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Early Evidence of Bone Marrow Dysfunction in Children with Indeterminate Fulminant Hepatic Failure Who Ultimately Develop Aplastic Anemia

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In children, aplastic anemia (AA) is a common complication associated with fulminant hepatic failure (FHF). The objective of this study was to determine whether specific pretransplantation clinical and laboratory characteristics can be used to distinguish between patients with FHF who are at higher risk of developing AA. We performed a retrospective case–control study to evaluate the clinical and laboratory characteristics of those patients who presented with evidence of FHF and eventually developed aplastic anemia. We identified nine patients with AA, and all had the indeterminate form of FHF and underwent liver transplantation (LTx). The AA patients were compared with a control group of 47 patients with indeterminate FHF that underwent transplantation and did not develop AA. We found that males were over-represented in the group of patients that developed AA ($p=0.01$). Furthermore, during the pretransplant period, the AA group had a significantly lower white count ($p=0.005$), absolute lymphocyte count ($p=0.004$), and platelet count ($p=0.019$) when compared with controls. We conclude that evidence of early bone marrow dysfunction is apparent before liver transplantation and the development of AA in a subset of patients with the indeterminate form of FHF.

Key words: Acute liver failure, hepatitis

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Introduction

Fulminant hepatic failure (FHF) is defined as severe liver injury with onset of encephalopathy within 8 weeks after the onset of jaundice and in the absence of preexistent liver disease (1,2). Fulminant hepatic failure may result from a variety of causes including infectious hepatitis, toxin or drug-induced injury, metabolic diseases, or an indeterminate (IFHF) form in which the etiology is unknown and could not be established (3). The causes of FHF vary geographically and are also age-dependent. In the United Kingdom the most frequent pediatric cause of FHF is acetaminophen toxicity, whereas most children in the United States have the indeterminate type (4–6). Liver transplantation (LTx) remains the only definitive mode of treatment for individuals with FHF who fail to recover spontaneously. Although LTx has drastically improved survival in patients with FHF with 1-year survival rates that approach 90%, various complications influence long-term morbidity and mortality (7–9).

Aplastic anemia (AA) is a well-described occurrence following LTx in patients with FHF, and typically presents within 3–4 months after the onset of the first clinical symptoms of FHF. Since the first report of AA after FHF in 1987 by Stock et al. (10), it has become increasingly apparent that it is primarily observed in children with the indeterminate type of FHF following LTx (11–13). A recent review of 31 case reports showed the mean incidence of AA following IFHF was $23\%$, with a mean age of 10 years and a male/female ratio of 4.6 (11). There is a high morbidity and mortality rate associated with these patients from bleeding and infection owing to pancytopenia. Unfortunately, AA in patients with IFHF remains poorly understood, as there are no published reports comparing these patients with age-matched controls. The objective of this study is to determine whether specific pretransplantation clinical and laboratory characteristics can be used to distinguish between patients with FHF who are at higher risk of developing AA.

Methods

In addition to our existing database of liver transplant patients, the International Classification for Diseases, Ninth Revision (ICD-9, code for acute liver failure (ICD-9 #570) was reviewed for all admissions of pediatric patients (0–18 years of age) in order to further identify any additional patients presenting with FHF to the UCLA Medical Center between January 1985 and December 2002. The UCLA human subjects’ review board approved the study.

The criteria used to diagnose patients with FHF were the development of abnormal liver synthetic function and hepatic encephalopathy within 8
weeks of jaundice, in the absence of known pre-existing liver disease (14). A series of laboratory tests were obtained in all patients described in this study. They included HA total and IgM, HBsAg and Ab, HBeAg Ab, HIV 1 and 2 Abs, CMV IgG/IgM, and EBV/CA IgG/IgM. HCV Ab has been assessed in all cases since the early 1990s and HCV qualitative blood RNA assay has been performed in recent years. Depending on the age of the patient, routine analysis for various infectious agents were generally performed including HSV 1 and 2 IgG/IgM, enterovirus and adenovirus culture, and CMV urine culture in most neonates. HHV-6 IgG/IgM and parvovirus B19 IgG/IgM were frequently performed.

Urine organic acid and serum amino acids were routinely assessed in neonates and most young infants, and galactosemia results were available as part of the state-wide screen. Serum lactate, pyruvate, ferritin, alpha-1 antitrypsin level and phenotype levels were routinely assessed in children during early infancy. Patients in early and late childhood ages were routinely assessed for serologic evidence of autoimmune hepatitis (ANA, anti-SM, pANCA, anti-KLM) and for evidence of Wilson’s disease (serum and urinary copper and ceruloplasmin). Serum acetaminophen and acetylsalicylic acid levels were routinely tested when clinically indicated, and careful assessment to exposure to a variety of drugs including valproic acid, isoniazid and halothane was generally obtained. Only in the absence of all the above clinical entities was the designation of IFHF used (5).

Patients diagnosed with AA following FHF were identified by searching for the ICD-9 code #284 for AA in discharge summaries of all hospitalized pediatric patients at the UCLA since 1985. Those patients were then cross-matched to our pre-existing UCLA database of pediatric patients who presented with FHF. All AA patients had hematological studies and bone marrow pathology that confirmed the diagnosis of AA as defined by the International Aplastic Anemia Study Group criteria: depression of two of three marrow pathology that confirmed the diagnosis of AA as defined by the International Aplastic Anemia Study Group criteria: depression of two of three peripheral blood counts (granulocytes < 0.5 x 10^9/L, platelets 20 x 10^9/L or reticulocytes < 1%) in the presence of hypocellular bone marrow (15). The date that the diagnosis of AA was established is reported as the period between LTx and the day that the CBC results met IAASG criteria for AA. All patients who met IAASG criteria underwent bone marrow biopsy, and the diagnosis of AA was only applied to those patients who had evidence of hypocellular bone marrow. Management of AA included administration of granulocyte-colony stimulating factor (G-CSF) and reduction of immunosuppression. Antithymocyte globulin (ATG) as well as a continuing steroid regimen was used in the management of several patients. Medications that had the potential to cause bone marrow suppression were discontinued when AA became clinically suspicious. Unless higher blood counts were clinically indicated, packed red blood cells and platelets were transfused to maintain a hemoglobin level > 7 g/dL and platelet count > 20 x 10^9/µL.

Medical records for each subject were reviewed and pertinent clinical and laboratory data were obtained, which included: sex, age, date of admission and discharge, date of transplant, and laboratory data. Laboratory data comprised of hepatic and hematologic parameters were obtained from each subject at the time of admission to the UCLA. In addition, hematologic parameters consisting of platelet and white blood cell counts were followed from the date of transplant to 4 weeks post-LTx.

**Statistics**

Univariate analysis was undertaken using a Student’s t-test for continuous variables, while categorical variables were analyzed using the Chi-square test. A p-value of 0.05 was considered statistically significant.

**Results**

**General characteristics**

Since 1985 a total of 272 children have been admitted to the UCLA Medical Center for evaluation and treatment of FHF. A total of 110 patients were diagnosed with the indeterminate form of FHF and 66 of these patients were transplanted with a survival rate of 86%. The nine patients who developed AA were all from this group of patients who underwent LTx for the indeterminate form of FHF. The control group consisted of 47 patients with IFHF who survived LTx beyond 4 weeks of transplant. Nine patients, who died within this time period, and one patient whose medical records were not available for review were excluded from the study. None of the nine patients who died within 4 weeks of LTx had evidence of pancytopenia.

Aplastic anemia developed in only 3.3% (9/272) of all patients with FHF, and in 8.2% (9/110) with the indeterminate form of FHF. Of the patients who were transplanted for IFHF, 13.6% (9/66) developed AA. Of the 44 patients with IFHF who were not transplanted, 45% survived. Aplastic anemia was not seen among the 20 patients who recovered from FHF in the absence of LTx. The association between LTx and the development of AA approached statistical significance (p = 0.07) when evaluated by Chi-square analysis.

**Aplastic anemia**

Aplastic anemia developed post-transplant in nine patients who underwent liver transplantation for IFHF. Table 1 shows the general characteristics of the patients in whom AA developed. Comparison was made between the aplastic anemia group (n = 9) and the control group (n = 47). All patients with AA were male and were therefore

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**Table 1: General characteristics of patients with aplastic anemia after acute liver failure**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Time to LTx (days)</th>
<th>Time from LTx to AA (days)</th>
<th>Season/year of presentation</th>
<th>Survival</th>
<th>BMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.9</td>
<td>M</td>
<td>10</td>
<td>23</td>
<td>Winter/1990</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>2</td>
<td>1.9</td>
<td>M</td>
<td>1</td>
<td>17</td>
<td>Winter/1993</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>3</td>
<td>4.8</td>
<td>M</td>
<td>19</td>
<td>12</td>
<td>Fall/2001</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>M</td>
<td>5</td>
<td>151</td>
<td>Summer/2000</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>5</td>
<td>1.4</td>
<td>M</td>
<td>4</td>
<td>26</td>
<td>Spring/2001</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>6</td>
<td>12.9</td>
<td>M</td>
<td>4</td>
<td>80</td>
<td>Winter/2001</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>7</td>
<td>2.3</td>
<td>M</td>
<td>1</td>
<td>37</td>
<td>Winter/1999</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>8</td>
<td>14.3</td>
<td>M</td>
<td>13</td>
<td>25</td>
<td>Fall/2001</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>9</td>
<td>1.3</td>
<td>M</td>
<td>5</td>
<td>13</td>
<td>Winter/2003</td>
<td>Y</td>
<td>N</td>
</tr>
</tbody>
</table>
overrepresented when compared with the control group (24/44, 54% males, \( p = 0.01 \)). The average age for those who developed AA was \( 6.2 \pm 1.7 \) years of age, compared with \( 6.0 \pm 0.8 \) years (\( p = 0.91 \)). The mean time between date of LTx and onset of AA in these patients who met the IAASG criteria for AA was \( 42 \pm 15.2 \) days post-LTx. Biopsies confirmed the presence of hypocellular bone marrow in all cases. The time from admission to LTx was similar between the two groups: \( 6.9 \pm 2 \) and \( 9.6 \pm 0.8 \) years (\( p = 0.91 \)).

Two patients died without ever entering remission for AA (Table 1). Patient 3 died of multisystem organ failure 25 days after undergoing his second LTx. Graft vs. host disease and acute cardiopulmonary arrest developed in patient 7 who died 82 days post LTx. Patients 1 and 4 received bone marrow transplants as treatment for their AA and both patients survived and recovered uneventfully.

**Hepatic and hematological markers on admission**

For both groups, we compared the hepatic and hematological laboratory studies obtained on the day of their initial admission to the UCLA for liver failure (Table 2). Hepatic markers included albumin, AST, ALT, total and conjugated bilirubin ammonia, fibrinogen, and prothrombin time. Hematological parameters examined were white blood cell count (WBC), platelets, hemoglobin, absolute neutrophil count (ANC), and absolute lymphocyte count (ANL). Reticulocyte count was rarely obtained in patients on the day of admission and was therefore not evaluated.

While the patients with FHF who developed AA had a lower ammonia level (\( p = 0.04 \)) on the day of admission, none of the other values in the liver panel from the first day of admission approached statistical significance between the two groups (Table 2). In contrast, the hematological parameters in the AA group displayed a significantly lower white blood cell count \( (6.2 \times 10^{3}/\mu L \text{ vs. } 10.5 \times 10^{3}/\mu L, \ p = 0.005) \) and platelet count \( (125 \times 10^{3}/\mu L \text{ vs. } 202 \times 10^{3}/\mu L, \ p = 0.019) \) on admission compared with the control group (Table 2 and Figure 1). Analysis of the mean absolute lymphocyte count on admission between the two groups revealed a significant difference \( (1.6 \times 10^{3}/\mu L \text{ vs. } 3.4 \times 10^{3}/\mu L, \ p = 0.004) \). Hemoglobin and absolute neutrophil counts were not found to be substantially different between the two groups.

**Trends in hematological markers**

We compared the mean WBC and platelet counts for the first 4 weeks after liver transplant in the AA-IFHF and IFHF groups (Figure 1A,B). A consistent elevation of the WBC count marked the initial (first week) post-transplant period in both groups. The mean WBC levels between the
Fulminant Hepatic Failure and Aplastic Anemia

Discussion

This study represents the first published case-control study evaluating the clinical characteristics of patients who develop aplastic anemia following acute liver failure. In our study, the development of AA after LTx for IFHF occurred generally in young children (mean age 6.2 years). This finding is similar to that reported previously where the mean age was approximately 10 years of age (11,16,17).

We provide evidence that male patients are at significantly higher risk for developing AA after transplant. While males represented 54% of the control group, all the patients in the AA group were male (p < 0.01), and 27.3% (9/33) of the males in the group transplanted for IFHF were diagnosed with AA. This finding is consistent with case reports that have described a predisposition of males among those patients who develop AA following LTx, however, gender differences have not been described in other established causes of AA (16,17).

It has been speculated that AA associated with IFHF is the result of an infectious etiology by a yet to be identified infectious agent. While parvovirus has been associated with the development of aplastic anemia, as well as FHF, we failed to identify any cases of parvovirus by serology in our patients. However, suggestive of a viral etiology is the fact that we have detected a recent increase in the number of patients that we have encountered over the last 2 years (Table 1). Of note is the overall distribution at time of presentation between the AA and control groups, demonstrating a recent clustering of patients presenting with AA over a 20-month period without a significant change in the control group.

In our study, aplastic anemia was diagnosed only in those patients who underwent LTx for IFHF. Of the patients who were transplanted for IFHF, 13.6% (9/66) developed AA. This finding is in contrast to the higher rate of occurrence reported by Tzakis et al. and Cattral et al., where 28% of 32 patients and 33% of 18 children transplanted for IFHF developed AA, respectively (16,17,17). In another series, three of 10 patients who survived LTx for IFHF had evidence of AA postoperatively (18). The question arises of whether or not there would have been further cases of AA that would have developed in those patients who did not survive LTx or did not survive long enough to be transplanted, especially considering greater than 50% of the patients from the nontransplanted group with IFHF died.

In our data series, there is no evidence of AA developing in patients transplanted for other causes of FHF or in the non-LTx patients with IFHF. All nine patients who developed AA had been transplanted for IFHF. A potential correlation between AA and liver transplant was observed in this study. Comparison of these nine patients with the 20 patients with IFHF who survived without requiring

Figure 1: (A) Mean platelet and (B) white blood cell counts post liver transplant for the aplastic anemia indeterminate fulminant hepatic failure (AA-IFHF) and IFHF groups. (C) Rate of change in WBC post liver transplant for the AA-IFHF and IFHF groups.

groups were significantly different during the entire 28-day follow-up period with the exception of day 2 and day 15, where the mean difference was not of statistical significance. Similarly, the trend in platelet counts between the two groups was significantly different over the same time period (Figure 1B). The platelet count in the control patients gradually increased to normal levels a week after the liver transplantation, while the platelet count in the AA group never improved during the immediate postoperative period. Post-operative absolute reticulocyte count was significantly lower in the AA group (3.1 $\times 10^4$ ± 1.2 $\times 10^4$) when compared with the controls (1.3 $\times 10^5$ ± 1.6 $\times 10^4$, p < 0.00003).

To determine the rate of change in WBC during the postoperative period, we analyzed the log-base 10 of the white blood cell counts for both groups during three different time periods. All time periods began from the initial date of transplant and ranged from 1–3 weeks post-LTx. Linear regression analysis was performed, generating a linear trend-line through the data points. The slope of each trend-line was calculated and the mean was compared between the AA and control groups. The aplastic anemia group was found to have a significant decline in WBC counts between 0 and 2 weeks (– 0.009 vs. 0.017, p = 0.023) and 0–3 weeks (– 0.023 vs. 0.003, p = 0.004) post-LTx (Figure 1C). No significant difference was observed during the first week post-LTx in the rate of decline.
transplantation approached statistical significance (p = 0.07) when evaluated by means of Chi-square analysis. While this may suggest that AA in this patient population may be a complication of the transplantation procedure, this study indicates that those patients who eventually develop AA have subtle evidence of bone marrow dysfunction before the time of liver transplantation. This implies that the process of transplantation is not causative to the development of AA although LTx and ensuing medical treatment may contribute to or hasten the development of AA. It is more likely that patients with IFHF who develop AA inherently go on to require transplantation. In other words, these patients who would eventually develop AA fail to improve their liver function spontaneously, therefore necessitating a liver transplantation. While this study did not identify significant differences in the hepatic function between the two groups at the time of admission, we have not evaluated trends in liver function between the two groups during the initial hospital stay.

In this study, AA was diagnosed by bone marrow biopsy at a mean time of more than 2 months after admission for FHF. Although liver panels on admission were not found to predict the development of AA, lower levels of both WBC and platelet counts were found to correlate with the risk of developing AA post-transplant. Interestingly, while there was no significant difference in the absolute neutrophil count, there proved to be a lower mean absolute lymphocyte count in the AA-IFHF group compared with the control group. This most likely is caused by the same etiologic agent or process that caused the fulminant liver failure to develop. It is unclear if this is owing to direct cytotoxic effects from a yet to be identified infectious agent or autoimmune mechanism induced at the time of liver failure. Our data strongly suggests this process is initiated before transplantation of the new liver, thus making it unlikely that the procedure or accompanying medications play anything more than perhaps a contributory role to the process. The effect on all three hematopoietic cell lines is suggestive of a cytotoxic effect on either stem cells or early pluripotent progenitor cells. If this is so, it would make the strategy of storing autologous marrow or peripheral blood stem cells before the liver transplant for future stem transplantation ineffective. However, as the vast majority of patients recover bone marrow function in the absence of bone marrow transplantation, residual stem cells must be present, albeit in insufficient levels to quickly repopulate the marrow. This study defines the general characteristics of children at risk of developing AA following FHF, and it may allow for either earlier intervention in the search for a potential bone marrow donor, or implementation of immunosuppressive therapy for treatment of AA. Nevertheless, further studies are needed to assess the characteristics of the various immature and mature lineages in the bone marrows of these patients.

The WBC and platelet counts were analyzed for a period of 4 weeks following LTx and the data demonstrated evidence of early bone marrow dysfunction before establishing the diagnosis of AA by bone marrow biopsy. During our analysis of the WBC data, we noticed a consistent elevation in the white counts during the immediate postoperative period for both groups of patients. The etiology of this elevation is likely of multifactorial origin, and may include stress, postoperative infections, and the use of steroids as part of the immunosuppressive regimen. Aside from this transient elevation, the white counts postoperatively were clearly different between the AA and control groups (Figure 1A,C). In contrast to the controls, the WBC count of the AA group did not return to baseline after the post-LTx elevation, but steadily declined over the 4-week period, dropping to less than 1000/μL. In fact, the rate of change of the WBC count during the initial weeks post-transplant was significantly different between the two groups. Specifically, the AA group had a progressive decline in the WBC counts, in contrast to the WBC counts in the control group after the first 2 and 3 weeks post-transplant.

In summary, IFHF is the most commonly diagnosed cause of fulminant hepatic failure in pediatric patients presenting to the UCLA, accounting for 40% of all cases since 1985. In children, AA is an occasional yet severe complication that frequently occurs concurrently in patients with IFHF. Moreover, the incidence may have been significantly higher than reported previously, as some of our patients may have died before clear laboratory evidence of AA developed. Young males appear to be at highest risk for the development of AA after transplantation for fulminant liver failure of the indeterminate type. In addition, we have identified early clinical trends such as the difference in the profile of WBC and platelet counts before and post-LTx in patients developing AA after IFHF. Our data suggests that levels of lymphocyte and platelet on admission, and during the immediate postoperative period, may help distinguish those patients at risk of developing subsequent AA. Further studies to identify additional laboratory and clinical predictors will facilitate early identification of ‘at risk’ individuals. This will allow for early initiation of the donor search process in anticipation of possible need for hematopoietic stem cell transplant.

References