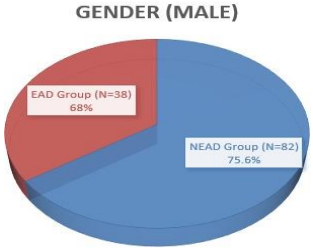

Figure 1: Represented the high percentage% of male in (EAD): Early allograft dysfunction and NEAD: Non- EAD (NEAD)

- The median age results show non-significant difference between two groups (p=0.246) as shown in figure 2

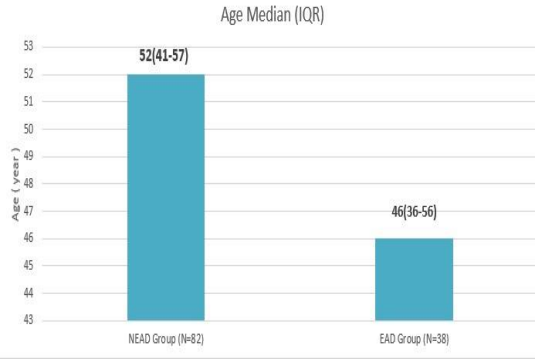

Figure 2: Median age in Early allograft dysfunction (EAD) and Non-EAD (NEAD), IQR: interquartile range

Platelet count:

Results of platelet count showed in (table 1, figure 4). PLT count in EAD group had significant difference on preoperative day (day0), (p=0.011) and highly significance on postoperative days from post day-1 to post day-6 ($p \leq 0.001$), showed extremely significance on post day-7(p=0.000) in compared to NEAD group.

Table 1. Platelet count on preoperative day (0) and postoperative days from (1-7) in patients with and without early allograft dysfunction.

Variable	NEAD group Median (IQR) (N=82)	EAD group Median (IQR) (N=38)	P value
PLT-0 (**K/ul**)	63(48 – 103)	56.7(32 – 75.95)	0.011*
PLT-1 (**K/ul**)	61.7(39 – 85)	47.95(30 – 63.2)	0.005**
PLT-2 (**K/ul**)	37.5(27 – 57)	30(20 – 51.37)	0.038*
PLT-3 (**K/ul**)	39.7(27 – 61)	30.5(20 – 38.4)	0.003**
PLT-4 (**K/ul**)	43(30.6 – 72)	34.4(19 – 52.8)	0.009**
PLT-5 (**K/ul**)	52.5(37 – 85)	32(22.7 – 58.2)	0.002**
PLT-6 (**K/ul**)	61.8(37 – 87)	36(21.9 – 64.3)	0.001**
PLT-7 (**K/ul**)	75(50 – 102)	44(26 – 68.37)	0.000***

EAD: Early allograft dysfunction, **NEAD**: non-EAD, **PLT**: platelets, *significant** highly significant, ***extremely significant

Neutrophil count:

Neutrophil count in EAD group compared to NEAD group were listed in (table 2), increase in neutrophil count had significant difference (p<0.05) on postoperative days from post day-4 to post day-6 and had highly significant difference between two groups on post-7.

Lymphocyte count:

Results of Lymphocyte count showed in (table 3), the decrease in lymphocyte count in EAD group had significant difference compared to NEAD group (p<0.05) on postoperative days (2,4,5,6) and highly significant difference on post days (3,7) (p<0.01).

Table 2. Neutrophil count on preoperative day (0) and postoperative days from (1-7) in patients with and without early allograft dysfunction.

Variable	NEAD group Median (IQR) N=82	EAD group Median (IQR) N=38	P value
NEU-0 **(K/ul)**	55(45 – 65.22)	58.9(47 – 69.7)	0.420
NEU-1 **(K/ul)**	86.9(81.7 – 90)	87(85.3 – 89.7)	0.333
NEU-2 **(K/ul)**	83(78.7 – 87.7)	85(79 – 89)	0.233
NEU-3 **(K/ul)**	79(74.6 – 86.6)	84(78.2 – 88.2)	0.058
NEU-4 **(K/ul)**	78.60(70 – 86)	81(76 – 86.9)	0.048*
NEU-5 **(K/ul)**	77.7(69.7 – 84)	79.6(71 – 86.7)	0.045*
NEU-6 **(K/ul)**	73(65.2 – 84.2)	79.4(70 – 87.5)	0.046*
NEU-7 **(K/ul)**	72.9(62.7 – 84)	82.7(69 – 34.5)	0.008**

EAD: Early allograft dysfunction, NEAD: non-EAD, NEU: Neutrophil count, *significant** highly significant.

Table 3. Lymphocyte count on preoperative day (0) and postoperative days from (1-7) in patients with and without early allograft dysfunction.

Variable	NEAD group Median (IQR) N=82	EAD group Median (IQR) N=38	P value
LYM-0 **(K/ul)**	26.7(16 – 37)	22.3(16.4 – 34.5)	0.311
LYM-1 **(K/ul)**	6.4(4.6 – 8.8)	5.75(3.8 – 7.8)	0.175
LYM-2 **(K/ul)**	7.9(5.4 – 10)	5.85(4.2 -9.5)	0.036*
LYM-3 **(K/ul)**	9.7(6.6 – 8.8)	6.4(5.1 – 10.3)	0.004**
LYM-4 **(K/ul)**	10.9(7 – 15.4)	8.6(5.2 – 13.57)	0.044*
LYM-5 **(K/ul)**	11.2(7.9 – 15)	8.4(5.4 – 14.95)	0.033*
LYM-6 **(K/ul)**	13.3(7.9 – 17)	9.35(7 – 15)	0.028*
LYM-7 **(K/ul)**	13.9(9 – 13.4)	9.3(6.4 – 12.6)	0.003**

EAD: Early allograft dysfunction, NEAD: non-EAD, LYM: lymphocyte, *significant, ** highly significant

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Neutrophil to Lymphocyte ratio:

Results of Neutrophil to Lymphocyte ratio listed in (table 4, figure 5), regarding comparative between two groups, NLR in EAD group had significant difference ($p<0.05$) on postoperative days from post day-4 to post day-6 and highly significant difference ($p<0.01$) on post days (2,3,7).

Table 4. Neutrophil to Lymphocyte ratio on preoperative day (0) and postoperative days from (1-7) in patients with and without early allograft dysfunction.

Variable	NEAD group Median (IQR) N=82	EAD group Median (IQR) N=38	P value
NLR-0 ratio	2(1.2 – 3.6)	2.4(1.2 – 3.7)	0.584
NLR-1 ratio	13.4(9.7 – 18)	14.9(11.1 – 23.4)	0.158
NLR-2 ratio	10.4(7.1 – 16)	15.9(8.8 – 23.3)	0.007**
NLR-3 ratio	8.4(5.8 – 13)	13.6(7.7 – 17.2)	0.003**
NLR-4 ratio	7(4.6 – 12.5)	11(6 – 16)	0.022*
NLR-5ratio	7(4.6 – 12.5)	8.4(5.1 – 14.8)	0.018*
NLR-6 ratio	5.7(3.7 – 10)	8.7(5.2– 14.3)	0.012*
NLR-7 ratio	5.2(3.1 – 9.9)	9.1(5.2 – 15.3)	0.002**

EAD: Early allograft dysfunction, **NEAD:** non-EAD, **NLR**: Neutrophil-to-lymphocyte ratio, *, significant, ** highly significant.

Diagnostic performance in EAD prediction

Platelets (PLT)

Count of PLT on preoperative day and postoperative days from post day-1 to post day-7 exhibited area under curves (AUC) ranged from 0.618 to 0.682 with (sensitivity from 65% - 81%, specificity from 52% - 59%, p=0.038 – 0.001) (table 5), the result on post day seven showed potential values in EAD prediction (figure 3). These results indicating that PLT may be a useful biomarker for predicting postoperative liver dysfunction.

Table 5. Area under curve (AUC), sensitivity, and specificity of PLT in preoperative day (0) and postoperative days from (1-7) in EAD prediction in patients underwent living donor liver transplantation.

Variable	AUC (95% CI)	Cutoff	Sensitivity %	Specificity%	P value
PLT-0	0.645 (0.538-0.752)	61.25	63	53	0.011*
PLT-1	0.658 (0.557 – 0.760)	55.80	68	61	0.005**
PLT-2	0.618 (0.506 – 0.730)	36.25	60	55	0.038*
PLT-3	0.666 (0.566- 0.767)	37.60	76	55	0.003**
PLT-4	0.650 (0.544 – 0.755)	39.85	63	55	0.009**
PLT-5	0.680 (0.577- 0.784)	48.55	71	57	0.002**
PLT-6	0.693(0.589- 0.0.797)	53.10	71	63	0.001**
PLT-7	0.735 (0.640 – 0.830)	67.25	76	59	0.000***

PLT: Platelet count, EAD: Early allograft dysfunction, **CI:** Confidence interval*significant** highly significant, ***extremely significant

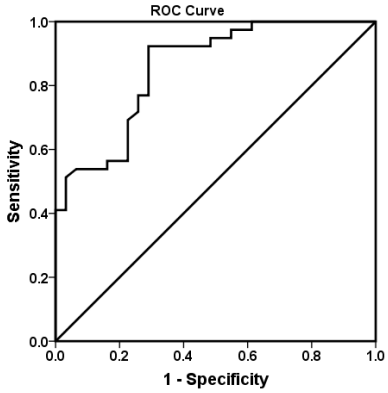

Figure (3): ROC curve for PLT on postoperative day-7 in prediction EAD

Neutrophils to lymphocyte ratio (NLR)

The ratio of NLR on postoperative days from post day-2 to post day-7 exhibited area under curves (AUC) ranged from 0.630 to 0.676 with (sensitivity from 68% - 81%, specificity from 52% - 59%, p=0.022 – 0.002). (table6, figure 4). The results of NLR indicating that it may be a useful screening tool in EAD prediction. Overall, these results suggest that NLR and PLT may be useful biomarkers for predicting EAD after surgery.

Table 6. Area under curve (AUC), sensitivity, and specificity of NLR in postoperative days from (2-7) in EAD prediction in patients underwent living donor liver transplantation.

Variable	AUC (95% CI)	Cutoff	Sensitivity %	Specificity%	P value
NLR-2	0.653 (0.549-0.758)	11.4	71	57	0.007**
NLR-3	0.669 (0.566-0.772)	8.7	73	58	0.003**
NLR-4	0.630 (0.525-0.735)	7.4	68	52	0.022*
NLR-5	0.655 (0.547-0.749)	6.5	72	55	0.018*
NLR-6	0.642 (0.537-0.747)	6.2	71	59	0.012*
NLR-7	0.676 (0.573-0.778)	5.2	81	52	0.002**

NLR: Neutrophil to lymphocyte ratio**, EAD:** Early allograft dysfunction, **CI:** Confidence interval, *significant** highly significant

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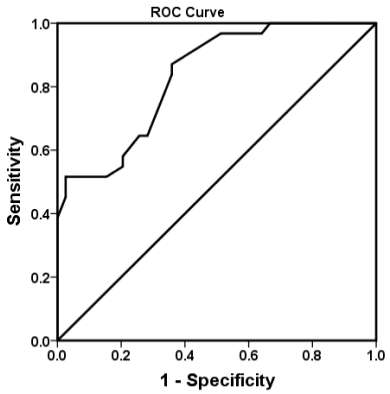

Figure (4): ROC curve for NLR on postoperative day-7 in prediction EAD

Discussion:

Liver transplantation (LT) attitudes as a life-saving curative pathway for individuals suffered from end-stage liver disease, acute liver failure, and a part of patients afflicted with primary and secondary hepatic malignancies **(Quaresima et al., 2023)**. Many previous studies have demonstrated that early allograft dysfunction (EAD) is a prospector step in the pathway to eventual graft loss **(Vos et al., 2014)**.

Thrombocytopenia is commonly observed

in advanced liver disease. It is sometimes thrombocytopenia, examinations of which conclude in the diagnosis of liver illness in the first place. Numerous causes are linked to this thrombocytopenia. Chronic liver disease-related platelet dysfunction and thrombocytopenia typically persist during the perioperative phase and have consequences in the early post-transplant phase **(Pathik, 2024)**.

In the present study PLT count in EAD group shows significant difference on preoperative day (p=0.011) and highly significance in postoperative days (from day-1 to day-7, $p \leq 0.001$) compared to NEAD group. Count of PLT on preoperative day and postoperative days from post day-1 to post day-7 exhibited area under curves (AUC) ranged from 0.618 to 0.682 with (sensitivity from 65% - 81%, specificity from 52% - 59%, p=0.038 – 0.001), these results indicated that PLT were associated with EAD development and may be a useful biomarker for predicting postoperative liver dysfunction. The results of PLT in present study agree with the previous studies, which found that platelet counts were correlated with the early and late outcomes after liver transplant. Platelet counts on post-operative day- 5 can predicted patient complications **(Lesurtel et al., 2014).** Thrombocytopenic patients had twice more common severe complications and three more common incidence of early allograft dysfunction **(Li et al., 2015)**, patients analyzed in other previous study showed that approximately half of patients developed persistent thrombocytopenia after liver transplantation and the most substantial decrease in platelet levels occurred on post operative day-7**(Ma et al., 2024)**.

Neutrophil lymphocyte ratio is calculated using neutrophil counts, which reflect innate immunity, and lymphocyte counts, which reflect adaptive immunity; the underlying mechanisms of the association between NLR and various outcomes are thought to be related the chronic inflammation or inflammatory microenvironment **(Motomura et al.,2013)**. In present study results of NLR in EAD group had significant difference ($p<0.05$) on postoperative days from post day-2 to post day-7 showed association with EAD development. On the other hand, the ratio of NLR on postoperative days from post day-2 to post day-7 exhibited area under curves (AUC) ranged from 0.630 to 0.676 with (sensitivity from 68% - 81%, specificity from 52% - 59%, p=0.022 – 0.002), indicating that it may be a useful screening tool in EAD prediction. Agree with previous studies, which demonstrated that, increasing NLR was proportionally associated with higher risk of EAD **(Kwon et al., 2019).** NLR is a reliable indicator of systemic inflammation in patients with end-stage liver disease, with prevalent systemic inflammation associated with poor prognosis **(Oweira et al.,2016)**, The change in NLR during living donor liver transplantation may be associated with pre- and intraoperative conditions and may independently predict graft failure regardless of an absolute value (**Park et al., 2021).**

Conclusion:

From the preset results it can concluded that, NLR and PLT in liver transplantation may indicate a significant association with EAD development and may be useful biomarkers for predicting EAD after surgery.

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الملخص العربي

عنوان البحث: تقييم نسبة الخلايا المتعادلة إلى الخلايا الليمفاوية وعدد الصفائح الدموية بعد زراعة الكبد

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خلفية الدراسة: ارتبطت زيادة معدل نسبة الخلايا المتعادلة إلى الخلايا الليمفاوية (NLR) بشكل متناسب بارتفاع خطر الإصابة بالخلل الوظيفي المبكر. كما أن الصفائح الدموية (PLT) قد تلعب دورًا مهمًا في اختلال وظيفة الكبد بعد عملية الزرع، الهدف من الدراسة: تهدف الدراسة الحالية إلى تقييم دور نسبة الخلايا المتعادلة إلى الخلايا الليمفاوية (NLR)، والصفائح الدموية (PLT) في

تطور خلل الطعم المبكر (EAD) بعد زراعة الكبد (LT). طريقة البحث: تم عمل هذه الدراسة على مائة وعشرين مريضًا خضعوا لعملية زرع كبد من متبرع حي في مركز جراحة الجهاز الهضمي بجامعة المنصورة. تم تقسيم الأشخاص الذين شملتهم الدراسة إلى المجموعات الآتية: المجموعة الأولى: مجموعة الخلل الوظيفي غير المبكر للزرع تشمل ٨٢ مريض. المجموعة الثانية: مجموعة الخلل الوظيفي المبكر للزرع تشمل ٣٨ مريض.. تم إخضاع جميع المرضى لتقدير العلامات الكيميائية والدموية المختلفة في اليوم السابق للزراعة ومن اليوم الأول إلى اليوم السابع بعد زراعة الكبد. النتائج: أظهرت نتائج عد الصفائح الدموية أن مجموعة الخلل الوظيفي المبكر لديهم فروق معنوية كبيرة سواء في اليوم السابق للزراعة أو خلال الأيام السبعة التالية بعدها مقارنة بمجموعة المرضى في المجموعة الأولى وأظهرت مساحة تحت المنحنيات (AUC) قيم تتراوح من ٠,٦١٨ إلى ٠,٦٨٢ مع حساسية من ٦٥% إلى ٨١%، خصوصية من ٥٢% إلى ٥٩%. كما كان لنتائج نسبة NLR فرق معنوي كبير بين المجموعتين ابتداء من اليوم الثاني إلى اليوم السابع بعد زراعة الكبد. تراوحت نسبة NLR في الأيام التي تلي الجراحة من اليوم الثاني إلى اليوم السابع قيم المساحة تحت المنحني (AUC) تتراوح من ٠,٦٣٠ إلى ٠,٦٧٦ مع حساسية من ٦٨% إلى ٨١%، وخصوصية ٥٢% إلى ٥٩%. الاستنتاج: يمكن الاستنتاج أن NLR وPLT قد يشيران إلى ارتباط كبير بتطور الخلل الوظيفي المبكر وقد يكونان بمثابة مؤشرات حيوية مفيدة للتنبؤ بـ EAD بعد زراعة الكبد.