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A Basic Evaluation of LIASAUTO™ P-FDP and LIASAUTO™ D-dimer Neo in the Automated Blood Coagulation Analyzer CN-6000

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Fibrin-fibrinogen degradation products (FDP) and fibrin degradation products (D-dimer or DD) are used in routine clinical laboratory testing for diagnoses of thrombosis and disseminated intravascular coagulation (DIC) and monitoring their course of treatment. In this study, we performed a basic evaluation of the LIASAUTO™ P-FDP (LiaFDP; Sysmex Corporation, Kobe Japan) and the LIASAUTO™ D-dimer Neo (LiaDD; Sysmex) in the automated blood coagulation analyzer CN-6000 (CN-6000; Sysmex). Evaluation results were compared to those of reference reagents in patients with abnormal levels of FDP.

The performance of within-run precision, linearity, limit of detection, and interference was acceptable, and had sufficient performance for daily use.

Regarding correlation with the reference reagent, good correlation was obtained for both FDP and DD, with noted discrepancies. In FDP, the reagents studied tended to be higher or lower than the reference reagent, which was caused by the difference in the reactivity of the antibodies used for both reagents to each fraction of fibrin/fibrinogen degradation product. In DD, there was a discrepancy in which the reagents studied also tended to be higher than reference reagent, which was caused by the difference in the reactivity of the antibodies used in both reagents to the small molecule DD/E fraction.

The reactivity of both reagents was almost the same in both cases of DIC with suppressed fibrinolysis due to sepsis and enhanced fibrinolysis with acute promyelocytic leukemia. We believe the degree of fibrinolytic activation can be estimated by observing the balance of LiaFDP and LiaDD.

FDP and DD may differ in reactivity between reagents and should be used with a full understanding of the reactivity of the reagents used.

Key Words Fibrin and Fibrinogen Degradation Products (FDP), Fibrin Degradation Products (D-Dimer), CN-6000

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INTRODUCTION

Fibrin–fibrinogen degradation products (FDP) and fibrin degradation products (D-dimer or DD) are used in routine clinical laboratory testing. They aid in the diagnosis of thrombosis and disseminated intravascular coagulation (DIC) and monitoring course of treatment for both conditions.[1, 2] DIC is a syndrome characterized by organ injury and bleeding tendencies due to an activated blood coagulation–fibrinolysis system caused by various underlying conditions, such as sepsis and leukemia. Although FDP and DD levels are important parameters in monitoring the course of DIC treatment, there are no standardized FDP or DD assays for this purpose. Currently, available tests are known to yield inconsistent results depending on the reagents used.[3] We evaluated the basic performance of the fibrin/fibrinogen degradation product kit LIASAUTO™ P-FDP (LiaFDP; Sysmex Corporation, Kobe, Japan) and the fibrin degradation product kit LIASAUTO™ D-dimer Neo (LiaDD; Sysmex) on the fully automated blood coagulation analyzer CN-6000 (CN-6000; Sysmex). Reagent evaluation results were then compared with those obtained from reference reagents selected for this study with respect to abnormally high values of FDP. Evaluation findings, case studies and product reviews will be discussed in this paper.

Notes: This article is based on current regulatory requirements in Japan. (as of June, 2022)
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MATERIALS AND METHODS

1. Study samples and reagents

We studied 283 patient-derived blood samples submitted to the Department of Laboratory Medicine at Osaka Medical and Pharmaceutical University (OMPU) Hospital for testing. They were treated with 3.2% sodium citrate and centrifuged at 2,000 g for 10 min to harvest plasma. This study was conducted with prior approval from the Ethics Committee of OMPU (approval number 2627).

2. Analyzers

The CN-6000 was used to conduct the evaluation. Analyzer A was used to perform assays with reference reagents.

3. Assay reagents

FDP levels and DD levels were analyzed with LiaFDP and LiaDD, respectively (study reagents), and with FDP reagent A and DD reagent A, respectively (reference reagents). For additional information, the following markers were assessed: prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen (Fbg), antithrombin (AT), α2 antiplasmin (α2AP), thrombin–antithrombin complex (TAT), soluble fibrin monomer complex (FMC), and plasmin-α2-plasmin inhibitor complex (PIC). ***Table 1*** summarizes the reagents used for given assays and parameters.

4. Evaluation

(1) Within-run precision

Plasma sample pools of low, medium, and high concentrations for FDP and DD were prepared and measured in 10 replicates. The percent coefficient of variation (CV%) was calculated from the mean and standard deviation (SD) of the measured values.

(2) Dilution linearity

An 11-point dilution series was prepared with 10-step dilutions from high-concentration samples using the fibrinolysis diluent supplied in the reagent kit. The resultant sample at each point was assayed in duplicate. The theoretical value was established as the mean of the observed values at the highest point within the range not exceeding the upper limit of the assay range set by the manufacturer. Based on this theoretical value, the percent deviation was calculated for the mean of the observed values obtained at each point.

(3) Limit of detection

A six-point dilution series was prepared with five-step dilutions from low-concentration samples using the fibrinolysis diluent. Ten replicate measurements were performed on the resultant sample at each point. The limit of detection (LOD) was established as the measurement at the lowest point, where the mean minus 2.6 SDs did not overlap with the mean plus 2.6 SDs of the zero-concentration sample value.

Table 1 *Reagents used*

Parameter	Analyzer	Reagent	Manufacturer/ distributor
FDP (under evaluation)	CN-6000	LiaFDP	Sysmex
FDP (control)	Analyzer A	FDP reagent A	Manufacturer A
DD (under evaluation)	CN-6000	LiaDD	Sysmex
DD (control)	Analyzer A	DD reagent A	Manufacturer A
PT	Analyzer A	PT reagent A	Manufacturer B
APTT	Analyzer A	APTT reagent A	Manufacturer B
Fbg	CN-6000	Thrombocheck Fib (L)	Sysmex
AT	CN-6000	Revohem AT	Sysmex
α2AP	CN-6000	Revohem α-2 antiplasmin	Sysmex
TAT	Analyzer A	TAT reagent A	Manufacturer A
FMC	CN-6000	Auto LIA FM	Sysmex
PIC	CN-6000	LIASAUTO PIC*	Sysmex

*This reagent is normally not intended for use on CN-6000.

(4) Influence of interfering substances

A six-point dilution series was prepared with five-step dilutions from the pooled plasma samples spiked with Interference Check A Plus (Sysmex; including hemolytic hemoglobin, bilirubin C, bilirubin F, and chyle). Measurements were made in duplicate at each point. The percent change in the measurement of each diluted sample from that of the unspiked sample was calculated to assess the influence of these interfering substances.

(5) Correlation

The patient-derived samples (n = 283) submitted to our laboratory for FDP or DD assay as routine testing were evaluated using the study reagents (LiaFDP and LiaDD) and reference reagents (FDP reagent A and DD reagent A). The aim was to determine the correlation of study reagent assay results with the reference reagent results. To validate differences in inter-reagent reactivity, the following markers were analyzed: PT, APTT, Fbg, AT, α2AP, TAT, FMC, and PIC. A western blotting assay was used on samples with poor correlation to confirm the presence of FDP and DD fractions.

(6) Confirmation of the study reagents' reactivity in different diseases

Of the samples tested for correlation, some longitudinally obtained samples from patients with different conditions were assessed for disease status and the time course of test marker values.

RESULTS

(1) Within-run precision

For both LiaFDP and LiaDD, the CV% of within-run precision for low-, medium-, and high-concentration samples was below 5% (***Table 2***).

(2) Dilution linearity

The observed values with LiaFDP were within ±10% of the theoretical range values up to approximately 120 μg/mL. These values were consistent with the upper measurement range limit of 120 μg/mL set by the manufacturer. The measured values with LiaDD were also within ± 10% of the theoretical values, which met the manufacturer's set limit of 100 μg/mL; however, the measurement at point 10/10 yielded > 115 μg/mL, which exceeded the upper limit of the calibration range (***Fig. 1***).

(3) Limit of detection

The LOD for LiaFDP was 0.7 μg/mL, which was consistent with the lower limit of the manufacturer's set measurement range of 2.5 μg/mL. The LOD for LiaDD was 0.4 μg/mL, which also met the manufacturer's set limit of 0.5 μg/mL (***Fig. 2***).

(4) Influence of interfering substances

As for LiaFDP, the percentage change in the measurement from the unspiked sample was below 5% for the following: hemolytic hemoglobin in the range up to 490 mg/dL, bilirubin C up to 21.2 mg/dL, bilirubin F up to 19.1 mL/dL, and chyle up to 1,610 FTU (Formazine Turbidity Unit). Measurements with LiaDD were not influenced by the presence of the following: hemolytic hemoglobin up to 490 mg/dL, bilirubin C up to 21.2 mg/dL, bilirubin F up to 19.1 mg/dL, and chyle up to 1,610 FTU. Percentage change-based assessment was not conducted for LiaDD because the measured values with this reagent were low ranging from 1.4 to 1.6 μg/mL, but differences from the unspiked sample values all remained within 0.2 μg/mL (***Fig. 3***).

Table 2 *Within-run precision*

Parameter	FDP		
Sample	Low conc.	Medium conc.	High conc.
Unit	μg/mL	μg/mL	μg/mL
N	10	10	10
Mean	2.65	22.87	34.92
SD	0.108	0.298	1.303
CV%	4.08	1.30	3.73

Parameter	DD		
Sample	Low conc.	Medium conc.	High conc.
Unit	μg/mL	μg/mL	μg/mL
N	10	10	10
Mean	1.32	14.61	40.98
SD	0.063	0.120	0.852
CV%	4.79	0.82	2.08

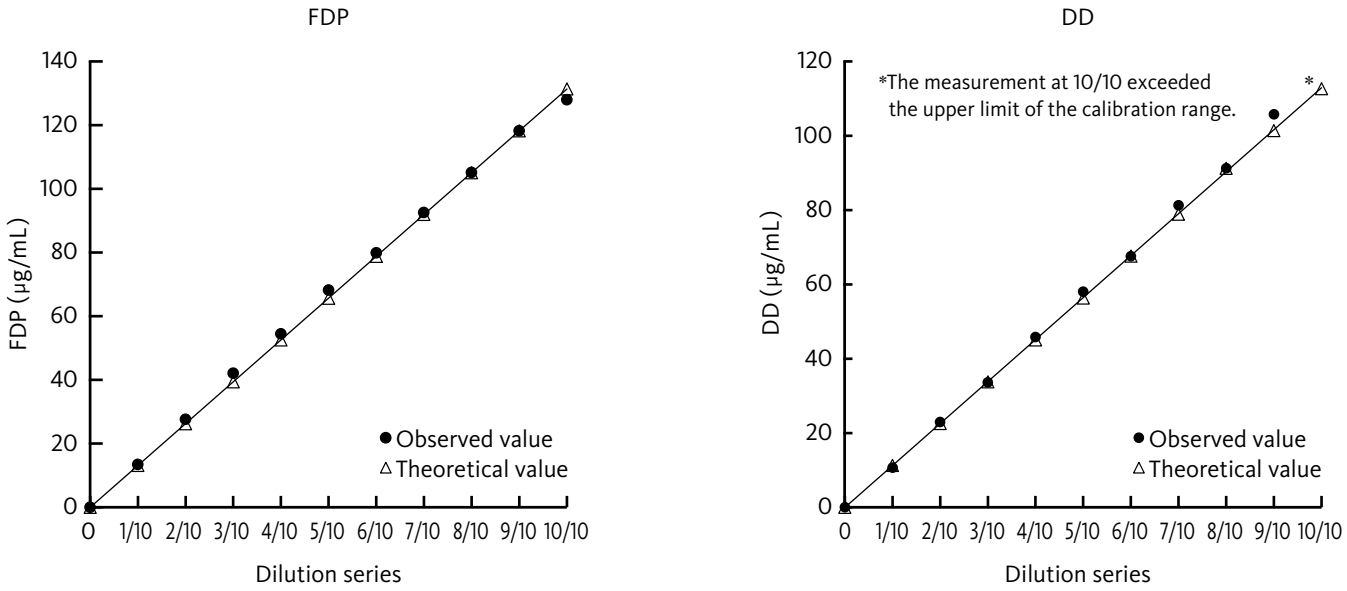


Fig. 1 *Dilution linearity*

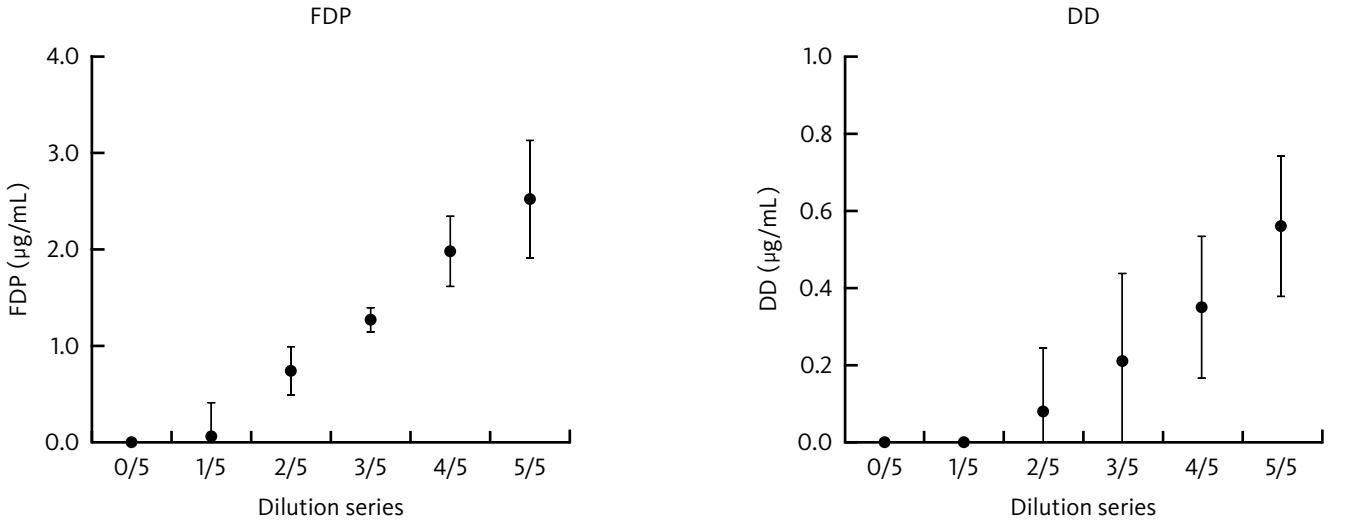


Fig. 2 *Limit of detection*

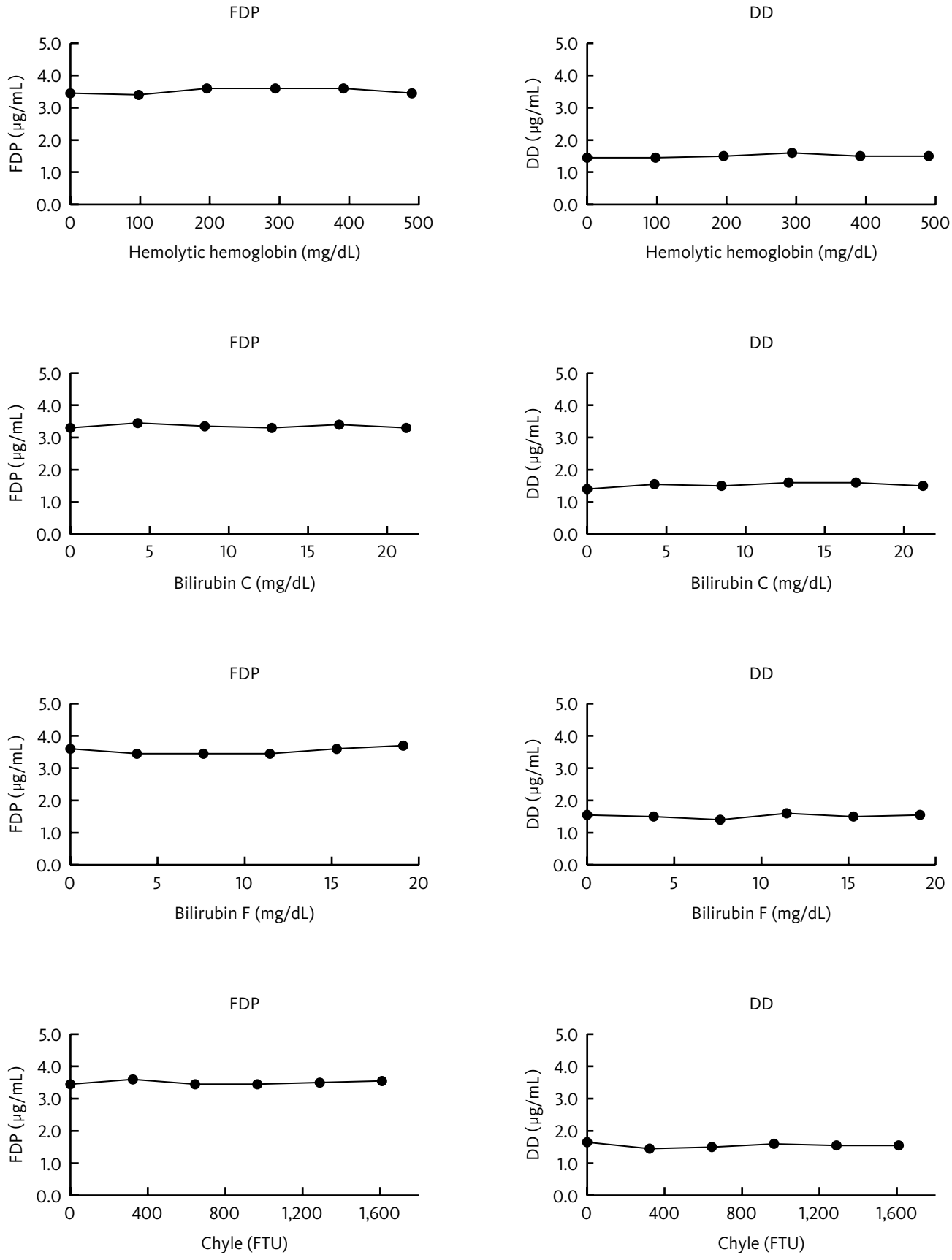


Fig. 3 *Influence of interfering substances*

(5) Correlation

For FDP, the correlation of the study reagent with the reference reagent was y = 1.12x − 5.65, r = 0.967 for all samples tested (n = 283). The correlation for the samples of FDP concentrations was ≤120 μg/mL (n = 244), y = 0.90x + 1.98, r = 0.962. Although the correlation coefficient was good at ≥ 0.96, some outliers were present (***Fig. 4***). For DD, the correlation of the study reagent with its control was y = 1.30x − 0.66, r = 0.964 for all samples tested (n = 283). The correlation for the samples of DD concentrations was ≤100 μg/mL (n = 270), y = 1.20x + 1.30, r = 0.961. The correlation coefficient was good at ≥ 0.96 but not without outliers (***Fig. 4***).

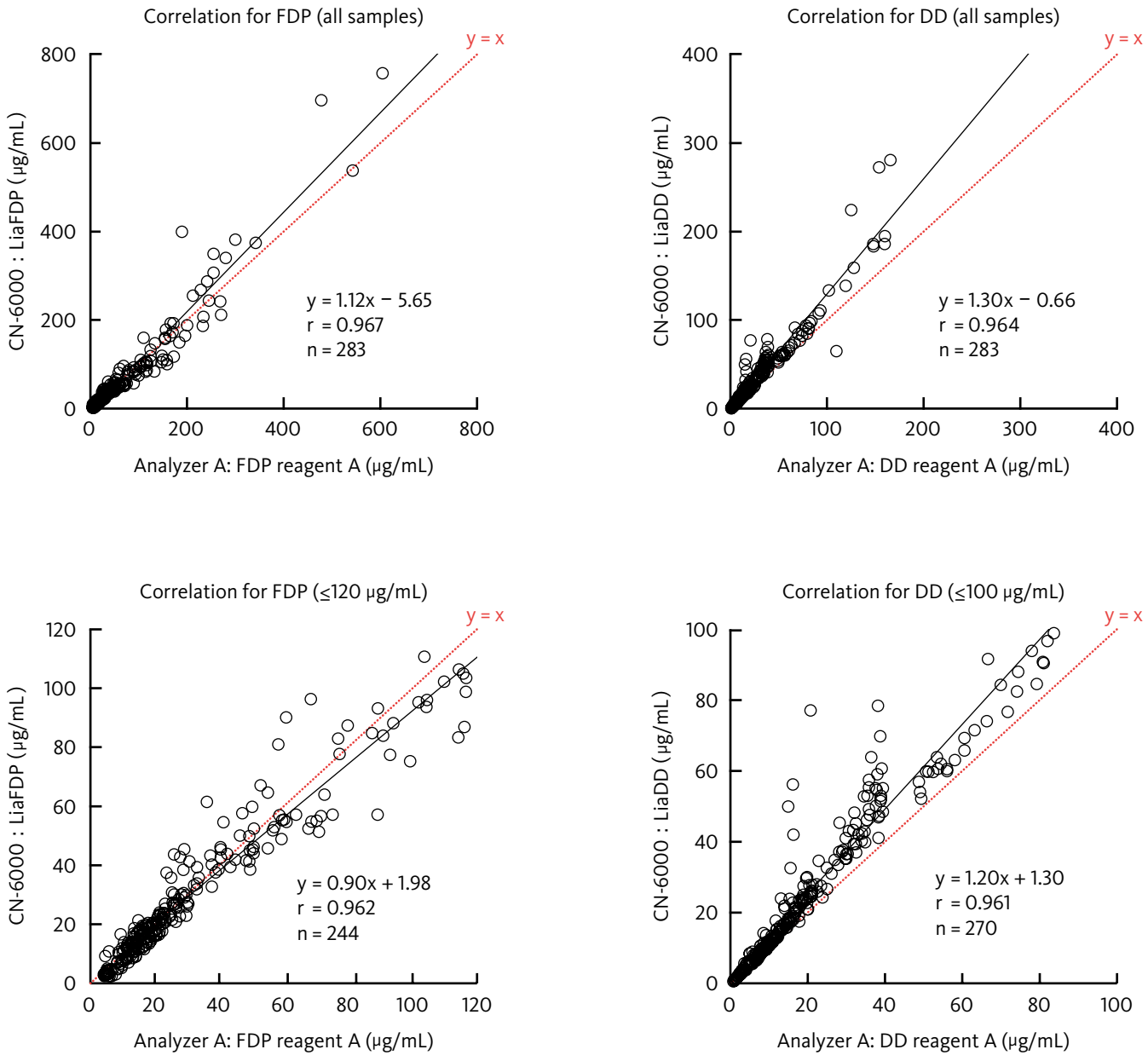


Fig. 4 *Correlation*

The correlation for FDP values between the study and the reference reagents was examined using the scoring system employed in the diagnostic criteria for DIC by the former Japanese Ministry of Health and Welfare (JMHW) and those by the Japanese Society on Thrombosis and Hemostasis (JSTH; 2017 version).[4] The scoring results were roughly in agreement with the two reagents in the range between 0 to 3 points (***Fig. 5***).

Fig. 6 provides detailed data on the outlier samples observed in the correlation analysis for FDP and DD (the numerals represent sample numbers). The outliers in the FDP assay included cases of both higher and lower measurement tendencies with LiaFDP not observed with the reference reagent. Samples exhibiting a tendency of higher values with LiaFDP were those from patients with various diseases including the following: thoracoabdominal aortic aneurysm and left thalamic hemorrhage (4-12), acute aortic dissection (17-2), deep-vein thrombosis (21-1), bladder cancer (34-2), stomach cancer (41-4, 41-5), abdominal aneurysm rupture (44-2), acute myelogenous leukemia (AML; 46-1), and myelodysplastic syndrome (longitudinal samples 47-1 and 47-3).

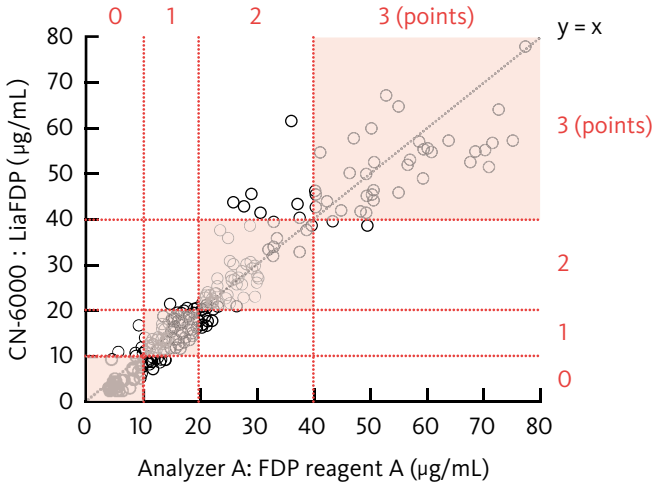


Fig. 5 *Correlation for FDP (comparison based on DIC diagnostic criteria scoring)*

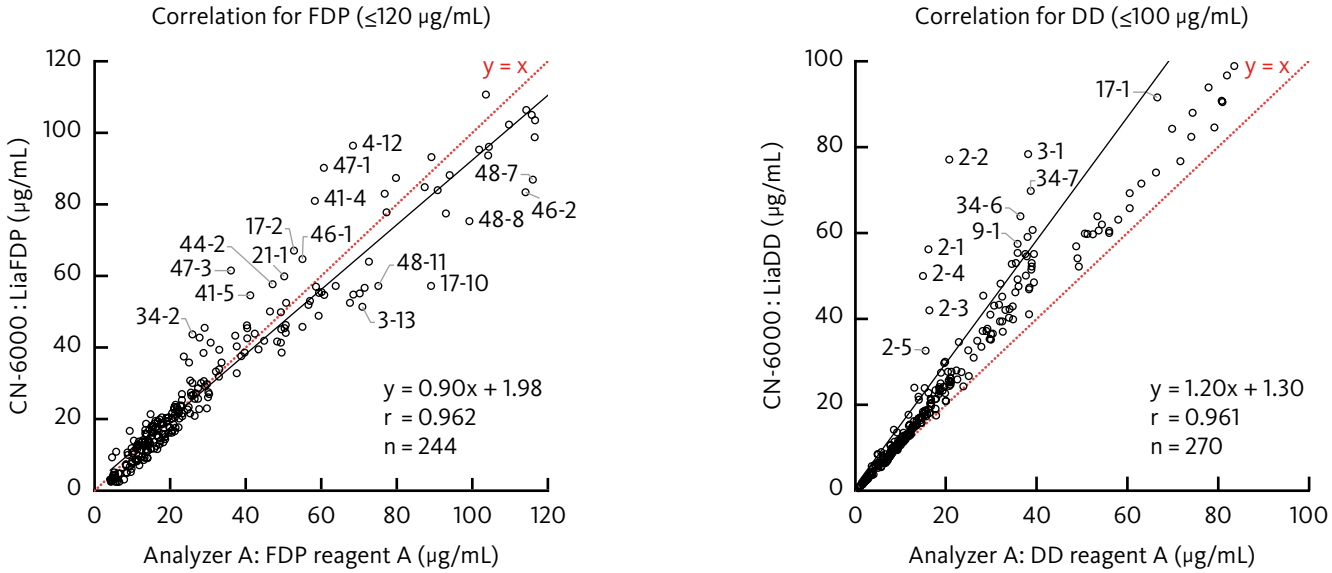


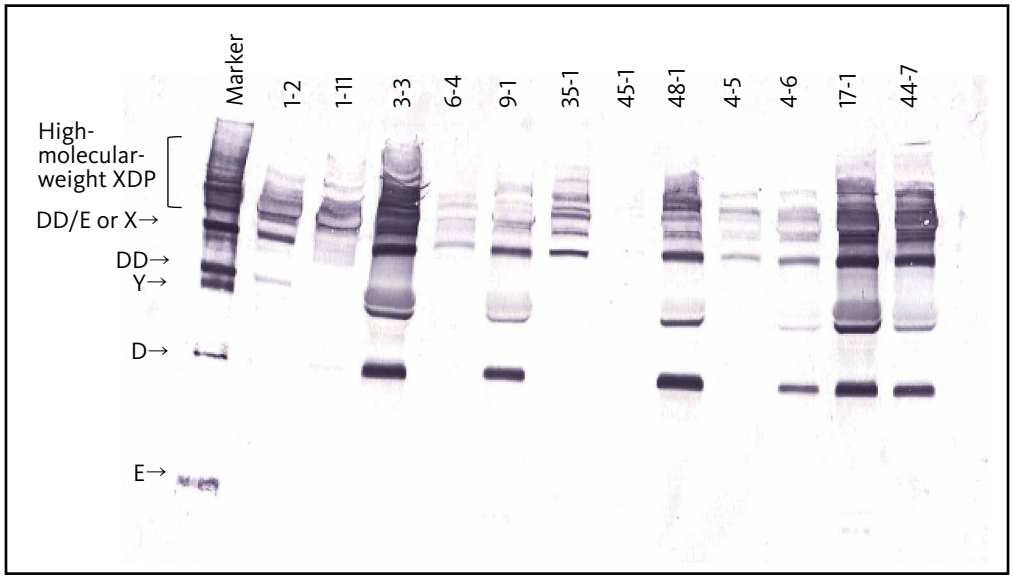
Fig. 6 *Details on some outlier samples*

Samples showing a lower measurement tendency with LiaFDP were those from patients with the following: sepsis (3-13), acute aortic dissection (17-10), AML (46-2), and acute promyelocytic leukemia (APL; longitudinal samples 48-7, 48-8, and 48-11). Samples 46-1 and 46-2 were from a patient with AML. Sample 46-1 was obtained 1 day earlier and displayed a higher measurement tendency with LiaFDP. With respect to outliers in DD measurements, assays with LiaDD yielded a higher result tendency compared to the reference reagent. Outliers included the following: 2-1 to 2-5 longitudinal samples from a patient with AML-M5, samples from patients with sepsis (3-1 and 9-1), acute aortic dissection (17-1), and longitudinal samples from a patient with bladder cancer (34-6 and 34-7). Most of these samples exhibited high values of TAT, FMC, and PIC (data not shown). Samples 9-1 and 17-1 were subjected to western blotting and the presence of low-molecular-weight DD/E fractions was confirmed (***Fig. 7***). Western blots also confirmed the presence of D fractions in other samples with sizable deviations between LiaFDP and LiaDD test values (3-3, 4-6, 9-1, 17-1, 44-7, and 48-1). These samples had a poor correlation between FDP and DD levels assayed with the reference reagents as well.

(6) Study reagent reactivity between different diseases

Case 1 (male in his 70s with sepsis)

The patient had pneumococcal pneumonia which was being managed by his previous doctor. The patient then developed respiratory distress syndrome and was urgently referred to OMPU Hospital. The intensive care unit provided respiratory and systemic control with the antimicrobials, meropenem + vancomycin + levofloxacin, upon admission to OMPU.



Parameter	Unit	1-2	1-11	3-3	6-4	9-1	35-1	45-1	48-1	4-5	4-6	17-1	44-7
LiaFDP	μg/mL	42.8	13.5	757.9	17.0	211.9	110.7	27.0	340.9	45.4	84.0	399.5	187.4
LiaDD	μg/mL	25.0	12.8	272.5	10.9	57.5	96.7	13.6	159.1	48.5	47.3	91.6	111.1
FDP reagent A	μg/mL	27.8	18.2	604.0	13.4	270.2	103.6	30.3	279.9	40.4	90.8	188.7	232.4
DD reagent A	μg/mL	18.8	11.2	153.6	9.7	35.8	82.0	9.2	127.5	39.4	38.4	66.6	92.9
PT	%	41.0	85.0	36.0	26.0	76.0	45.0	101.0	62.0	93.0	90.0	64.0	69.0
APTT	sec	360.0	33.9	No data	51.0	36.7	65.8	29.8	33.4	28.5	24.2	229.9	No data
Fbg	mg/dL	375.3	588.0	99.8	133.9	397.1	634.8	165.4	127.6	458.8	469.2	129.6	238.0
AT	%	35.4	66.6	39.4	73.8	57.5	46.2	62.3	98.4	87.7	97.2	62.6	51.2
α2AP	%	53.1	98.5	42.6	50.5	88.5	99.1	57.5	48.4	100.8	103.8	56.6	46.3
TAT	ng/mL	82.2	1.6	83.4	12.1	14.6	13.0	5.3	65.6	6.5	>90	>90	18.7
FMC	μg/mL	>150	6.0	>150	10.0	>150	94.2	35.4	>150	15.0	>150	>150	>150
PIC	μg/mL	1.3	2.7	16.7	3.7	15.7	2.4	2.4	23.7	3.0	9.1	46.9	14.2

Fig. 7 *Details on some outlier samples (listing of western blotting assay values and marker values)*

Fig. 8 gives the longitudinal courses of his marker values with day 0 as the hospital admission day. For FDP and DD, study and reference reagent assays both yielded high values, which remained elevated above their respective reference ranges through day 30. The study and the reference reagents showed similar trends. The patient's PIC values stayed near 2.0 μg/mL (reference range < 0.8 μg/mL), and α2AP remained above 80%. His TAT and FMC values were high up to day 2 and then stayed near the upper limit of their respective reference ranges thereafter.

Case 2 (male in his 30s with APL)

The patient was referred to the hematology department at OMPU Hospital for intraoral hemorrhage, upper limb purpura, and pancytopenia.

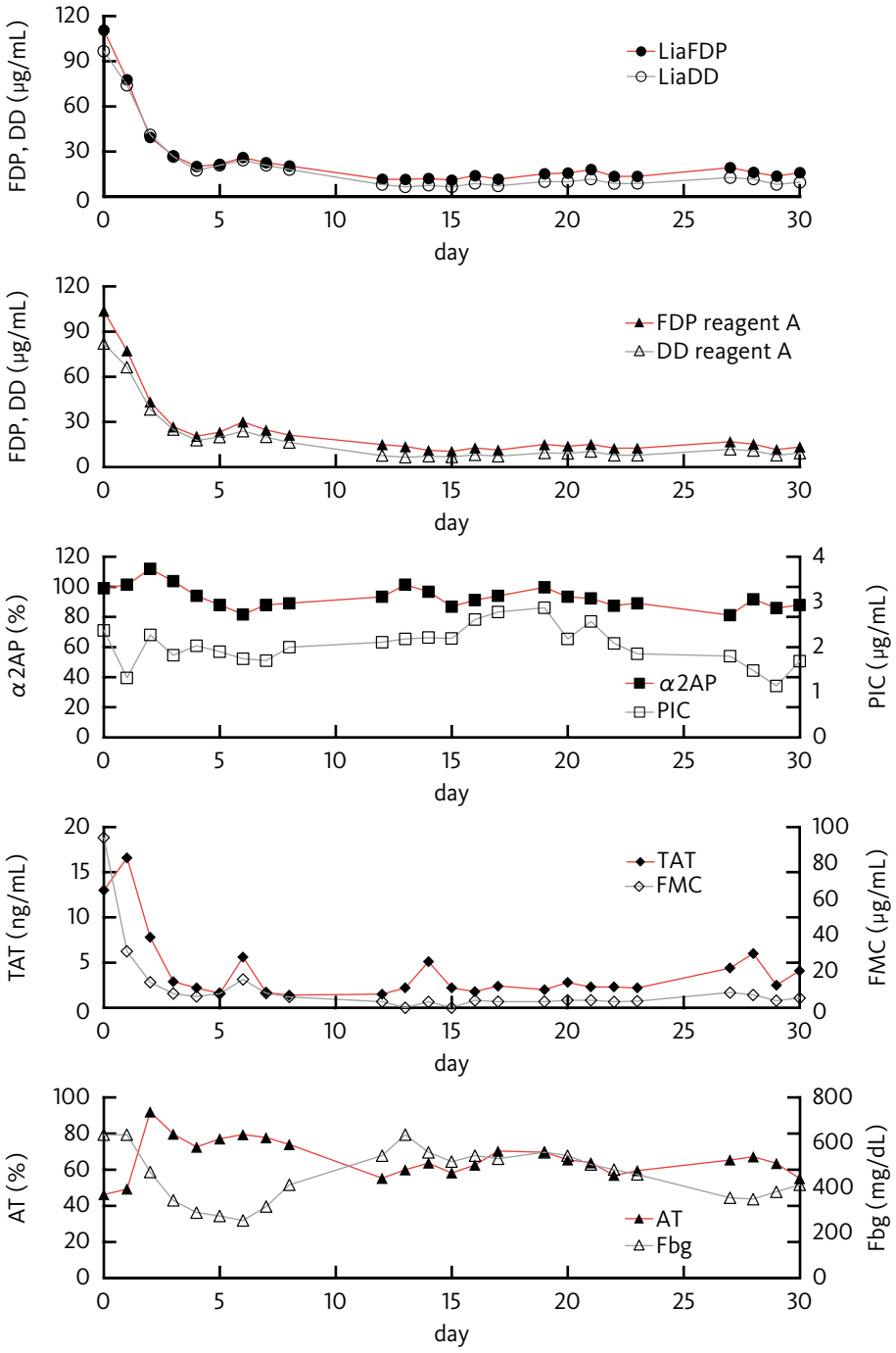


Fig. 8 *Comparison of reagent reactivity (Case 1: male in his 70s with sepsis)*

Fig. 9 displays the longitudinal course of his marker values with day 0 as the hospital admission day. On day 1, all-trans retinoic acid therapy was initiated. On day 5, fresh frozen plasma and platelet transfusions were administered. His FDP and DD values remained markedly increased since day 0 with differing trends up to day 14. Decreased tendencies followed, and the study and reference reagents exhibited similar patterns thereafter. His PIC values were especially high through day 14 and they remained elevated beyond the recommended reference range up to day 25. His α2AP values, measured along with the PIC, stayed below their reference range up to day 14. On day 15, abnormal cells in his peripheral blood were no longer present. His TAT and FMC values remained elevated up to days 14 and 19, respectively.

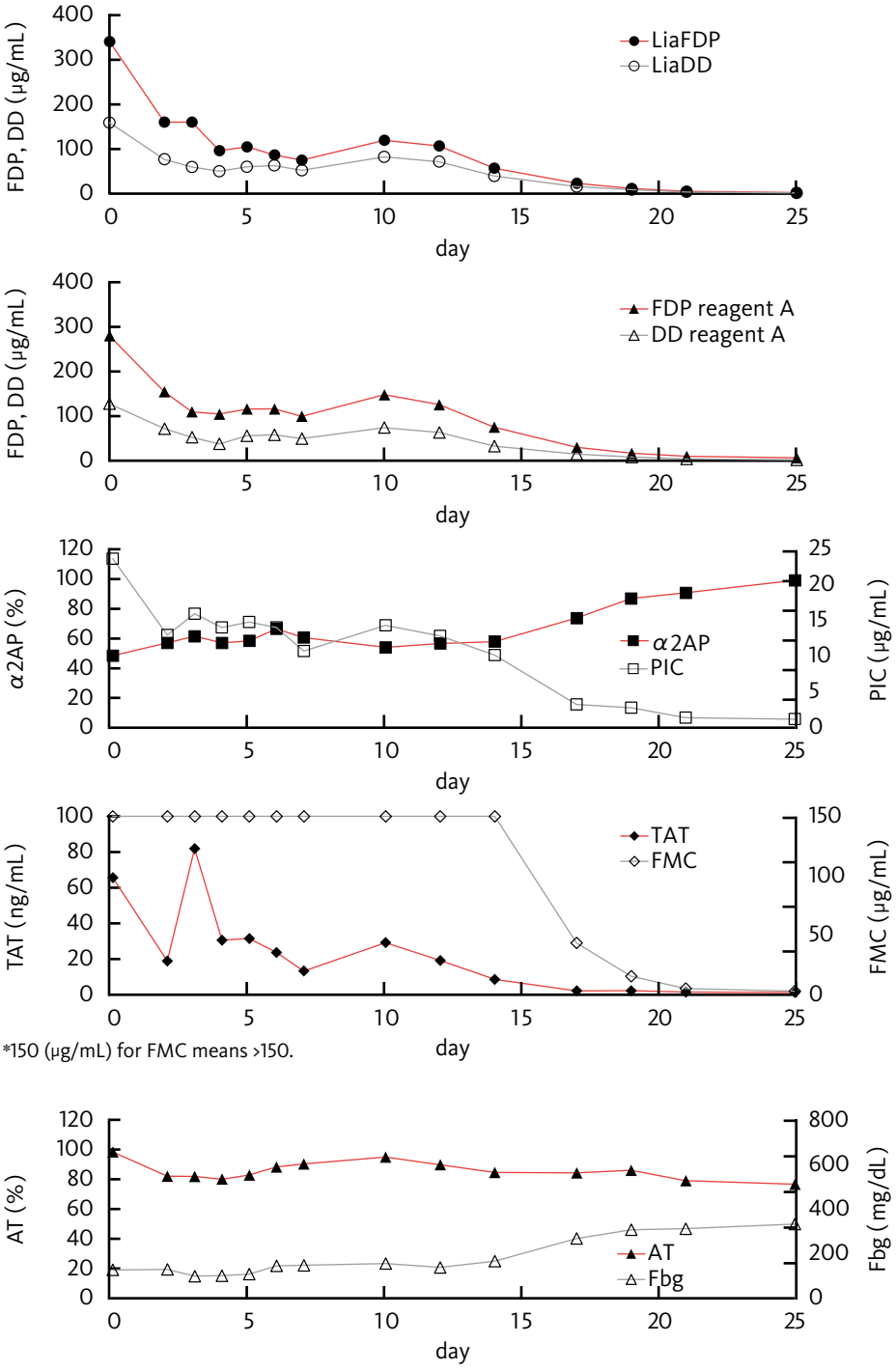


Fig. 9 *Comparison of reagent reactivity (Case 2: male in his 30s with APL)*

DISCUSSION

In this basic performance study for LiaFDP and LiaDD on the CN-6000 analyzer, the products exhibited good within-run precision, dilution linearity, and LOD. Findings showed no evidence of influence by interfering substances. Both reagents demonstrated sufficient performance in routine testing tasks.

The correlation of FDP values between the study reagent assay and the reference reagent assay was $y = 1.12x - 5.65$, $r = 0.967$ for all samples tested ($n = 283$). The correlation of FDP sample concentrations was ≤120 μg/mL ($n = 244$), $y = 0.90x + 1.98$, $r = 0.962$. Although this correlation is not a standardized parameter, the findings support the possible utility of the study reagent in the diagnosis of DIC. The assay results of the study reagent and the reference reagent were relatively close and consistent with DIC diagnostic criteria scoring proposed by the former JMHW and by the JSTH (2017 version). The inter-reagent correlation was good overall. Several outlier cases were identified with LiaFDP results showing either higher or lower values than the reference reagent.

The 2017 version of the JSTH's DIC diagnostic criteria mentions possible difficulties for some reagents to detect plasma FDP in cases of highly activated fibrinolysis with advanced fibrin/fibrinogen degradation levels. The detection issue is due to reagent reactivity variations to plasma FDP.[4] The outlier samples noted in the present study all exhibited elevated values of TAT, FMC, and PIC, suggesting a state of enhanced coagulation–fibrinolysis with advanced fibrin/fibrinogen degradation. Depending on the samples tested, LiaFDP exhibited different tendencies, i.e., higher and/or lower value tendencies compared to results with the reference reagent. This was attributed to differences in the reactivity (potency) of antibodies in each reagent to fractions of FDP that vary based upon sample type and content percentage. Ota and associates reported high reactivity of LiaFDP to the D and E fractions as a reason for higher measurement results with this reagent. They also reported that some manufacturers' reagents have enhanced reactivity to the X fraction and/or with high responsiveness to the D fraction. The investigators identified the need for users to be aware of such different characteristics of reagents.[5] Given these findings, reagent reactivity differences may account for the poor correlation with some sample observed in the present study.

For DD, the correlation in assay results between the study reagent and the reference reagent was $y = 1.30x - 0.66$, $r = 0.964$ for all samples tested ($n = 283$). The correlation of DD sample concentrations was ≤100 μg/mL ($n = 270$), $y = 1.20x + 1.30$, $r = 0.961$. Several samples showed higher LiaDD values compared to reference reagent values, exceeding the regression line. Findings showed most of these samples exhibited increased TAT, FMC, and PIC values suggesting an enhanced coagulation–fibrinolytic state with these samples and the possible presence of DD fractions, even those of low-molecular-weight. LiaDD has been shown to react with the fraction of high-molecular-weight crosslinked fibrin degradation products (XDP) and with the low-molecular-weight DD/E fraction. As a result, higher DD values were obtained compared with the reference reagent when samples contain low-molecular-weight fractions that are artificially induced using plasmin degradation products of fibrin clots.[6] In the present study, samples with poor inter-reagent correlation were subjected to western blotting and some were found to contain low-molecular-weight DD/E fractions. These findings suggest that the LiaDD assay may yield higher DD values than the reference reagent assay when the sample is in an enhanced fibrinolytic state.

Inter-reagent reactivity comparison specific to different diseases were reviewed in several case studies. In Case 1 (patient with sepsis), FDP and DD levels remained high and exceeded their respective reference ranges from day 0 to day 30. The trends of FDP and DD values obtained from the study reagents almost matched those of the reference reagents. The FDP and DD values were consistent with both the study and reference reagents and followed similar time courses. PIC values, which exceeded recommended reference ranges, remained consistently low at 2.0 μg/mL, whereas α2AP stayed within the expected reference range. These observations suggest suppressed-fibrinolytic-type DIC in this case example.

Case 2 (patient with APL) exhibited markedly increased FDP and DD from day 0 with significant differences between these two values through day 14. PIC was markedly elevated while α2AP was below its reference range, both up to day 14. In this case, enhanced-fibrinolytic-type DIC was suspected. Compared with Case 1, Case 2 had greater differences between LiaFDP and LiaDD test values. In such cases, even if molecular markers, e.g., PIC and α2AP, are not analyzable at in-hospital clinical laboratories, it can be presumed that the sample is in an enhanced fibrinolytic state based on the imbalance between FDP and DD values.

CONCLUSIONS

LiaFDP and LiaDD exhibited good performance that was sufficient for routine laboratory testing. Since reactivity to FDP and DD vary depending on reagents, users must choose reagents based on a full understanding of their characteristics.

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