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Case Report

Three Cases of Down Syndrome with Transient Abnormal Myelopoiesis who Underwent Liver Biopsy before Induction of Low-Dose Cytarabine

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Among patients with transient abnormal myelopoiesis (TAM) associated with Down syndrome, approximately 20% die within 6 months from multiorgan failure, especially liver fibrosis. We experienced three children with TAM who had low white blood cell counts but increased bilirubin levels. Here, we discuss the detailed clinical courses of these patients, including the pathological findings of liver biopsies. Our cases, together with previous literature, suggest that liver biopsy can be performed safely and provides useful information, especially regarding disease activities, and that low-dose cytarabine is a reasonable option to prevent early death in TAM patients with liver dysfunction.

Key words: liver biopsy, transient abnormal myelopoiesis, Down syndrome, low-dose cytarabine

Transient abnormal myelopoiesis (TAM) is a unique clonal myeloproliferative disorder occurring in 5-10% of neonates with Down syndrome and characterized by a proliferation of immature megakaryoblasts [1]. Although TAM regresses spontaneously in most patients, approximately 20% of TAM patients die from multiorgan failure, especially liver fibrosis, within the first 6 months of life. TAM is usually diagnosed at birth, but the timing of onset is variable, and case reports of stillbirth [2] or hydrops fetalis [3] suggest that TAM begins in utero, and may even reach its peak during the fetal period in some cases.

Risk factors of early death in TAM patients, such as higher white blood cell (WBC) counts (especially >100,000 /μL), ascites, preterm delivery, coagulopathy, failure of spontaneous remission, and elevated direct bilirubin levels, have been discussed previously [1,4-6]. Additionally, early initiation of low-dose cytosine arabinoside (LD-CA) in high-risk cases has been associated with better prognoses [7,8]. Prednisolone (PSL) is also an effective drug for some patients.

Previous autopsy reports of TAM patients have revealed residual extramedullary hematopoiesis and severe hepatitis with diffuse fibrosis, suggesting that liver failure in TAM is the result of inflammation caused by the cytokines produced by TAM blasts, such as transforming growth factor-β1 or platelet-derived growth factor-β [9-11]. Historically, TAM-affected infants with lower WBC counts and lower blast counts at birth have been considered to have a better prognosis [1,5]. In contrast, some patients show increased bilirubin as peripheral blasts disappear, progressing to liver fibrosis, which eventually becomes fatal. However, there are no

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clear criteria for the induction of therapy in these cases.

We experienced three cases of TAM with WBC counts less than 100,000 /μL at birth (normal, 9,000-30,000 /μL). Peripheral blasts disappeared in two patients, but their direct bilirubin was elevated, and hepatosplenomegaly progressed. We performed liver biopsies to determine the indication for LD-CA and PSL therapy. Here, we discuss the use of liver biopsy and LD-CA therapy in TAM cases with less extreme white blood cell elevation.

Case Reports

Case 1. A 2,832-g boy born at 36 weeks of gestation was diagnosed with TAM at birth, with a WBC count of 16,000 /μL and blast count of 17% (normal, 0%; see Table 1A). There was no severe coagulopathy (Table 1B). He showed hepatosplenomegaly and patent ductus arteriosus. Trisomy 21 was detected by karyotyping analysis. Patent ductus arteriosus spontaneously resolved, but hepatosplenomegaly remained, and direct bilirubin increased over his first weeks of life. He was admitted to our hospital on day 27. Laboratory tests showed hyperbilirubinemia and increased liver fibrosis markers on admission (Table 1A). His anemia and thrombocytopenia progressed rapidly, and he required several transfusions. Liver biopsy was performed on day 30, revealing cholestasis, hepatocyte swelling, and periportal and centrilobular fibrosis surrounding hepatocytes (Fig. 1A). Megakaryocytes were also present, which we considered to be residual TAM blasts. We started LD-CA (0.5 mg/kg/day) for 7 days from day 35. Because laboratory indicators and hepatosplenomegaly did not improve, we added PSL (1 mg/kg/day) from day 40 and a second course of LD-CA from day 54. After that, his bilirubin gradually decreased. Peripheral blasts disappeared on day 61. He experienced Common Terminology Criteria for Adverse Events (CTCAE) v4.0 grade 4 neutropenia from day 49 to 90, but it recovered spontaneously without febrile neutropenia or any infection. After day 77 he required no more transfusions. On day 106, his direct bilirubin had normalized, and he was discharged on day 115. During follow-up, his platelet count decreased again,

Table 1A Laboratory data of the three TAM cases

		WBC	Blast	Plt	Hb	T. bil	D. bil	AST	ALT	type Ⅳ colagen 7S	hyaluronic acid
		/μL	%	$\times 10^3$/μL	g/dL	mg/dL	mg/dL	U/L	U/L	ng/mL	ng/mL
	at birth	16,000	17	163	14.5	4.9	1.5	39	43	–	–
Case 1	on admission	4,410	21.5	47	8.9	6.29	3.52	74	32	56	117.7
	at discharge	2,440	0	85	8.3	0.61	0.16	34	23	16	52.7
	at birth	74,600	32	354	19.4	11.0	1.5	88	112	–	–
Case 2	on admission	7,190	0	100	11.2	13.61	9.82	340	344	62	1,130
	before death	50	0	10	9.2	18.43	13.77	316	631	26	58,709
	at birth	34,880	0.7	117	13.8	4.22	2.90	44	23	–	–
Case 3	before therapy	5,690	0	62	9.2	5.18	3.52	109	63	87	3,479
	at discharge	9,600	0	346	10.2	0.56	0.33	87	98	–	–

WBC, white blood cell; Plt, platelet; Hb, hemoglobin; T. bil, total bilirubin; D. bil, direct bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Table 1B Coagulation tests on admission/at liver biopsy

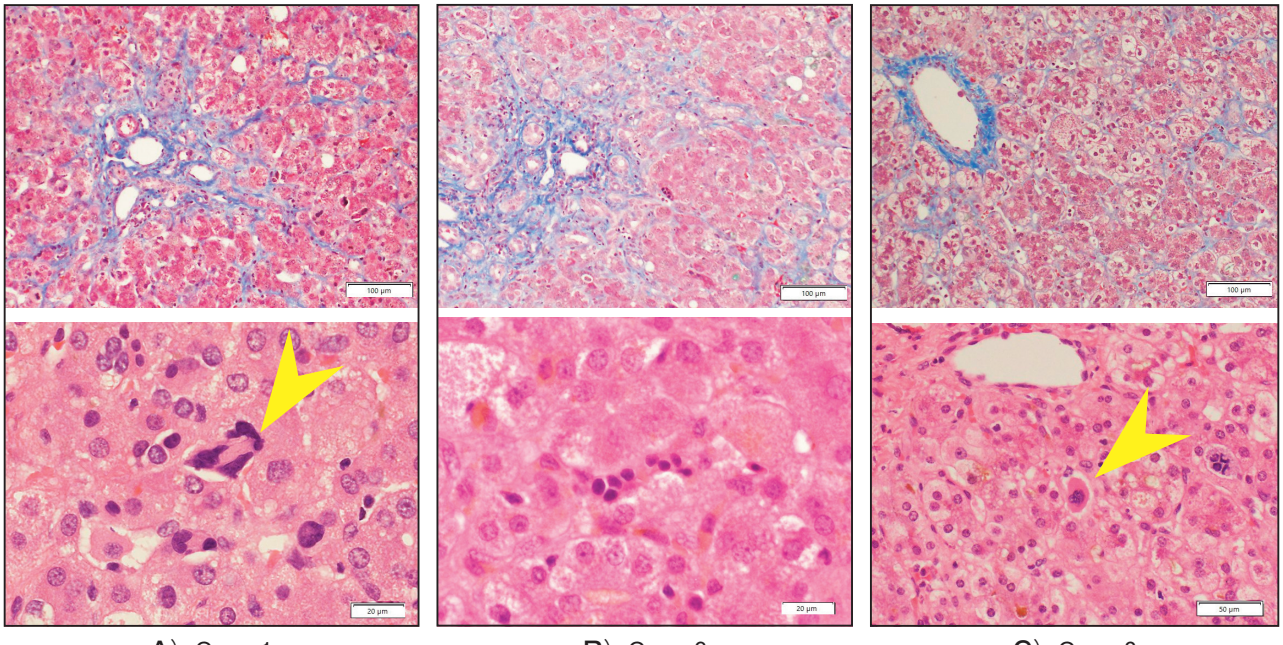
	days after birth	APTT	PT%	PT-INR	Fibg	ATⅢ	FDP	DD
		sec	%	n.d	mg/dL	%	μg/mL	μg/mL
Case 1	27	31.6	91	1.04	136	61	3	0.8
Case 2	86	30.7	80	1.1	202	70	n.d	2.3
Case 3	2	47.8	67	1.22	187	36	9.7	6.9

APTT, activated partial thromboplastin time; PT, prothrombin time; Fibg, fibrinogen; ATⅢ, antithrombin Ⅲ; FDP, fibrin/fibrinogen degradation products; DD, D-dimer.

and when he was 1 year and 3 months old, the percentage of blasts detected in his bone marrow was approximately 10% (normal, <5%). He received chemotherapy for myeloid leukemia with Down syndrome (ML-DS) and achieved complete remission. He has remained healthy for 7 years after being diagnosed with TAM.

Case 2. An 1,818-g boy born at 37 weeks of gestation was diagnosed with TAM at birth, with a WBC count of 74,600 /μL and blast count of 32% (normal, 0%; see Table 1A). He showed hepatosplenomegaly and a large ventricular septal defect. Trisomy 21 was detected by karyotyping analysis. Peripheral blasts disappeared by day 36, but thrombocytopenia, hepatosplenomegaly, and liver dysfunction progressed. Direct bilirubin and liver fibrosis markers gradually increased. He was admitted to our hospital on day 86, and laboratory tests showed hyperbilirubinemia and liver dysfunction on admission (Table 1A). His antithrombin III was 70% (normal, >80%), but no other coagulopathies were observed (Table 1B). Liver biopsy was performed on day 88, revealing cholestasis, extensive hepatocyte damage with swelling and ballooning, giant multinucleated hepatocytes, and diffuse fibrosis (Fig. 1B). Myeloblasts were observed, suggesting residual extramedullary hematopoiesis in his liver, but no megakaryocytes were detected. We started PSL 1 mg/kg/day on day 91. One week later, blood tests showed no response, and we increased the PSL dose to 2 mg/kg/day. We added LD-CA on day 106, but there was no improvement in his blood tests or general condition. Pancytopenia, multiorgan failure, and disseminated intravascular coagulopathy progressed, and he died on day 127.

Case 3. A 1,750-g girl born at 35 weeks of gestation suffered from dyspnea at birth. She had congenital heart disease and aproctia and was transferred to our hospital on day 0. On day 1, her laboratory tests revealed a WBC count of 34,880 /μL and blast count of 0.7% (normal, 0%), but she showed hyperbilirubinemia and extremely high liver fibrosis markers (Table 1A). Therefore, we diagnosed her with TAM. Her coagulation factors were normal for her age (Table 1B).



A) Case 1 B) Case 2 C) Case 3

Fig. 1 Upper: Masson trichrome staining. Lower: Hematoxylin-eosin staining. **A**, Liver biopsy performed on day 30 in case 1. Periportal and centrilobular fibrosis is observed with hepatocyte swelling. Megakaryocytes are present in the sinusoidal space (arrow); **B**, Liver biopsy performed on day 88 in case 2. Extensive hepatocyte damage with hepatocellular swelling, ballooning, and giant multinucleated hepatocytes are shown. Myeloblasts are observed, but no megakaryocytes are evident; **C**, Liver biopsy performed on day 11 in case 3. Mild perisinusoidal fibrosis compared with the other two cases is shown. Cholestasis and mild hepatocyte swelling are observed. Megakaryocytes are present in the sinusoidal space (arrow). Scale bars: 100 μm (upper A, B, C); 20 μm (lower A, B); 50 μm (lower C).

She also showed hypereosinophilia (62.3% of WBCs). An artificial anus was constructed for aproctia on day 1. Trisomy 21 was detected by karyotyping analysis. Peripheral blasts disappeared rapidly, but eosinophilia did not improve. She showed neutropenia and thrombocytopenia from day 7, and she required several transfusions during the clinical course. Liver biopsy was performed on day 11, revealing cholestasis, mild hepatocyte swelling, and mild periportal fibrosis (Fig. 1C). Megakaryocytes were observed; we considered these residual TAM blasts. We administered LD-CA from day 15. Eosinophils in peripheral blood decreased to the normal level by day 18, but direct bilirubin increased to 3.9 mg/dL. Therefore, we added PSL 1 mg/kg/day on day 19. Three days later, laboratory indicators started to improve. She experienced CTCAE v4.0 grade 4 neutropenia, but it recovered spontaneously by day 22 without febrile neutropenia or any infection. On day 56, her blood tests had almost normalized, and she was discharged (Table 1A). Surgery for congenital heart disease was performed when she was 4 months old, and she remains alive and healthy 13 months after her TAM diagnosis.

Discussion

In TAM patients, higher WBC counts, ascites, preterm delivery, coagulopathy, failure of spontaneous remission, and elevated direct bilirubin levels are well-established risk factors of early death [1,4-6]. From previous reports, sinusoidal megakaryocyte infiltration in the liver is specific to TAM patients, often accompanied by extramedullary hematopoiesis. TAM blasts are known to produce cytokines, such as platelet-derived growth factor or transforming growth factor-β1, which are assumed to be the cause of severe organ fibrosis in TAM patients [1,2,4,9,10,12]. Therefore, we consider the megakaryocytes accompanied by fibrosis in the liver of TAM patients as TAM blasts. Fibrosis is considered the main cause of liver failure in TAM patients.

In several studies, early induction of LD-CA resulted in better outcomes in high-risk TAM patients; however, it remains unclear which patients should be treated with chemotherapy [7,8]. Historically, WBC counts >100,000 /μL have been considered almost an absolute indication for LD-CA therapy in TAM patients, but some studies have discussed risks in lower WBC count cases. Previous studies revealed that TAM cases with relatively low WBC counts (<100,000 /μL) or relatively low blast percentages (peak blast <10%; LBP-TAM) generally showed a more favorable prognosis without therapy [1,8]. Even in these groups, some cases develop liver fibrosis and/or become fatal. Yamato *et al.* [1] reported that liver biopsy in an LBP-TAM case with liver fibrosis revealed diffuse fibrosis without megakaryocytes. They concluded that even though patients with LBP-TAM generally have a good prognosis, careful monitoring of their liver function is needed.

There are two studies in which LD-CA was administered to TAM patients, including relatively-low-WBC-count cases, and both demonstrated the effectiveness of LD-CA. Klusmann *et al.* [6] reported a prospective observational study of TAM, and they recommended LD-CA for some patients (Table 2). They concluded that the cumulative incidence of death was significantly lower in the treatment group compared with that in the non-treated group. Flasinski *et al.* [7] conducted the TMD Prevention 2007 study, which was a prospective study of LD-CA for TAM patients with risk factors that aimed to prevent ML-DS after TAM. Their criteria for LD-CA administration are shown in Table 2. They compared the results with those of historical controls, and the cumulative incidence of death was extremely low in their group. They concluded that LD-CA intervention did not prevent ML-DS after TAM but improved the prognosis of TAM patients, suggesting that this therapy was effective in preventing early death.

In this paper, we present three cases of TAM at our hospital. All cases showed WBC counts <100,000 /μL on laboratory tests at diagnosis, but they had hepatosplenomegaly, and their direct bilirubin increased during the disease course. Liver biopsy revealed hepatitis with fibrosis and residual extramedullary hematopoiesis, and the pathological findings were more severe when the biopsy was delayed. Namely, in case 3, whose biopsy was performed on day 11, the fibrosis or hepatocyte swelling was mild compared with that in the other two cases, whose liver biopsy was performed on day 30 (case 1) or day 88 (case 2). Pathological findings were most severe in case 2, who showed extensive hepatocyte damage, giant multinucleated hepatocytes, and massive fibrosis. ALL patients were administered LD-CA and PSL; case 2 died of multiorgan failure, including severe liver fibrosis, but the other two recov-

ered and are alive. In cases 1 and 3, we administered LD-CA first because of residual TAM blasts in their liver, as LD-CA has been reported to be highly effective against them [4]. However, we could not detect megakaryocytes in the liver of case 2, suggesting there were no residual TAM blasts and only hepatitis progression. Therefore, we selected PSL before LD-CA; unfortunately, both were ineffective. The clinical course of case 2 suggests two potential explanations for his death: 1) LD-CA might have been ineffective after TAM blasts disappeared from the liver, or 2) LD-CA side effects increased because his liver function had declined so greatly. After we initiated PSL and LD-CA therapy, his liver dysfunction and coagulopathy progressed rapidly, and his renal function declined. He showed severe bone marrow suppression, and his WBC decreased to almost 0 /μL (Fig. 2). Some studies have suggested that LD-CA is not effective in TAM patients with end-stage liver dysfunction [4-8]. This case suggests the importance of starting chemotherapies before liver damage becomes irreversible, and placing strict limits on the "wait and watch" strategy recommended by some authors for cases with less extreme WBC elevation.

Here, we suggest that although LD-CA cannot prevent ML-DS after TAM, a strategy to prevent early death in TAM patients is needed, and determining sufficient criteria for LD-CA or PSL administration to these patients is important. Even in TAM cases with low WBC counts or low blast counts at diagnosis, liver fibrosis may progress if there are residual TAM blasts in the liver. Although liver biopsy is an invasive proce-

Table 2 Cytosine arabinoside administration criteria from a previous report

reference No.	CA administration criteria
[6] Klusmann *et al*. (Blood, 2006)	WBC > 50,000 /μL
	Plt < 100,000 /μL
	D. bil > 1.5 mg/dL
	AST/ALT > 2 times of SD
	Hepatomegaly (confirmed by ultrasound; more than 3 cm above the age adjusted norm
[7] Flasinski *et al*. (Blood adv, 2018)	**(1) at diagnosis**
	WBC > 100,000 /μL
	ascites
	liver dysfunction (hepatomegaly & elevated liver enzymes and/or cholestasis)
	hydrops fetalis
	(2) at wk8
	MRD (GATA1-PCR or FCM) +
	(3) no adequate response after (1) or (2)
	detectable blast and/or MRD + after treatment
	*LD-CA added up to 3 courses when it is not effective in 1) or 2) cases

WBC, white blood cell; Plt, platelet; Hb, hemoglobin; T. bil, total bilirubin; D. bil, direct bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; MRD, minimal residual disease; GATA-1, GATA binding protein 1; PCR, polymerase chain reaction; FCM, flow cytometry; LD-CA, low-dose cytosine arabinoside.

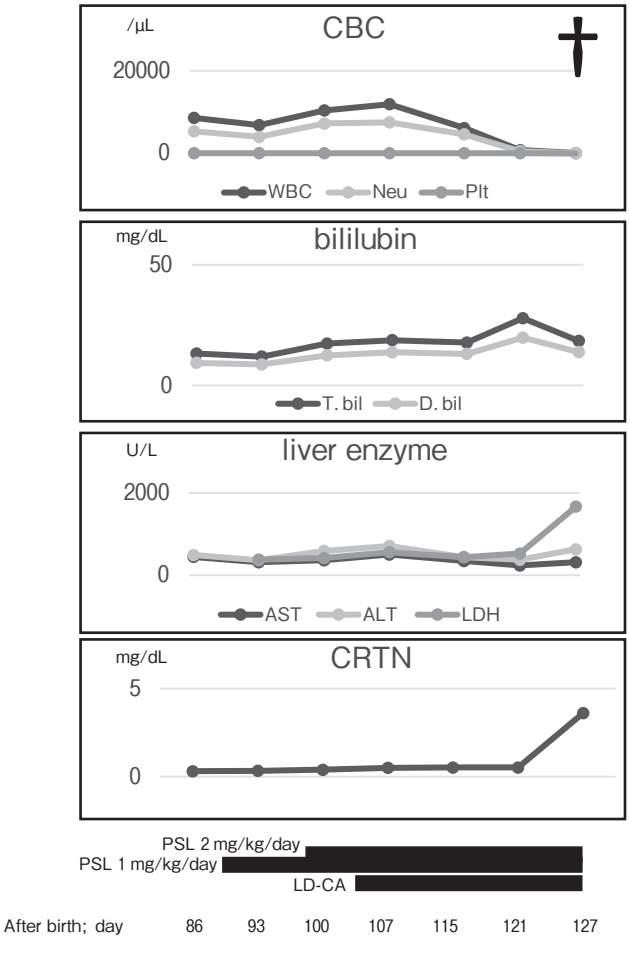


Fig. 2 Clinical course of case 2.

dure, and clinicians must beware of bleeding from thrombocytopenia or coagulopathy associated with TAM or liver dysfunction, it certainly provides useful information. For example, 1) liver biopsy may show residual megakaryocytes or extramedullary hematopoiesis, which suggest smoldering hepatitis in TAM, or 2) it can help rule out other liver diseases, such as non-syndromic paucity of interlobular bile ducts, neonatal hepatitis, biliary atresia, or hemochromatosis [13]. It is important to perform the surgery safely, and of course, performing biopsies for patients in severe conditions is particularly difficult. Therefore, administering LD-CA without liver biopsy to these patients is a better option. LD-CA may be ineffective for progressed or irreversible liver dysfunction but may possibly improve the prognosis of TAM patients when it is administered early. LD-CA can also be administered safely even in patients in severe conditions, such as hydrops fetalis or respiratory distress caused by TAM [3, 9]. Accordingly, LD-CA therapy provides a reasonable option for TAM patients with signs of liver dysfunction, even if their WBC count is lower than 100,000 /μL.

In summary, we experienced three TAM cases with WBC counts < 100,000 /μL at birth who developed liver fibrosis. Liver biopsy revealed residual TAM blasts or extramedullary hematopoiesis and suggested smoldering hepatitis caused by TAM blasts in their liver. We administered LD-CA and PSL to all three; one patient, for whom this treatment was delayed until 91, died from multiorgan failure, but two survived. As a strategy to prevent the early death of TAM patients, early administration of LD-CA should be considered for patients with liver dysfunction.

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