Continuous variables are expressed as the mean (range), and categorical variables were expressed as the number (%). For comparison between patients with hemophilia and those without hemophilia among the HCV-positive recipients, Wilcoxon's test and Fisher's exact test were used for continuous and categorical variables, respectively, as appropriate. Patient survival was compared using the log-rank test. A p value of less than 0.05 was considered to indicate statistical significance. All calculations were performed with SPSS statistical software (ver 22.0 for Windows, Chicago, IL, USA). This retrospective study was approved by the institutional review board.

Results

Perioperative characteristics

Grafts were procured from living donors in all cases, nine with a right liver graft and one with a left liver graft. The donors were female in seven and male in three recipients, with a mean age of 51 (35 - 62) years. The relationship was parent in five, spouse in two, sibling in two, and aunt in one. The donor of recipient #5, the mother of the patient, was a carrier, and recipient #9 was revealed to have low level of inhibitor to factor VIII (0.61 Bethesda Unit/ml), which required no bypassing agent. The mean amount of clotting factor intraoperatively administered was 9145 (4800 -17388) U. The mean intraoperative blood loss was 6833 (1900 - 26780) ml, comparable to the mean of 5775 (920 - 34800) ml among 134 HCV-monoinfection recipients without hemophilia who underwent liver transplantation at our institution during the same observation period (p=0.45).

Chronologic changes in clotting factor activity and the serum activated partial thromboplastin time

Chronologic changes in the clotting factor activity and serum activated partial thromboplastin time (APTT), and the dose of clotting factor replacement are presented in Figure 2, case-by-case. Changes in the serum total bilirubin levels are also plotted in the same figures. Continuous infusion of the clotting factor was successfully stopped per the protocol in seven patients, while

three required an extended period of continuous infusion or an additional bolus. The clotting factor activity and activated partial thromboplastin time were controlled within the target level without replacement treatment after liver transplantation in all cases except for case #5.

Postoperative course and long-term outcome

There was no surgical mortality, meaning all 10 patients survived more than 90 days after liver transplantation. Reoperation for bleeding was required in three cases (30%), a rate comparable to that among 134 HCV-monoinfection recipients without hemophilia (27/134, 20%; p=0.46). Hemostasis was achieved in these patients with an additional bolus injection of the clotting factor at the time of the reoperation. On the other hand, none of the patients experienced a thromboembolic episode due to the administration of excessive clotting factor. The mean total amount of the perioperative clotting factor replacement was 25450 (14200 - 41200) U.

Overall patient survival was 60% , and the cumulative survival at 1, 3, and 5 years after liver transplantation was 80%, 80%, and 67%, respectively, which was comparable to that of 134 HCV-monoinfection recipients without hemophilia who underwent liver transplantation at our institution during the same observation period (90%, 84%, and 81%, respectively; $p = 0.15$).

Discussion

Herein, we reported 10 consecutive case series of LDLT for patients with hemophilia, which is the largest cohort reported to date. The short-term result was satisfactory in terms of the management of clotting factor replacement in hemophilia recipients.

Several guidelines for the management of hemophilia patients, including intraoperative replacement therapy, have been published and indicate the desired level and duration of coagulation factors according to the type of hemorrhage or interventional/surgical procedure(5). These guidelines suggest only the desired trough level of clotting factors, however, and thus seem insufficient for major abdominal surgery, especially with respect to liver surgery. To the best of our knowledge, this is the first study to demonstrate the practical management of

perioperative clotting factor replacement among 10 consecutive adult LDLT recipients.

Adult LDLT for hemophilia patients may be highly challenging for hepatic surgeons, as meticulous preservation of the native vessels in the recipient often results in significant intraoperative bleeding and a partial graft from a living donor is not adequate for production of the clotting factor in the early postoperative days. On the other hand, excessive administration of the clotting factor may impair the hemostatic re-balance, resulting in thrombosis, which has a higher rate in LDLT than in DDLT. There are several reports of case series demonstrating the perioperative clotting factor replacement in DDLT for hemophilia patients (2, 3), in which the combination of the fixed bolus injection (i.e. [100-baseline] x body weight x 0.5) and maintenance continuous infusion (2 IU/kg/h) with meticulous monitoring of both coagulation panels and clotting factor level are recommended. According to these reports, the intraoperative hemostasis was well controlled, and continuous infusion was commonly discontinued within 24 hours with sufficient production of the clotting factor by the graft. On the other hand, the guidelines recommend that dosing decisions for coagulation factors should be made on an individual patient basis (5), and herein, we optimized the prophylaxis dosing based on individual pharmacokinetics. According to guidelines, it is recommended that the trough level of the clotting factor should be near 100% preoperatively in major surgery. Therefore we set the targeted trough level at 120% during extirpation of the native liver, however after reperfusion, the targeted trough level was set at 60% in fear of thrombotic complication due to excessive administration of clotting factors. This reduction of the targeted trough level after reperfusion may result in the slight increase in APTT in most cases presented in Figure 2, which recovered to normal along the next 24 hours. In addition, considering the relatively small size of the partial graft, continuous infusion was gradually tapered off within 72 hours after liver transplantation. While massive intraoperative blood loss (>20L) was encountered in two cases in the early period, intraoperative hemostasis was generally well controlled, and the clotting factor level and coagulation panel were maintained within a targeted level in the majority of cases. Generally, APTT values were kept stable near to the normal limit in the majority of cases during the postoperative period, however, the values of the activity of clotting factors were unstable. Many factors such as tapering of infusion dose, consumption, and the recovery of the synthetic capacity of the regenerating graft may influence the instability of clotting factor activity.

In conclusion, preoperative planning for the individualized optimization of clotting factor replacement strategy presented here resulted in a satisfactory perioperative outcome after LDLT for hemophilia patients. Our protocol for clotting factor replacement may improve the safety and efficacy of liver transplantation for ESLD patients with hemophilia.

Disclosures:

The authors have none to be disclosed.

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Figure legends

Figure 1. Protocol for perioperative clotting factor replacement

Abbreviation: BW, body weight; IVR, in vivo recovery;

LDLT, living-donor liver transplantation; POD, postoperative day

Figure 2. Changes in the clotting factor activity, serum activated partial thromboplastin time, and serum total bilirubin level. Amount of clotting factor administered (both bolus injection and continuous injection) is also presented case-by-case.

Abbreviation: APTT, activated partial thromboplastin time; FVIII, factor VIII; FIX, factor IX; LDLT, living-donor liver transplantation; POD, postoperative days; T-Bil, total bilirubin

Figure 1

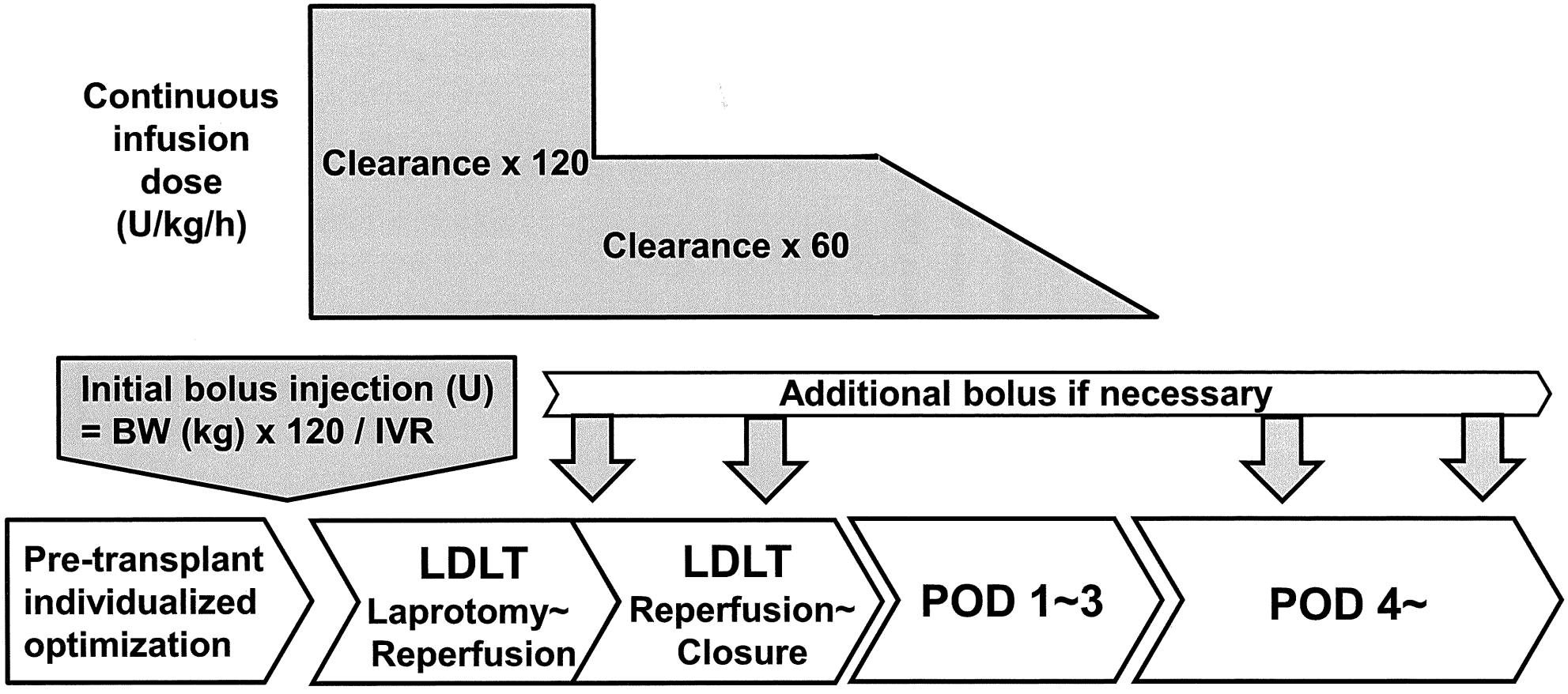
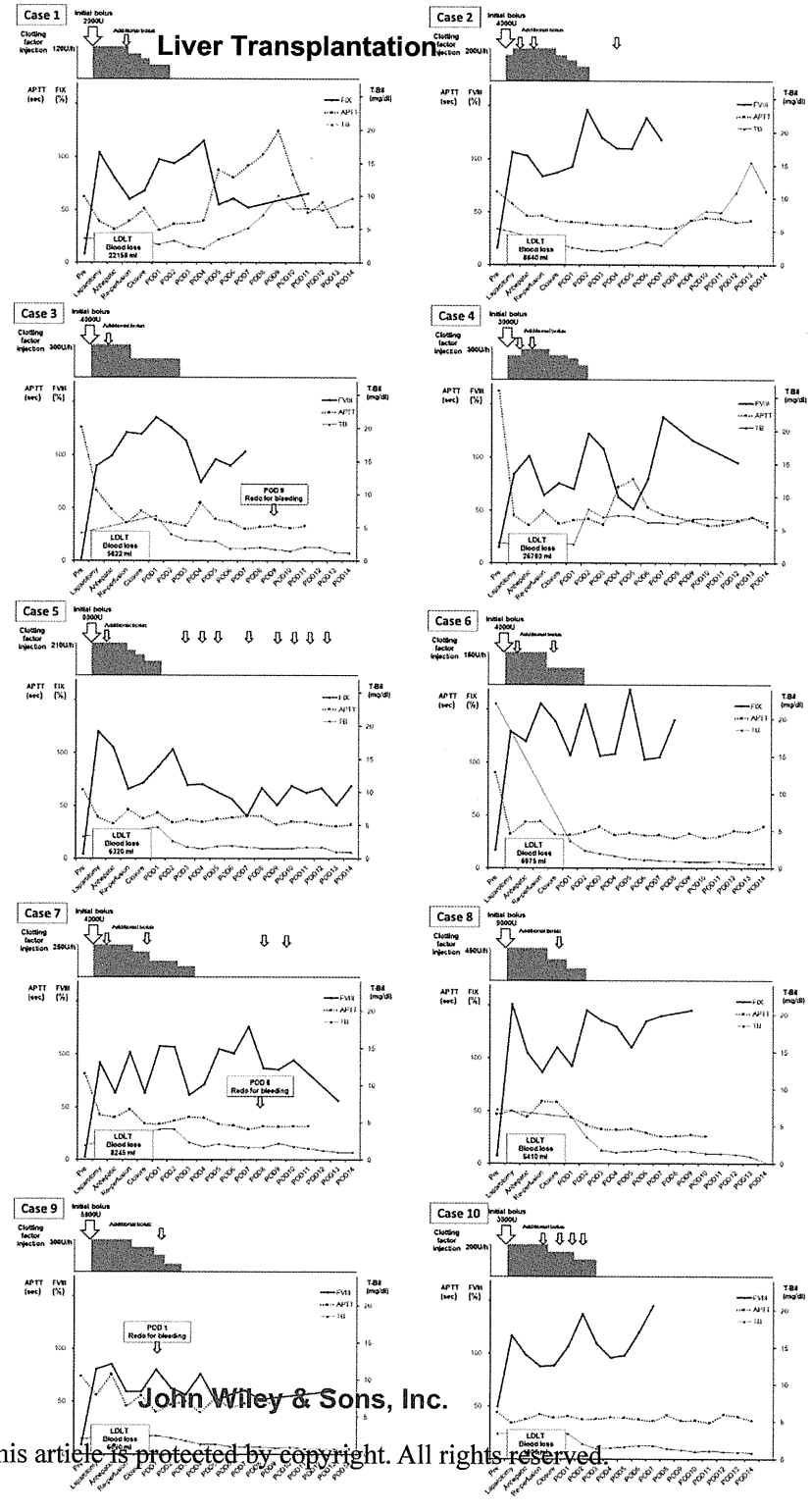
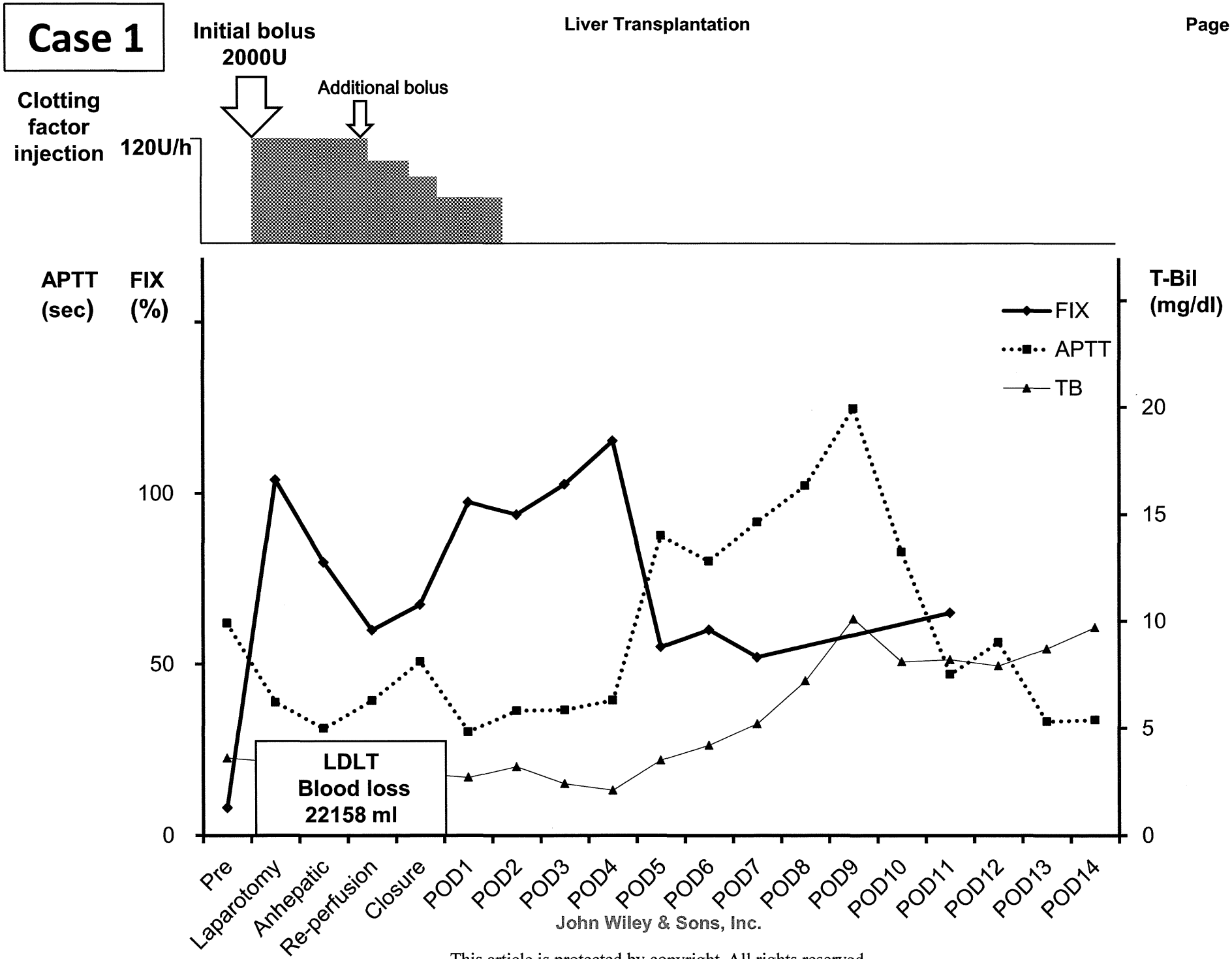
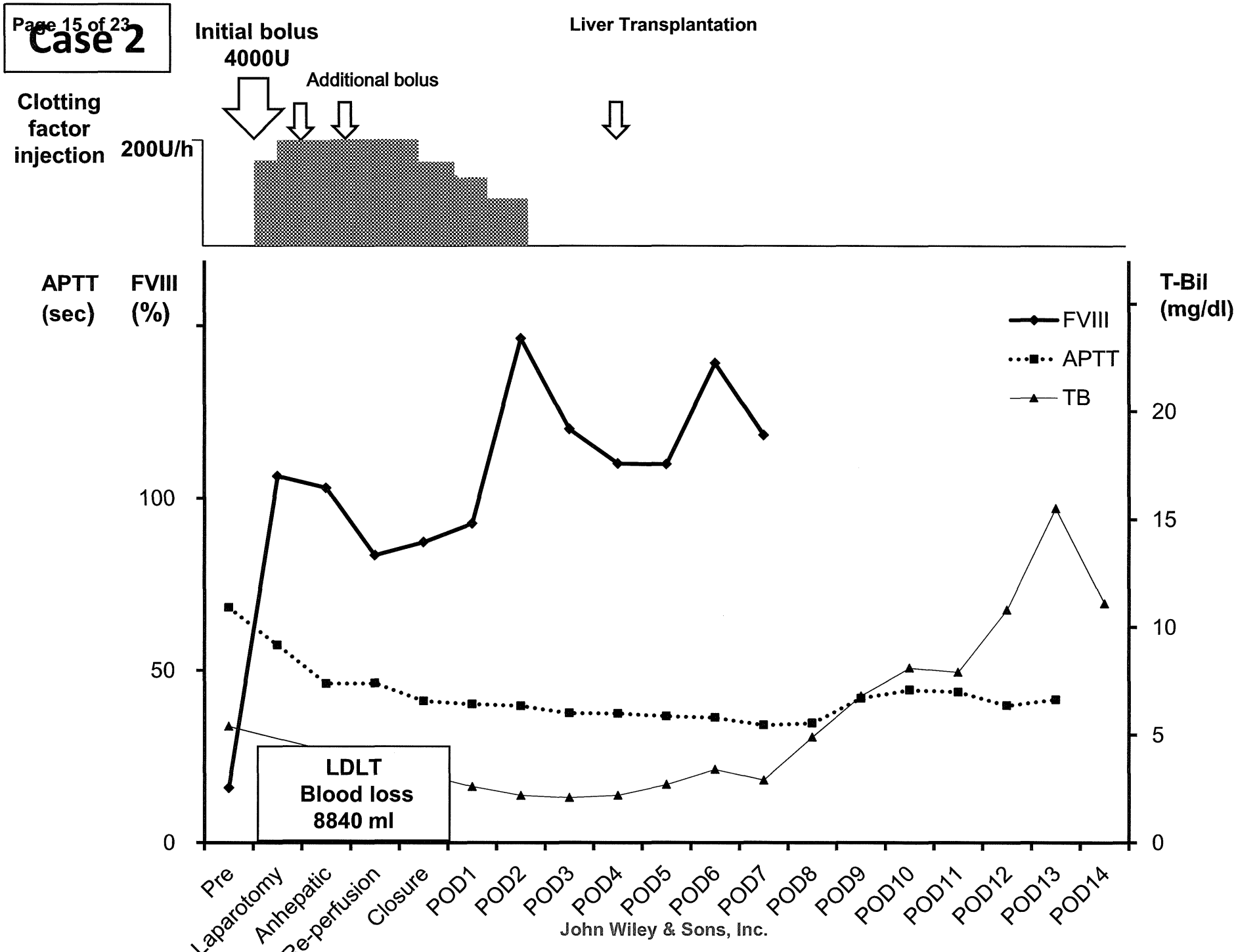


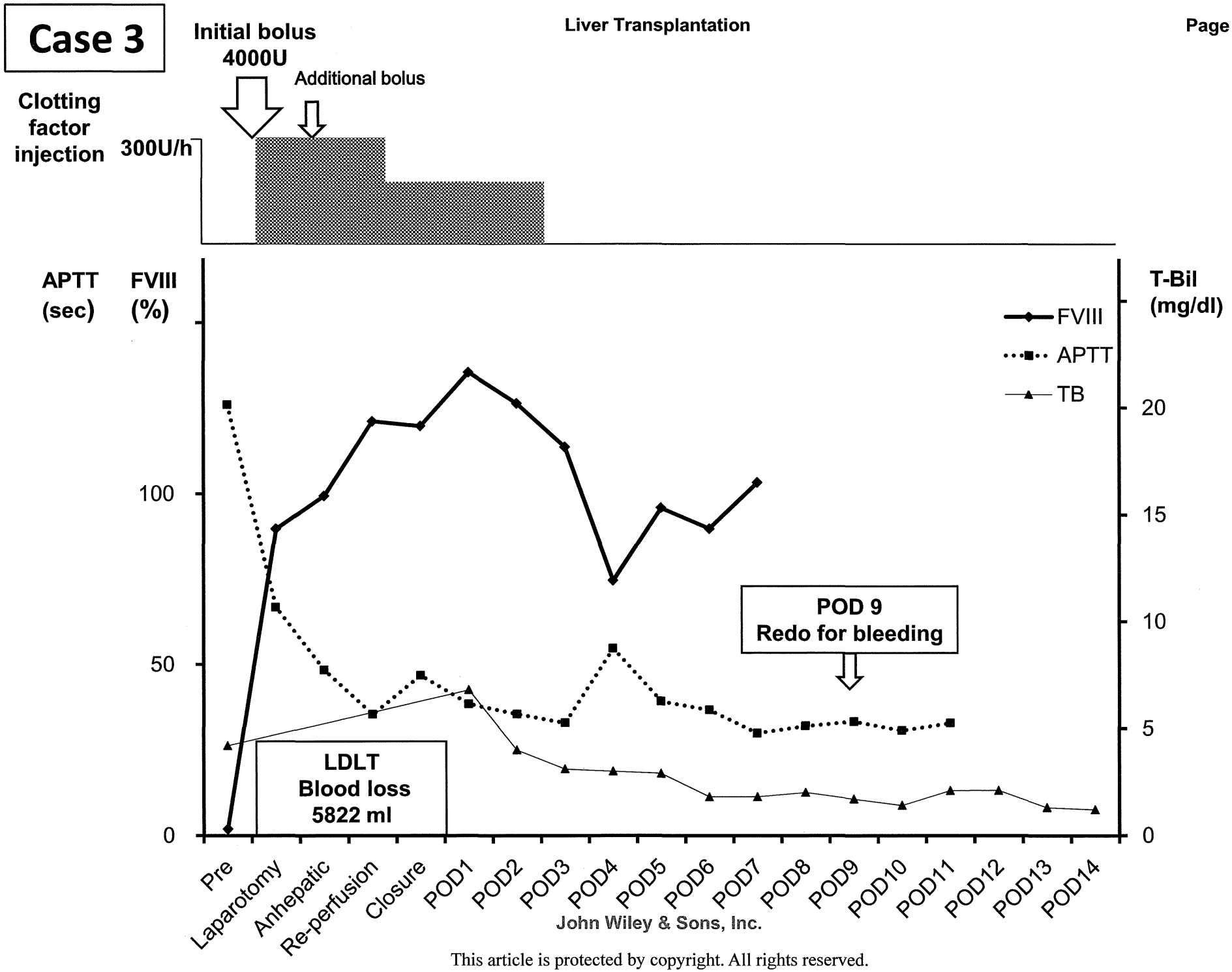
Figure 2





Case 2





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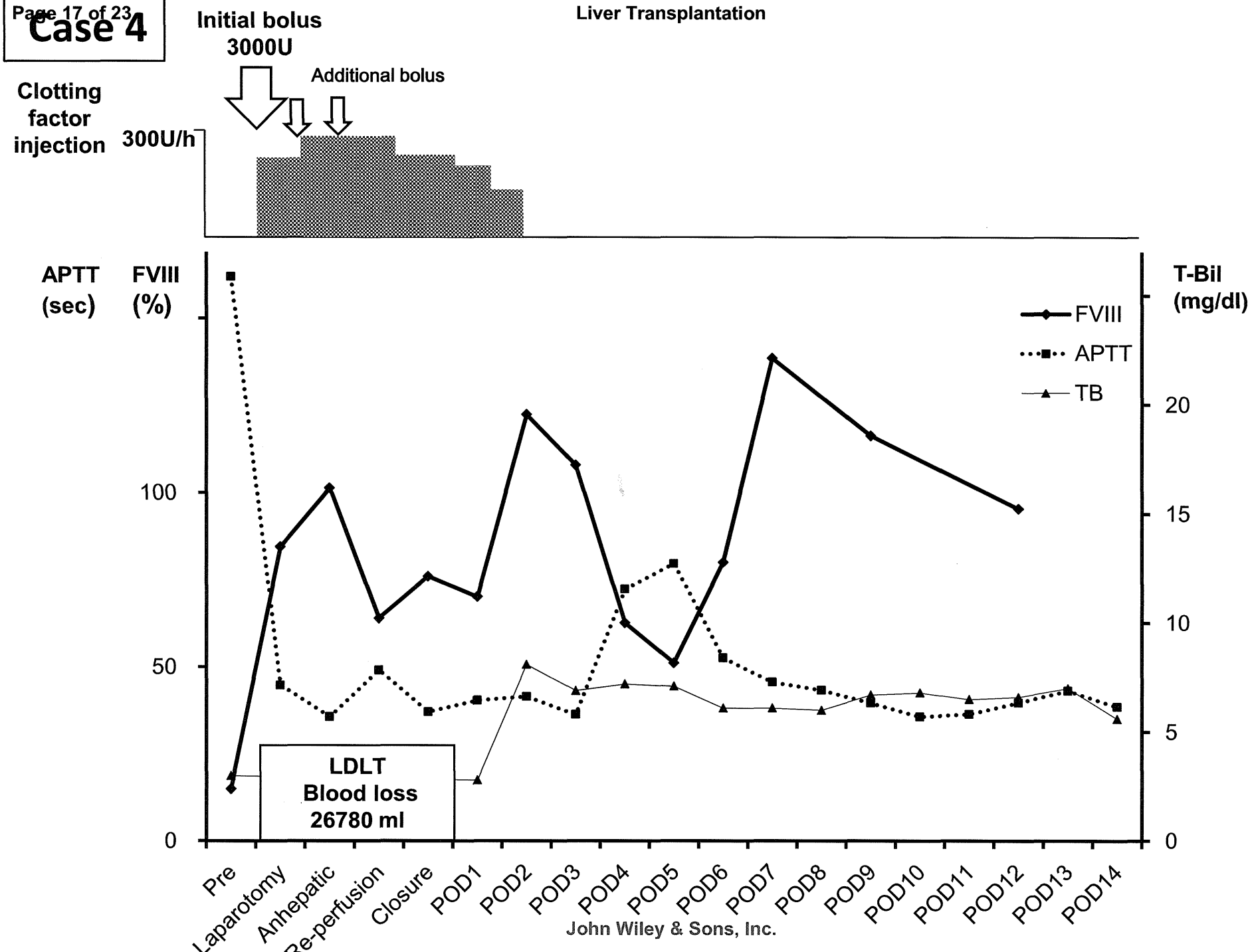
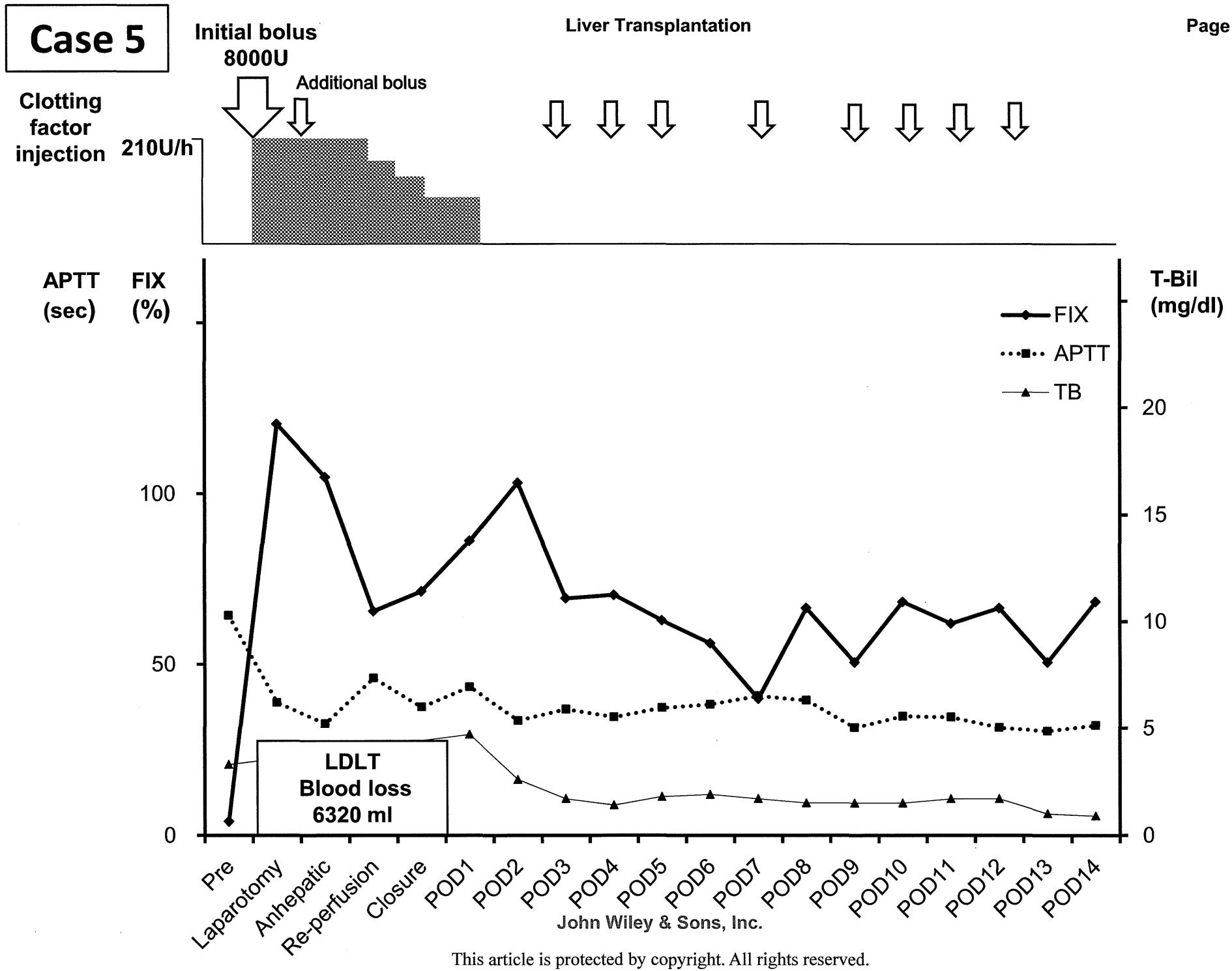
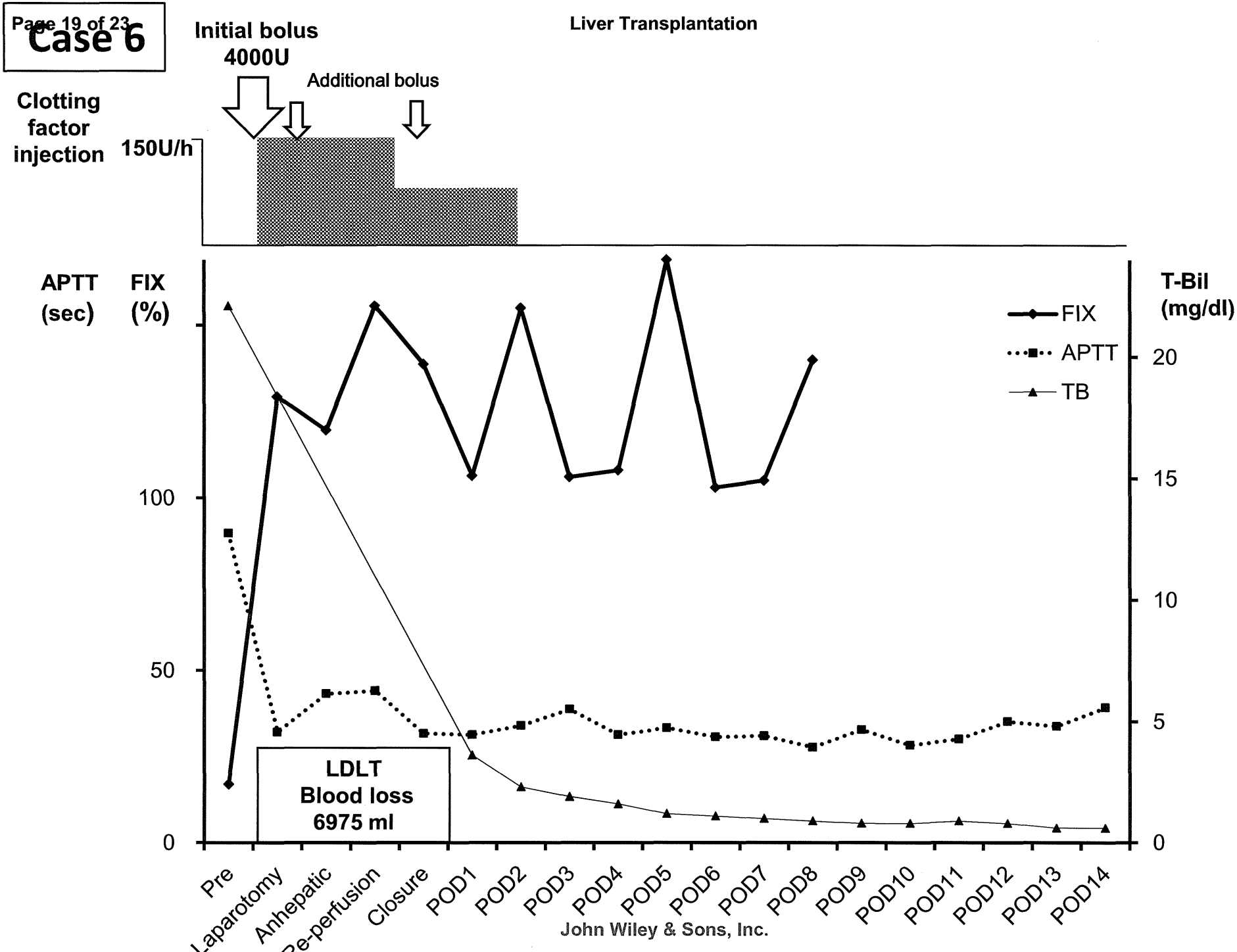
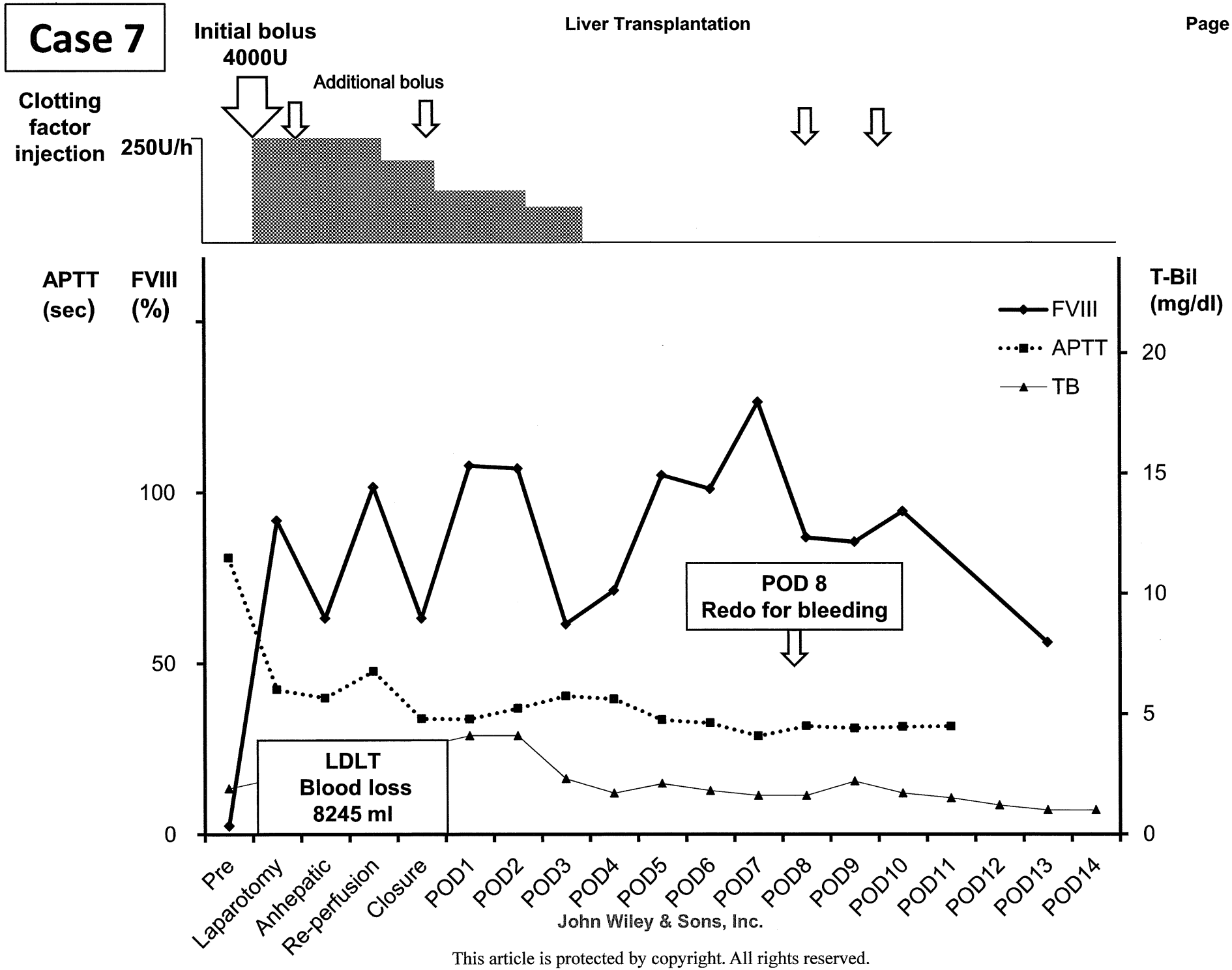




Case 6

Liver Transplantation





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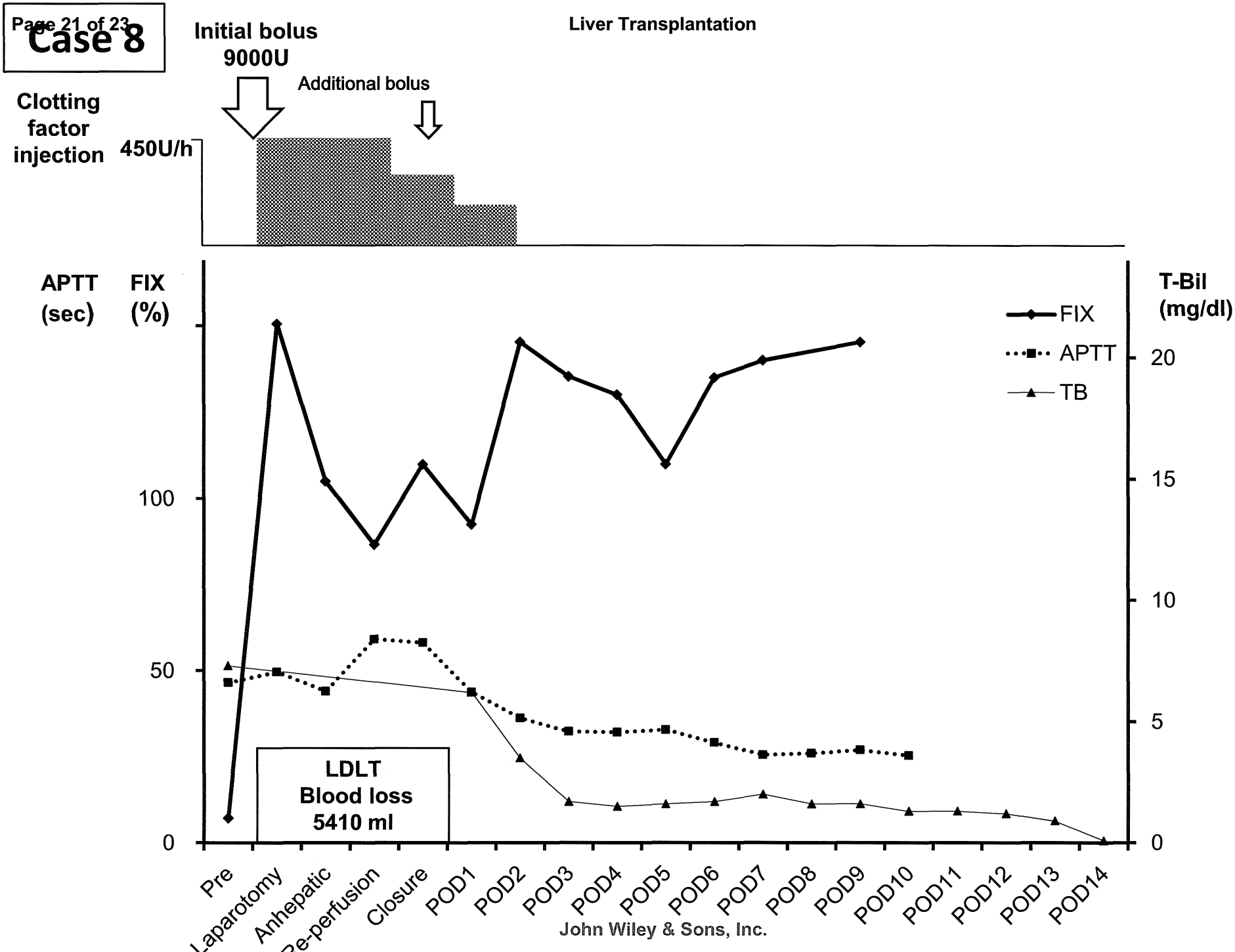
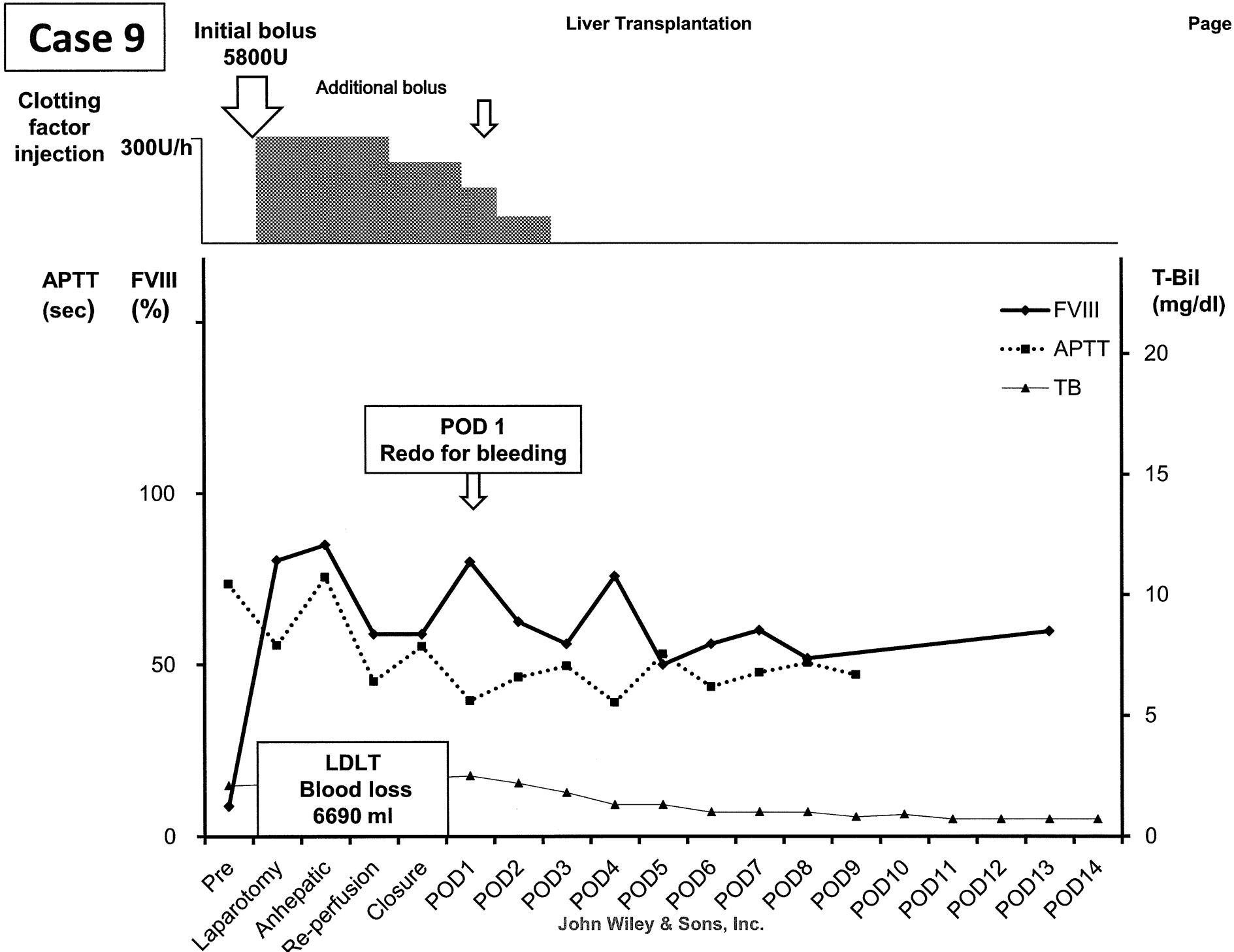
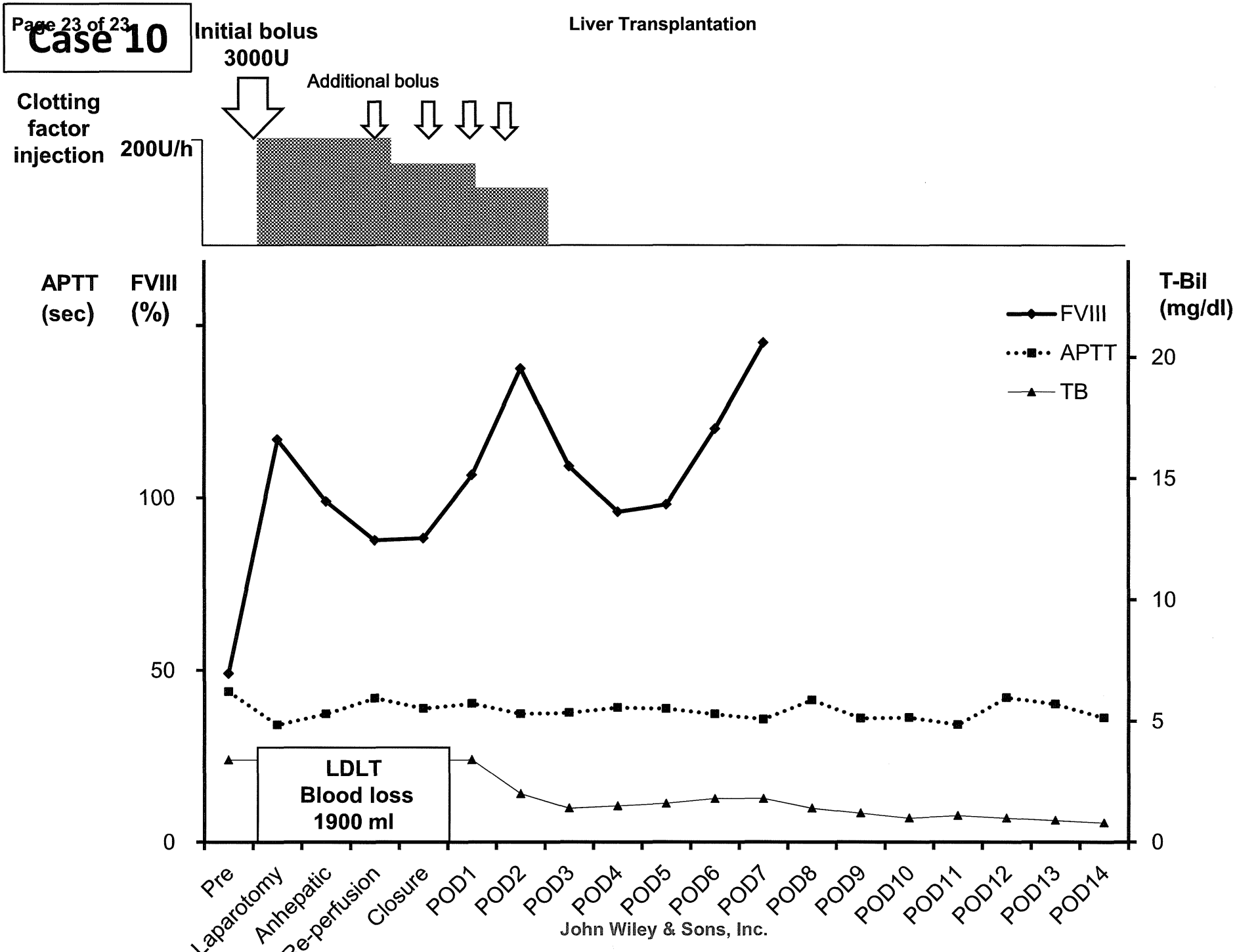




Case 10



Hepatology Research

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Original Article

Significance of miRNA-122 in chronic hepatitis C patients with serotype 1 on interferon therapy

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Aim: Peginterferon (PEG IFN) and ribavirin combination therapy is a curative treatment for chronic hepatitis C virus (HCV) infection, and virological response to IFN therapy has been strongly associated with genetic variation in *IL28B* single nucleotide polymorphisms (SNP). Recently, miRNA122 (miR-122), which is the most abundant miRNA in the liver, has been reported to be important for the replication of HCV RNA. Therefore, we investigated the correlation of miR-122 expression with virological response to IFN and other clinical data.

Methods: A total of 51 patients with HCV infection who were treated with IFN therapy at Nagasaki University Hospital from 2006 to 2011 were included in this study. We investigated the correlation of miR-122 expression in liver biopsy specimens with virological response to IFN therapy and other predictors of response, including IL28 SNP.

Results: miR-122 expression did not correlate with IL28 SNP. However, a significant difference was observed in miR-122 expression between patients who showed a sustained virological response (SVR) and those who did not ($P < 0.05$). Multivariate analysis indicated that miR-122 is an independent predictor of SVR.

Conclusion: miR-122 expression could be a marker for predicting the outcome of IFN therapy. Therapies targeting miR-122 may have positive effects not only by directly inhibiting viral propagation but also by ameliorating cholesterol and lipid abnormalities.

Key words: chronic hepatitis C, interferon, miRNA-122

INTRODUCTION

HEPATITIS C VIRUS (HCV) is a positive-strand RNA virus that has infected 170 million people worldwide. Once infected, 70–80% of patients experience persistent infection leading to repeated division of hepatocytes and ultimately fibrosis, cirrhosis and occasionally progression to hepatocellular carcinoma.[1,2] Therefore, the development of effective antiviral therapies against HCV is important. Treatment of chronic hepatitis C (CHC) infection has progressed from interferon (IFN) monotherapy to combination therapy with peginterferon (PEG IFN) and ribavirin (RBV), and direct-acting agents (DAA).[3–7] Such advances have improved the rate of sustained virological response (SVR) from 10% to 80% among CHC genotype 1 patients. Development of novel DAA is expected to further improve the prognosis of CHC patients. However, because of their severe adverse effects, not all patients can adapt to DAA. In the near future, we hope to be able to use newly developed DAA that do not have such severe side-effects. But such drugs have a problem for drug-resistance and possibility of viral mutation. So, despite the low SVR, combination therapy with PEG IFN and RBV may remain the one means of CHC treatment. To increase the cure rate as much as possible, factors capable of predicting SVR to CHC treatment should be identified. Numerous virus- and host-related factors are known predictors of SVR.[8–13] In 2009, a single nucleotide polymorphism

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(SNP) near *IL28B* was reported to be strongly associated with response to CHC treatment.[14]

miRNA are endogenous, small, non-coding RNA of approximately 21–22 nucleotides that have important gene regulatory functions in animals and plants; they bind to mRNA of protein-coding genes to direct their post-transcriptional repression.[15–17] miRNA are implicated in numerous biological processes and diseases, including viral infections and cancers.[18] They typically mediate their regulation by inducing mRNA destabilization or translational repression by binding to complementary sequences in the 3′-untranslated region (3′-UTR) of target mRNA.

miR-122 is a highly abundant, liver-specific miRNA that constitutes 70% of the total liver miRNA content. It positively modulates replication,[19] translation and virion production[20,21] by HCV by binding to the complementary target sequences in the 5′-UTR of HCV RNA.[19,22,23] Furthermore, sequestration of miR-122 in hepatoma cells by antisense oligonucleotides has been shown to decrease HCV replication and translation.[24,25]

Numerous *in vitro* studies have been performed, but few have examined the correlation of miR-122 with response to IFN therapy. A recent study of 42 patients who were seropositive for HCV RNA found that miR-122 was decreased in the livers of HCV patients, and that miR-122 correlated with clinical response to PEG IFN-α but not the HCV load.[26] We investigated whether miR-122 is a contributing factor to IFN treatment of CHC patients, and determined the correlation of miR-122 expression with virological response to IFN and other clinical parameters.

METHODS

Patients and clinical samples

CLINICAL DATA COLLECTED from the patients are listed in Table 1. Our study included 51 consecutive CHC patients who were treated with IFN therapy at Nagasaki University Hospital from January 2006 to April 2011. Forty-six patients in this study received PEG IFN-α-2b and RBV combination therapy, while five patients underwent PEG-IFN-α-2a and RBV combination therapy. Treatment duration ranged 48–72 weeks.

Twenty-two patients (43%) reduced IFN dose by neutropenia, and 21 patients (41%) reduced RBV dose by hemolytic anemia. Every case could continue IFN and RBV therapy. Adherence to IFN ranged 42–170% (mean, 98%) and adherence to RBV ranged 35–168% (mean, 98%). Twenty-one patients (41%) could achieve IFN adherence over 80%. Forty-eight patients (94%) could achieve RBV adherence over 60%.

Table 1 Clinical characteristics

Characteristics	Mean, number	Standard deviation (SD)
Age (years)	57.4	9.26
Sex (F/M)	20/31	
BMI (kg/m^2)	23.2	3.42
WBC (cells/μL)	4762	1292
PLT (×10^4 platelets/μL)	17.41	5.765
AST (IU/L)	56.09	32.70
ALT (IU/L)	80.27	58.11
γ-GT (IU/L)	52.51	52.57
Albumin (g/dL)	4.102	0.294
TC (mg/dL)	174.08	23.94
TG (mg/dL)	100.68	38.92
LDL-C (mg/dL)	96.43	21.10
HDL-C (mg/dL)	51.64	12.45
FFA (mEq/L)	0.438	0.409
PreAlb (mg/dL)	19.52	4.708
HbA1c (%)	5.409	0.948
HCV RNA (logIU/mL)	6.2	1.15
Staging (F0/1/2/3/4)	8/18/12/8/4	
Activity (A0/1/2)	2/37/7	
IFN adherence (%)	98 (42–170)	
IFN adherence, >80%	21 (41%)	
RBV adherence (%)	98 (35–168)	
RBV adherence, >60%	48 (94%)	
IFN response (SVR/TVR/NR)	29/12/10	
IL28B rs8099917 (TT/TG/GG)	38/13/0	

Normal values in laboratory tests: body mass index (BMI) calculated as bodyweight (kg)/height (m)^2; white blood cell count (WBC, cells/μL), 3500–9000; platelets (PLT, ×10^4 platelets/μL), 12–33; aspartate aminotransferase (AST, IU/L), 10–40; alanine aminotransferase (ALT, IU/L), 5–40; γ-glutamyltransferase (γ-GT, IU/L), <70 in males, <30 in females; albumin (Alb, g/dL), 4.0–5.0; total cholesterol (TC, mg/dL), 128–220; triglyceride (TG, mg/dL), 38–150; low-density lipoprotein cholesterol (LDL-C, mg/dL), 70–139; high-density lipoprotein cholesterol (HDL-C, mg/dL), 40–80; free fatty acid (FFA, mEq/L), 100–800; pre-albumin (preAlb), 22–40; hemoglobin A1c (HbA1c), <5.8%.
HCV, hepatitis C virus; IFN, interferon; NR, undetectable HCV RNA; RBV, ribavirin; SD, standard deviation; SVR, sustained virological response.

The study conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Informed consent was obtained from each patient. Thereafter, a liver specimen was obtained by echo-guided liver biopsy. All liver biopsy tissue specimens were examined using hematoxylin–eosin, Azan–Mallory and silver reticulum staining. The specimens were assessed by one reviewer