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Leukocytosis is Common Following Pediatric Liver Transplant for Biliary Atresia but Should be Interpreted with Caution

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Abstract

Infection is a leading cause of short-term morbidity and mortality in pediatric patients after liver transplant (LT). Diagnosing infection in this population can be challenging, requiring consideration of laboratory results and clinical context. The prevalence and significance of post-operative leukocytosis has not yet been explored in children after LT. Our goals were to characterize post-transplant leukocytosis in pediatric patients after LT for biliary atresia (BA) and evaluate the relationship between post-LT leukocytosis and infection. Retrospective review of patients aged 0-18 years who underwent LT between 2012-2022 for BA. Clinical data were extracted from electronic medical records. Infectious outcomes were characterized as organism-confirmed infection (OCI), presumed infection, and no apparent infection. Differences between groups were assessed using two-sample t-tests and Fisher's Exact tests. 60 children met criteria for inclusion (mean age at LT 33.7 ± 50.9 months; 65% female). Forty-

four (73.3%) had leukocytosis in the 14 days after transplant. There was no association between leukocytosis in general and OCI (p = 0.67) or presumed (p = 0.71) infection. Only leukocytosis >30,000/µL was associated with OCI (p = 0.008). Leukocytosis after LT for BA is common, although only white blood cell (WBC) count >30,000/µL was associated with organism-confirmed infection. This study is the first to describe the prevalence of leukocytosis in children after LT and emphasizes the importance of considering the multifactorial nature of leukocytosis when evaluating for and treating infections in this population.

Keywords
Infection; cholangitis; antibiotics; blood culture

1. Introduction

Infection is a leading cause of short-term morbidity and mortality in pediatric patients after liver transplant (LT) [1]. Most of the literature regarding the prognostic value of various laboratory metrics in the post-transplant period has been in the adult population, including post-operative leukocytosis [2-7]. Post-operative stress, steroids, and infection are likely contributors to leukocytosis after LT [2], though adult literature suggests the cause of post-operative leukocytosis may vary depending on the timing after transplant. In adult LT recipients, leukocytosis in the first 14 days is most commonly attributable to a postoperative response or an unidentified etiology, whereas leukocytosis after day 14 is most commonly attributable to infection [2]. A potential driver of non-infectious leukocytosis is resolution of pre-transplant hypersplenism, with the release of sequestered splenic granulocytes after LT [2].

Given the serious nature of infection in pediatric LT patients, it is critical to maintain a high level of vigilance in identifying and treating post-LT infection. However, clinicians must distinguish between infection and other causes of leukocytosis to avoid unnecessary exposure to antibiotics and invasive testing in this population. There are variable data on whether other markers of infection such as procalcitonin (PCT), C-Reactive Protein (CRP), and even fever are sensitive or specific enough to differentiate infection from other causes of leukocytosis in the immediate post-LT period, which further complicates this evaluation [3-7].

This study aimed to quantify the prevalence of post-transplant leukocytosis in pediatric patients with biliary atresia (BA) at our center, investigate its association with identified infection, and explore practice patterns of antibiotic use associated with the finding of leukocytosis. By detailing our center's experience, we hope to provide a framework for clinicians to consider the usefulness of leukocytosis in the larger clinical context of the high-risk post-transplant period.

2. Methods

2.1 Study Design, Participants, and Statistical Analysis

This is a retrospective review of sixty children aged 0-18 years who underwent LT between 2012-2022 for BA at Children's Hospital Colorado. We chose to include only children with BA, as opposed to all transplant indications, to remove the potential confounding variable of underlying disease. A

review of the electronic medical records before and after LT was performed to extract relevant clinical data. Differences between groups were assessed using two-sample t-tests (for continuous variables) and Chi Square or Fisher's exact test (for categorical variables). 3-way ANOVA was used to assess differences between multiple groups. Prism GraphPad software was utilized for data analysis.

2.2 Ethics Statement

This study was approved by the Colorado Multiple Institutional Review Board (#22-2286) and deemed to be IRB Exempt on March 14th, 2023.

2.3 Leukocytosis and Infection Classification

Leukocytosis was defined as a white blood cell count (WBC) that is elevated for age based on laboratory standards and was further categorized by WBC count <20,000/µL, 20,000/µL-30,000/µL and >30,000/µL. Presumed infection was defined as a clinically diagnosed, culture-negative cholangitis, whereas organism-confirmed infection (OCI) was defined as bacteremia, pneumonia, PCR-positive viral respiratory illness or gastroenteritis, culture-positive cholangitis or peritonitis. Fever was defined as temperature >38.0 Celsius. The immediate period post-LT was defined as days 0-4, while the early period was defined as day 5-14 post-LT.

2.4 Immunosuppression and Anti-Microbials

Data was obtained regarding steroid use on post-operative days zero, three and seven. Antimicrobial use was defined as a new course of antibiotics, antifungals, and/or antivirals, separate from what is used for peri-operative prophylaxis. This included new antimicrobial courses and/or broadening of existing antimicrobials. Antimicrobials which started before post-operative day zero for known infections pre-LT were not included. Antimicrobials started for ≤2 days were defined as "rule outs" while antimicrobials used for >2 days were considered full antimicrobial courses. One patient was excluded from analysis given it was their second LT.

2.5 Length of Hospital Stay and Intensive Care Unit Length of Stay

Length of hospital stay was defined as post-operative day zero until day of discharge. Intensive Care Unit (ICU) length of stay was defined as post-operative day zero until day of transfer to the medical floor, including additional ICU days for those patients who were transferred back to the ICU after initially transferring to the medical floor.

3. Results

In this patient cohort, a large portion of children exhibited leukocytosis in both the immediate and early periods (48.3% and 73.3%, respectively) after transplant (Table 1). Combined, 78.3% of patients experienced leukocytosis at some time in the 0-14 days following transplant, with 25% experiencing a WBC count >30,000/µL. Leukocytosis was further stratified by degree of WBC count elevation and analyzed in three groups: WBC <20,000/µL, WBC 20,000/µL to 30,000/µL, and WBC >30,000/µL. All patients included in this study received methylprednisolone 30 mg/kg (max

1000 mg/dose) at the time of transplant for induction immunosuppression, followed by 3 days of methylprednisolone 10 mg/kg (max 1000 mg/dose). On post-operative day seven 56/60 patients were on 1 mg/kg of steroids (max 60 mg/dose) while 3/60 were still on 10 mg/kg (max: 1000 mg/dose) due to concern for acute rejection, and 1 patient was deceased.

Table 1 Patient Demographics.

	Leukocytosis (n = 46)	No Leukocytosis (n = 14)	p-Value
Age at transplant (months)	33.7 ± 48.7	50.6 ± 74.4	p = 0.82
Sex	Female: 27 (59%) Male: 19 (41%)	Female: 12 (86%) Male: 2 (14%)	p = 0.13
Avg length hospital stay (days)	19.7 ± 13.6	11.9 ± 8.3	p = 0.02
Avg length ICU stay (days)	9.3 ± 10.2	6.4 ± 7.7	p = 0.26
OCI	12 (26%)	3 (21%)	p = 0.93

Controlling for patient age, sex and steroid regimen, there was no significant difference in rate of OCI between patients with leukocytosis and those without (p = 0.67), nor was leukocytosis significantly associated with presumed infection (p = 0.71). Only leukocytosis >30,000/µL was found to be associated with OCI (p = 0.008), which was true at all timepoints within the 14 post-operative days (Figure 1). Children with leukocytosis of any level during the 14 days post-LT were started on antibiotics at higher rates than children without leukocytosis (p < 0.001). Ten total patients received treatment for presumed infection. Of these ten patients, nine had leukocytosis-five (55%) with a WBC count between 20,000-30,000/µL and four (44%) with a WBC count >30,000/µL.

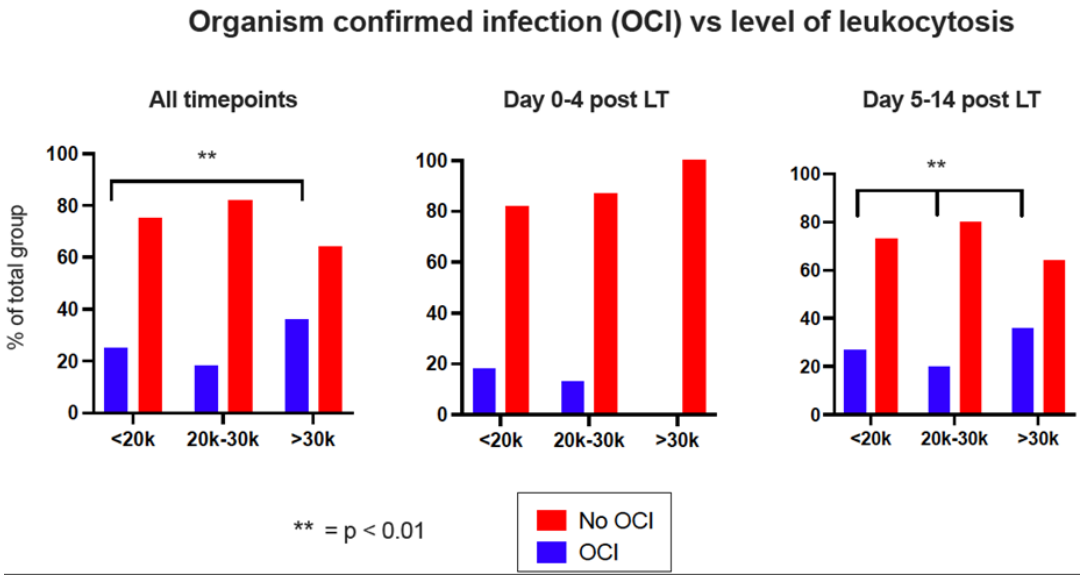

Figure 1 Organism Confirmed Infection, Level of Leukocytosis & Timepoint Post-Transplant The rate of OCI was significantly higher in patients with leukocytosis >30,000/µL across all post-transplant timepoints studied compared to those with lower levels of WBC elevation or no WBC elevation. In post-operative days 5-14, the rate of OCI was significantly higher in patients with leukocytosis >30,000/µL than those with lower levels of leukocytosis.

All children in this study with a positive PCR test for respiratory illness had fever. The presence of both leukocytosis and fever was highly correlated with OCI ($p < 0.001$) although when positive respiratory viral illness results were excluded from the OCI group this correlation was far more modest ($p = 0.02$). There was no significant difference in rate of OCI when stratified by presence of fever and level of leukocytosis: children with leukocytosis >30,000/μL and fever were not more likely to have an OCI than those with WBC count <20,000/μL or 20,000-30,000/μL and fever ($p = 0.7$).

Children with fever were significantly more likely to have antibiotic initiation by clinicians regardless of leukocytosis ($p < 0.001$) and were more likely to receive a full course of antibiotics instead of a rule-out ($p < 0.001$). The duration of antibiotics (48-hour "rule out" versus full antibiotic course) did not change based on degree of leukocytosis ($p = 0.79$). All ten patients with suspected cholangitis received a full course of antibiotics. Length of hospitalization was longer for patients with any degree of leukocytosis ($p = 0.05$) and for those who received antibiotics ($p = 0.008$). There was no correlation between presence of leukocytosis and mortality ($p = 0.09$), though only 2 of 60 patients died in the 0-14 days post-LT so survival analysis was not possible.

4. Discussion

This study highlights that post-LT leukocytosis is a common phenomenon in children with end-stage liver disease secondary to BA, as a large portion of patients in our cohort exhibited leukocytosis in both the immediate and early transplant period. Prevalence of leukocytosis after transplant did not differ significantly by sex, age, or steroid dose in this patient population. While leukocytosis is a common finding in pediatric patients post-LT, the etiology is not well-elucidated and is likely multifactorial, including a non-specific inflammatory response, resolution of hypersplenism, corticosteroid effect, and presence of infection. These factors are commonly experienced by post-LT patients, which is likely the reason for the high frequency of leukocytosis seen in this patient population.

"Non septic post-operative leukocytosis" is a non-specific inflammatory response to surgery that has been well documented across a variety of surgical specialties and patient populations, mostly in adult literature [8-12]. Landmann et al. demonstrated that granulocytes increase significantly with physical stress, and the stress of a multi-hour surgery with anesthesia such as liver transplant likely causes high levels of physiologic stress, therefore increasing granulocytes and subsequently WBC count [13]. It is likely that children exhibit similar pathophysiology after surgery, contributing in part to the leukocytosis seen in our cohort.

An additional suggested cause of leukocytosis in adult liver transplant recipients is resolution of pre-transplant hypersplenism [2]. In patients with cirrhosis and portal hypertension, splenic sequestration can cause margination of granulocytes leading to leukopenia. With transplantation and resolution of portal hypertension these sequestered splenic granulocytes are released, ultimately leading to an increase in circulating white blood cells [2]. This phenomenon may also be true in children with BA who typically have significant hypersplenism prior to transplant.

All patients in our study received steroids for induction immunosuppression, which is common practice in pediatric LT. Leukocytosis and neutrophilia are known sequelae of corticosteroid use, attributable to the enhanced release of cells from the bone marrow and inhibition of neutrophil apoptosis [14]. This leukocytosis is usually noted within hours of administration of steroids and typically reaches peak values within two weeks of the initial dose, although this peak can appear

sooner with higher doses [14-16]. We believe that steroid use is likely a contributor to the post-transplant leukocytosis seen in our patient population, though at our institution, post-transplant steroid dosing is protocolized, and nearly all our participants received the same dose of steroids on both day 3 and day 7. Therefore, we did not have enough variation to examine the correlation between steroid dose and leukocytosis. A 2018 study by Frenkel et. al attempted to account for the component of steroid induced leukocytosis in patients with acute infection and found that patients receiving steroids with confirmed acute infection had significantly higher average WBC count than those not receiving steroids [17]. Similar logic may explain the correlation between leukocytosis of >30,000/µL and proven infection in our study, suggesting that clinicians may benefit from placing increased importance on particularly elevated WBC count when monitoring children for infection after LT.

Another commonly used medication for immunosuppression in our transplant population is tacrolimus, which we did not include in this study because it is not associated with leukocytosis. However, it is important to note that other medications used to treat post-transplant patients may affect the bone marrow. Though these were not examined in our study due to infrequent usage, it is important for providers to consider all medications a patient is on when trying to understand leukocytosis in a post-LT patient.

One of the most important potential causes of leukocytosis is infection, given the significant morbidity and mortality associated with post-transplant infections [1]. Our analysis showed that post-transplant leukocytosis in general was not significantly associated with OCI, nor was presence of leukocytosis associated with presumed infection. Only a WBC count >30,000/µL was correlated with OCI when controlling for age, sex and steroid dose. These data mirror findings in adult solid organ transplant recipients, which show that leukocytosis is present in less than half of patients with true bacteremia and is a common finding in those without systemic infection [18]. Additionally, in adults post-LT, infection accounts for only 28% of episodes of leukocytosis [2].

Not surprisingly, clinicians in our study were significantly more likely to initiate or broaden antimicrobial agents for any level of leukocytosis during the two weeks after transplant. Furthermore, the duration of antibiotic course (48-hour “rule out” versus full antibiotic course) did not change based on degree of leukocytosis. Given the lack of correlation between OCI and leukocytosis in general, we must rely on the complete clinical scenario when making decisions about the need for antimicrobial initiation. Clinicians should weigh the fragility of recently transplanted patients with the known side effects of antibiotic use - especially resistance and prolonged hospital stays - to determine if antibiotics are appropriate. If the clinical suspicion for infection is low but patients have mild-moderate leukocytosis, our data suggest that antibiotic initiation may be reasonably deferred in that setting. On the contrary, the association between very high WBC counts (e.g., >30,000/µL) and infection in this study may indicate that leukocytosis to that degree warrants at least an antibiotic “rule-out”. Additionally, the co-occurrence of fever and leukocytosis was significantly associated with OCI, supporting the use of antibiotics in that clinical scenario. Other signs and symptoms of infection or sepsis should be considered when making the decision to start antimicrobials but is often challenging in the post-transplant patient who may have tachypnea, tachycardia, fever, and leukocytosis (SIRS Criteria) or respiratory support, vasoactive medications, coagulopathy, or neurologic dysfunction (Phoenix Criteria for Pediatric Sepsis) due to a multitude of reasons. This emphasizes the need to consider the entire clinical picture when making such decisions.

Post-transplant leukocytosis did impact hospital utilization, as patients with any degree of leukocytosis had longer hospital stays than those without. We hypothesize that this is related to the fact that patients with leukocytosis were more likely to be started on antibiotic regimens despite not having OCIs or an identified presumed infection, prolonging duration of admission both for monitoring and administration of antibiotics. Not only do increased hospital stays post-transplant increase financial cost on families and the healthcare system, but they also can have detrimental effects on patients and their families through lack of returning to work or school, disrupting sleep/wake cycles, and impacting child development [19, 20]. This should be considered when choosing treatment and diagnostic testing for post-transplant patients with leukocytosis. The number of deaths (n = 2) was too low to make statistically meaningful conclusions regarding mortality in this patient population.

The study design (retrospective chart review) limits our ability to fully understand the clinical decision-making that guided the management of post-LT leukocytosis, namely the decisions surrounding initiation of antimicrobials. Additionally, our sample size was relatively small and as such, we cannot make broad generalizations regarding the management of post-LT leukocytosis. Since this is the first study to our knowledge to examine post-LT leukocytosis in pediatric patients, there is ample room for future directions of research to understand how outcomes in this vulnerable population can be improved. Further research is needed to determine the predictive ability of inflammatory markers such as CRP and PCT in distinguishing infection from non-infectious causes of leukocytosis in children following LT. Furthermore, our study only included children transplanted for biliary atresia. Future studies including a larger number of children with a variety of liver diseases would help determine how variations in leukocytosis predict infection among different transplant indications.

5. Conclusion

This study is the first to characterize the prevalence of leukocytosis in pediatric patients with BA after undergoing liver transplant, and to describe the significance of this finding in relation to identified infection and initiation of antibiotics. We demonstrated that leukocytosis is common in the first two weeks after transplant for BA but found leukocytosis to be significantly associated with OCI only when WBC count was >30,000/µL. However, clinicians were more likely to initiate antibiotics in patients with elevated WBC counts regardless of degree of leukocytosis. Given the known pathophysiology of non-infectious post operative leukocytosis as well as the near universal use of steroids in this patient population, this study suggests that clinicians must rely on the complete clinical scenario to gauge the level of concern for infection and the need for adding or changing antimicrobial treatment.

Author Contributions

A. Isabella Shanker, Lauren Maloney and Julia Boster designed the project, analyzed the results, and contributed to the writing and editing of the manuscript, and agree to be accountable for all aspects of the work represented herein.

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Competing Interests

The authors declare that no competing interests exist.

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