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transfusions should be avoided in TTP patients with a severe ADAMTS13:AC deficiency, but that platelet transfusions must be done if patients experience overt bleeding.

In our 17 childhood patients with acquired TTP, 15 patients were promptly treated with PE and corticosteroid therapy, and 16 children (94%) achieved a first remission. Recently, McDonald et al. [63] reported that the number of PE courses to first remission was higher in children (median, 22.5; range, 10–30) than in adults (median, 15.5; range, 3–93) [64], suggesting that childhood TTP may be more resistant to treatment. By contrast, our results indicated that patients with acquired TTP and a severe ADAMTS13:AC deficiency responded well to PE (median number of PE courses, 5.5; range, 2–39), but two patients (2/17, 11.8%) relapsed and one (1/17, 5.9%) died. Furthermore, in this study, we observed that the children with a high ADAMTS13:INH titer (> 5 BU) tended to require more frequent PE courses to achieve remission.

Fakhouri et al. [65] recently reported that adulthood TTP patients with high-titer ADAMTS13:INH could be successfully treated with a combination of PE and rituximab, a chimeric monoclonal antibody to CD20. The efficacy of rituximab in such patients is apparently due to a reduction in anti-ADAMTS13 IgG antibodies by depleting the patient's B-lymphocytes [65,66]. Recently, there have been many successful cases [67–69], and to date, no significant adverse effects have been reported. In our registry, only one childhood TTP patient (7 years old) with acquired TTP with ADAMTS13:INH was successfully treated with PE followed by rituximab, as shown in table II. However, the best choice or combination in regard to immunosuppressants for treating children with acquired TTP and a severe ADAMTS13:AC deficiency needs to be carefully determined in future studies.

## Conclusion

The discovery of ADAMTS13 provided a breakthrough in our understanding of the mechanism of platelet thrombus formation under high shear stress and directly linked this enzyme to TTP pathogenesis in humans. Subsequently, the recent development of rapid and sensitive ADAMTS13 assays and their utilization in clinical practice have shown that the early- and late-onset phenotypes of USS are not different diseases and are likely affected by both acquired endogenous and exogenous circumstances. Furthermore, we have presented a novel category of ai-TTP that occurs during very early childhood (less than 2 years of age), which was perhaps totally overlooked or misdiagnosed before 2002 [39]. Thus, TTP should be recognized as a life-threathening generalized disease that not only occurs in adulthood, but also in childhood, causing a paradigm shift in our clinical understanding of TTP since the first discovery by Moschcowitz in 1924.

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# Prospective evaluation of three different diagnostic criteria for disseminated intravascular coagulation

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## Summary

There are three different diagnostic score systems for disseminated intravascular coagulation (DIC) established by the Japanese Ministry Health and Welfare (JMHW), the International Society on Thrombosis and Haemostasis (ISTH) and the Japanese Association for Acute Medicine (JAAM). The JMHW criteria are still used in Japan. In the present study, all three diagnostic criteria were used to prospectively evaluate 413 patients with different underlying diseases of DIC who were treated at the Mie University Hospital (JMHW, n= 166; ISTH, n=143; JAAM, n=291). The odds ratio (95% confidence interval) for death was 1.88 (1.22 – 2.90) in JMHW, 2.55 (1.65 – 3.95) in ISHT and 1.99 (1.19 – 3.32) in JAAM. The platelet count, prothrombin time, fibrin and fibrinogen degradation products and fibrinogen were significantly important for diagnosis of DIC by all three diagnostic criteria. Haemostatic molecular markers were significantly high in all patients and were useful for the diagnosis of DIC. The JAAM diagnostic criteria displayed a high sensitivity for DIC and the ISTH overt-DIC diagnostic criteria displayed a high specificity for DIC. All three diagnostic criteria for DIC were related to a poor patient outcome.

## Keywords

DIC, Japanese Ministry Health and Welfare, ISTH, haemostatic markers, mortality, resolution rate

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## Introduction

Disseminated intravascular coagulation (DIC) is a life-threatening disease that is often associated with severe organ failure and a bleeding tendency (1–4). Recent clinical trials for severe sepsis (5–7) revealed a high mortality rate in the patients with severe sepsis. The frequency of DIC in patients with severe sepsis was reported to be 40.7% in the KyberSept trial for antithrombin (AT) (5) and 22.4% in the PROWESS study for activated protein C (APC) (6). Such patients tend to have a poor outcome.

Several diagnostic criteria for DIC have been established. These include those of the Japanese Ministry Health and Welfare (JMHW) (8), the International Society on Thrombosis and Haemostasis (ISTH) (3), and The Japanese Association for Acute Medicine (JAAM) (9). These diagnostic criteria adopt global coagulation tests such as prothrombin time (PT), platelet count, fibrinogen and fibrin and fibrinogen degradation products (FDP) or D-dimer in scoring for haemostatic abnormalities. Both the JAAM DIC criteria (9) and non-overt-DIC diagnostic criteria established by ISTH (3) have adopted the rate of change in global coagulation tests.

A study in which the efficacy of DIC treatment in relation to the JMHW DIC score was compared at the beginning of treatment showed that greater efficacy was achieved in late-onset DIC patients than in DIC patients (10). The outcome was poorer with an increasing DIC score, thus suggesting that both an early diagnosis and early treatment for DIC are important. The late-onset DIC is considered to be within a week before the onset of DIC (11) or non-overt DIC (3, 12).

This study prospectively evaluated the JMHW, ISTH and JAAM diagnostic criteria for DIC and examined the usefulness of haemostatic molecular markers for the diagnosis of DIC.

## Materials and methods

A total of 413 patients [female: male; 173: 242, age (median; 64.0 years old, 25%-75% tile; 49.0–73.0 years old)] with diseases associated with DIC who were treated from January 1, 2005 to December 31, 2008 at Mie University Hospital and associated hospitals were

prospectively enrolled in the study. There were 136 patients with solid cancers (45: 91, 63.5 years old; 53.0 –72.0 years old), 94 with haematopoietic tumour (44: 50, 61.5 years old: 44.0 – 72.0), 78 with infectious disease (30: 48, 67.5 years old; 57.0 –72.0 years old), 31 with aneurysm (12: 19, 73.0 years old; 68.0 – 76.8 years old), 25 patients with trauma or burn (12: 13, 67.0 years old; 47.0 – 74.3 years old), 12 patients with cardiovascular disease (4: 8, 69.0 years old; (64.0 – 75.3 years old), 10 patients with gastrointestinal disease (7: 3, 58.0 years old; 22.0 – 63.0 years old), 8 with autoimmune disease (5: 3, 58.0 years old; 22.0 – 63.0 years old), 8 with obstetrical disease (8: 0, 33.0 years old; 30.5 – 34.5 years old) and 11 with other diseases (6: 5, 53.5 years old; 42.0 – 68.0 years old). The inclusion criteria were the observation of more than one abnormal finding according to the laboratory tests (platelet count; < 120/ x$10^3$/μl, FDP > 10 μg/ml, fibrinogen < 1g/l, PT ratio > 1.25) in addition to the presence of disease(s) associated with DIC. Any patients demonstrating associations with heparin-induced thrombocytopenia (HIT), thrombotic thrombocytopenic purpura (TTP), antiphospholipid syndrome (APS) or severe liver injuries were excluded. APS was diagnosed according to the Sapporo criteria (13), but one patient with symptoms of APS and antiphospholipid antibodies was excluded from this study after two months without undergoing any tests. Organ failure and inflammatory conditions were evaluated by the sepsis-related organ failure assessment (SOFA) (14) and the systemic inflammatory response syndrome (SIRS) score (15), respectively. The study protocol was approved by the Human Ethics Review Committee of Mie University School of Medicine, and a signed consent form was obtained from each patient.

DIC was diagnosed on the day of registration using the JMHW, ISTH and JAAM diagnostic criteria (▶Table 1) (8). The onset of DIC within a week after the registration was defined as late-onset DIC. The DIC score using platelet count, FDP, fibrinogen and PT was thereafter checked in all patients not diagnosed with DIC every day after registration. Haemostatic molecular markers such as thrombin-AT complex (TAT), fibrin monomer complex (FMC), D-dimer, plasmin plasmin inhibitor complex (PPIC), thrombomodulin (TM) and AT were measured at registration. No DIC treatment was administered prior to the diagnosis of DIC.

PT, fibrinogen, platelet count and FDP were measured as previously described (16, 17). TAT, FMC, D-dimer, PPIC, TM and AT activity were measured by SRL Inc. (Tokyo, Japan). TAT and TM were measured by an enzyme immunoassay (EIA) using TAT [S] (TFB, Tokyo, Japan) and TM Banasera (Fujirebio, Tokyo, Japan), respectively. FMC, D-dimer and PPIC were measured by a latex immune agglutination (LIA) test using Auto LIA FM (Roche Diagnostic, Tokyo, Japan), LATECLE D-dimer (Kainos, Tokyo, Japan) and LPIA-ACE PPI II (Mitsubishi Chemical Medicine Corporation, Tokyo, Japan), respectively. AT activity was measured by means of heparin cofactor activity using the Testchyme S ATIII kit (Sekisui Medical, Tokyo, Japan).

## Statistical analysis

The data are expressed as the median (25%-75% percentile). The differences between the groups were examined for statistical significance using the Mann-Whitney U test. A p-value < 0.05 was considered to be significant. A chi-square statistical analysis demonstrated an odds ratio (OR) of 95% confidence interval (CI) for the mortality, resolution rate from DIC, and the cut-off value of haemostatic parameters. All statistical analyses were performed using the SPSS II software package (SPSS Japan, Tokyo, Japan).

**Table 1: Three different diagnostic criteria for DIC established by the Japanese Ministry of Health and Welfare (JMHW), the International Society on Thrombosis and Haemostasis (ISTH), and the Japanese Association for Acute Medcine (JAAM).**

| Establish | Points | JMHW | ISTH | JAAM |
|---|---|---|---|---|
| Underlying disease | 1 | 1 point | necessary | necessary |
| Clinical symptoms | 1 | bleeding* | - | SIRS 1 point |
| | 1 | organ failure | | |
| Platelet counts (x$10^3$/μl) | 1 | >80 but < 120 * | >50 but < 100 | >80 but < 120 #1 |
| | 2 | >50 but < 80 * | < 50 | 80< #2 |
| | 3 | < 50* | | |
| Fibrin-related marker | 1 | FDP (μg/ml) | FDP, SF or D-dimer | >10 but < 25 |
| | 2 | >10 but < 20 | Moderately increased | >25 |
| | 3 | >20 but < 40 | Markedly increased | |
| | | >40 | | |
| Fibrinogen (g/l) | 1 | >1 but < 1.5 | < 1 | - |
| | 2 | <1 | | |
| PT, PT ratio, | 1 | >1.25 but <1.67 | Prolongation of PT | >1.2 |
| Prolongation of PT | 2 | >1.67 | >3 but 6< | |
| Diagnosis of DIC | points | ≥7 | ≥5 | ≥4 |

JMHW, Japanese Ministry of Health and Welfare; ISTH, International Society on Thrombosis and Haemostasis; JAAM, Japanese Association for Acute Medcine. *: 0 points in patients with hematopoietic malignancy. #1: or a 30% reduction in the platelet count. #2: or a 50% reduction in the platelet count.

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Table 2: Diagnostic rate according to three diagnostic criteria for DIC.

| Underlying disease | JMHW | ISTH | JAAM |
|---|---|---|---|
| Solid cancer | 47 (34.6%) | 45 (33.1%) | 95 (69.9%) |
| Haematopoietic tumor | 39 (41.5%) | 30 (31.9%) | 71 (75.5%) |
| Infectious disease | 36 (46.2%) | 32 (41.0%) | 60 (76.9%) |
| Aneurysm | 15 (48.4%) | 11 (35.5%) | 22 (71.0%) |
| Trauma/Burn | 7 (28.0%) | 6 (24.0%) | 19 (76.0%) |
| Cardiovascular disease | 5 (41.7%) | 6 (50.0%) | 9 (75.0%) |
| Gastrointestinal disease | 8 (80.0%) | 7 (70.0%) | 9 (90.0%) |
| Autoimmune disease | 1 (12.5%) | 0 | 5 (62.5%) |
| Obstetrics disease | 6 (75.0%) | 5 (62.5%) | 5 (62.5%) |
| Other disease | 2 (18.2%) | 1 ( 9.1%) | 5 (45.5%) |
| Total | 166 (40.2%) | 143 (34.6%) | 291 (70.5%) |

JMHW, Japanese Ministry of Health and Welfare; ISTH, International Society on Thrombosis and Haemostasis; JAAM; Japanese Association for Acute Medcine.

## Results

Of the 413 patients, 166 (40.2%), 143 (34.6%) and 291 (70.5%) were diagnosed for DIC by the JMHW, ISTH and JAAM criteria, respectively (▶Table 2). The JAAM and ISTH overt-DIC diagnostic criteria diagnosed the highest and lowest numbers of patients, respectively. The high number of patients associated with DIC was evident for the cases of solid cancer, haematopoietic tumour and infectious disease. The prevalence of late onset of DIC was 12.1%, 13.3% and 13.9% using the JAAM, ISTH overt-DIC, and JAAM diagnostic criteria, respectively (▶Table 3). The mortality rate was 35.5%, 40.6% and 31.7% in the patients diagnosed using the JMHW, ISTH overt-DIC and JAAM diagnostic criteria, respectively. The sensitivity for death was the highest using the JAAM criteria (80.9%), and the specificity for death was the highest using the ISTH overt-DIC diagnostic criteria (71.4%). The OR for death (95% CI) was 1.88 (1.226|2.90, p< 0.005), 2.55 (1.65 – 3.95, p< 0.001) and 1.99 (1.196|3.32, p< 0.001) using the JMHW, ISTH overt-DIC and JAAM criteria, respectively.

Abnormalities of the global coagulation tests such as platelet count, PT ratio, FDP and fibrinogen were significantly higher in the patients with DIC than those without DIC using all three diagnostic criteria (▶Table 4). Platelet count was significantly lower in the DIC patients diagnosed using the JMHW criteria than in those patients diagnosed using the ISTH overt-DIC or JAAM criteria, and the platelet count was significantly higher in the patients without DIC diagnosed using the JAAM criteria than in those patients diagnosed using the ISTH overt-DIC diagnostic criteria. The PT ratio was significantly higher in the patients with DIC diagnosed using the ISTH overt-DIC diagnostic criteria than in those patients diagnosed using the JMHW criteria and JAAM criteria. The FDP was significantly lower in the patients without DIC diagnosed using the JAAM criteria than in those patients diagnosed using the ISTH-overt DIC criteria. The fibrinogen level was significantly higher in the patients with DIC diagnosed using the JAAM criteria than in those patients diagnosed using the JMHW and ISTH overt-DIC criteria.

The D-dimer, FMC, TAT, AT and TM abnormalities were significantly higher in the patients with DIC than those patients without DIC who were diagnosed using all three diagnostic criteria (▶Table 5). There were no significant differences in D-dimer, FMC, TAT, PPIC, AT and TM levels of the patients with or without DIC diagnosed using the JMHW and ISTH criteria (▶Table 5). The D-dimer, PPIC and AT levels abnormalities in the patients with DIC were significantly less using the JAAM criteria than using either the JMHW or ISTH criteria. The D-dimer, FMC, TAT and PPIC abnormalities in the patients without DIC were significantly less using the JAAM criteria than using the JMHW or ISTH criteria.

## Discussion

Hitherto, the three diagnostic criteria for DIC have not been simultaneously evaluated. The present study prospectively evaluated the JMHW, ISTH and JAAM DIC diagnostic criteria in patients treated at Mie University Hospital and associated facilities. These diagnostic criteria use the same global coagulation tests but their

Table 3: Relationship between mortality and the diagnostic criteria.

| | JMHW | ISTH | JAAM |
|---|---|---|---|
| DIC | 166 (40.2%) | 143 (34.6%) | 291 (70.5%) |
| Without DIC | 247 | 270 | 122 |
| Late onset of DIC* | 30 (12.1%) | 36 (13.3%) | 17 (13.9%) |
| Mortality in DIC | 35.5% (59/166) | 40.6% (58/143) | 31.7% (92/291) |
| Sensitivity for death | 51.3% | 50.4% | 80.0% |
| Specificity for death | 64.9 | 71.4% | 33.2% |
| Odds ratio for death | 1.88 (1.22 – 2.90) | 2.55(1.65 – 3.95) | 1.99 (1.19 – 3.32) |
| | P< 0.005 | P< 0.001 | P< 0.001 |

Late onset of DIC: The patients were not diagnosed at registration but they were diagnosed to have DIC within one week.


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Table 4: Global coagulation tests in the patients with DIC, those with late-onset DIC and those without DIC.

| | | JMHW | ISTH | JAAM |
|---|---|---|---|---|
| Platelet | DIC(+) | & 4.3 (2.6~7.0) | & 6.3 (3.5~9.9)** | & 5.6 (3.2~7.7)* |
| ($X10^4/\mu l$) | DIC(-) | & 9.6 (6.1~16.6) | & 9.4 (6.2~16.7) | & 15.5 (9.3~22.7)## |
| | Late | 7.9 (5.8~12.4) | 6.1 (3.0~8.3)* | 12.6 (9.2~15.8)*## |
| PT ratio | DIC(+) | & 1.39 (1.16~0.76) | & 1.48 (1.28~1.95)* | & 1.27 (1.11~1.52)**## |
| | DIC(-) | & 1.12 (1.02~0.24) | & 1.12 (1.02~1.23) | & 1.08 (1.02~1.17) |
| | Late | 1.21 (1.10~0.32) | 1.18 (1.09~1.30) | 1.19 (1.03~1.42) |
| FDP | DIC(+) | & 43.0 (21.7~64.3) | & 38.0 (23.1~61.2) | & 31.9 (19.2~58.0) |
| (μg/ml) | DIC(-) | & 20.1 (11.2~37.2) | & 20.2 (12.0~40.2) | & 16.4 (9.4~26.0)# |
| | Late | 21.0 (16.7~30.7) | 21.2 (15.1~42.7) | 18.5 (10.4~21.8) |
| Fibrinogen | DIC(+) | & 191 (120~345) | & 203 (115~334) | & 254 (150~365)*# |
| (mg/dl) | DIC(-) | & 314 (236~398) | & 312 (212~397) | & 352 (245~446) |
| | Late | 318 (192~378) | 320 (191~370) | 303 (125~382) |

Data represent the median (25%tile – 75%tile). DIC(+): patients with DIC, DIC(-): patients without DIC, Late: patients with late onset DIC. && : $p< 0.01$ between DIC (+) and DIC (-). **, or *; $p< 0.01$ or $p< 0.05$ in comparison to DIC, without DIC or Late onset established by JMHW. ##, or #; $p< 0.01$ or $p< 0.05$ in comparison to DIC established by ISTH.

Table 5: Haemostatic molecular markers in the patients with DIC, those with late-onset DIC and those without DIC.

| | | JMHW | ISTH | JAAM |
|---|---|---|---|---|
| D-dimer | DIC(+) | & 22.8 (11.9~45.0) | & 21.2 (11.2~38.3) | & 19.0 (9.7~35.6)* |
| (μg/ml) | DIC(-) | & 10.3 (5.9~20.7) | & 12.0 (6.6~26.6) | & 8.6 (4.5~13.4)*## |
| | Late | 21.3 (8.8~28.4) | 17.3 (8.8~28.5) | 16.1 (9.8~25.5) |
| FMC | DIC(+) | & 112.0 (18.2~235.0) | & 110.0 (16.1~224.8) | & 70.8 (16.1~210.0) |
| (μg/ml) | DIC(-) | & 33.0 (7.6~134.0) | & 36.6 (9.4~156.0) | & 16.7 (6.5~94.4)# |
| | Late | 57.9 (14.0~151.3) | 79.0 (12.3~182.0) | 18.0 (8.9~79.6) |
| TAT | DIC(+) | & 25.6 (13.3~90.0) | & 29.2 (13.2~90.0) | & 23.1 (10.8~62.8) |
| (ng/ml) | DIC(-) | & 13.6 (6.8~32.3) | & 16.9 (7.5~40.9) | & 10.1 (5.1~28.0)# |
| | Late | 18.1 (11.2~32.6) | 17.3 (11.0~24.6) | 18.9 (13.1~30.7) |
| PPIC | DIC(+) | N 2.3 (1.0~6.8) | N 1.4 (0.9~6.2) | & 2.4 (1.2~6.2)# |
| (μg/ml) | DIC(-) | S 2.0 (1.1~4.1) | S 2.3 (1.2~4.6) | & 1.6 (0.9~3.2)## |
| | Late | 2.2 (1.4~3.6) | 2.5 (1.4~5.7) | 2.1 (1.1~2.6) |
| AT | DIC(+) | & 63.9 (44.0~83.0) | & 53.6 (38.7~76.0) | & 68.0 (47.1~85.0)## |
| (%) | DIC(-) | & 77.9 (56.0~99.8) | & 75.4 (55.7~95.3) | & 84.9 (59.5~102.0) |
| | Late | 66.5 (51.7~83.0) | 79.9 (61.1~89.8) | 85.4 (62.0~120.0) |
| TM | DIC(+) | & 5.3 (3.5~8.2) | & 5.6 (3.8~8.5) | & 4.9 (3.2~6.9) |
| (ng/ml) | DIC(-) | & 3.9 (2.7~5.2) | & 4.2 (2.7~5.9) | & 3.6 (2.6~4.8) |
| | Late | 4.1 (3.2~7.4) | 4.0 (3.0~6.7) | 4.3 (2.5~6.8) |

Data represent the median (25%tile – 75%tile). Late onset: Late onset DIC. && or NS: $p< 0.01$, $p< 0.05$, or not significant between DIC (+) and DIC (-). **, or *; $p< 0.01$ or $p< 0.05$ in comparison to DIC established by JMHW. ##, or #; $p< 0.01$ or $p< 0.05$ in comparison to DIC established by ISTH.

cut-off values are different. The JAAM diagnostic criteria have been considered to have a high sensitivity for the diagnosis of DIC, and the ISTH overt-diagnostic criteria to have a high specificity for the diagnosis of DIC. The possibility of progression from the JAAM DIC to the ISTH DIC was reported (18). The latter study also reported the JMHW diagnostic criteria for DIC to be more sensitive than ISTH overt-DIC diagnostic criteria. A high number of associations with DIC were observed in cases of solid cancer, haematopoietic tumour and infectious disease. The frequency of DIC by the three diagnostic criteria in these underlying diseases was similar to that of the total patients, but the JMHW and ISTH criteria tended to display a low sensitivity for DIC in the patients with trauma and burn injuries. A late onset of DIC was observed in 13.9% of patients without DIC using the highly sensitive JAAM diagnostic criteria for DIC. This value was similar to that of the patients without DIC using either the JMHW criteria or ISTH criteria, thus suggesting that all of three diagnostic criteria might miss the early stage of DIC, since these criteria adopt same global coagulation tests which were not sensitive or specific for early stage of DIC. Haemostatic molecular markers such as TAT and SF might therefore be a sensitive indicator for the early phase of DIC (20).

The diagnostic criteria for DIC by JAAM, ISTH and JAAM were related to a poor outcome. In several trials of sepsis (6, 21), the patients associated with DIC displayed a poor outcome. In the pres-

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## What is known about this topic?

- There are three variations of diagnostic criteria for disseminated intravascular coagulation (DIC) established by the Japanese Ministry of Health and Welfare (JMHW), the International Society on Thrombosis and Haemostasis (ISTH) and the Japanese Association for Acute Medicine (JAAM).
- Three diagnostic criteria have been considered to be useful for the diagnosis of DIC.
- DIC patients are considered to have a poor outcome.

## What does this paper add?

- Three diagnostic criteria were evaluated simultaneously.
- The JAAM diagnostic criteria have a high sensitivity for DIC and the ISTH diagnostic criteria have a high specificity for DIC.
- All three diagnostic criteria are related to a poor outcome.

ent study, the mortality of JAAM, ISTH and JAAM DIC was more than 30%. These data also proved the diagnosis of DIC by three diagnostic criteria to be related with a poor outcome. Furthermore, it is important to prove that DIC treatment improves the outcome of DIC and that the sensitivity of DIC diagnostic criteria for poor outcome is also important. The JAAM diagnostic criteria have the highest sensitivity, but the lowest specificity for poor outcome. Future studies should prospectively examine the effect of intervention for DIC treatment.

In this study all global coagulation tests such as platelet count, PT ratio, FDP and fibrinogen levels were significantly abnormal in the patients with DIC diagnosed by all three criteria, and these markers tended to be less abnormal in those patients with DIC who were diagnosed by JAAM criteria than in those patients diagnosed by the ISTH or JMHW diagnostic criteria. Of the haemostatic molecular markers, only PPIC was not useful for the diagnosis of DIC using all three diagnostic criteria. The observation that the D-dimer, FMC, TAT and AT markers also tended to be less abnormal in the patients without DIC who were diagnosed by the JAAM criteria, suggests that the JAAM criteria can detect mild haemostatic abnormalities. The values of TM and AT are reported to be worse in patients with poor outcome than in patients with a better outcome (22), thus suggesting that TM and AT may therefore be useful as markers of injured vascular endothelial cells. In the critical care field, a scoring system that includes the platelet count and PT has a prognostic value in severe sepsis (23).

In conclusion, all three diagnostic criteria for DIC are associated with a poor outcome and miss late-onset DIC at the time of admission. As a result, there are no useful markers for the late onset of DIC.

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ORIGINAL ARTICLE

# Elevated Von Willebrand factor propeptide for the diagnosis of thrombotic microangiopathy and for predicting a poor outcome

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**Abstract** Thrombotic microangiopathy (TMA) is associated with vascular endothelial cell injury and is sometimes linked with poor outcome. Von Willebrand factor (VWF) propeptide (VWFpp) is considered to be a marker of vascular endothelial cell injury. The plasma levels of VWF, VWFpp, and thrombomodulin (TM) were evaluated for their use in the diagnosis of TMA in 75 patients with TMA. There were 30 TMA patients with marked decreases in ADAMTS13 (TMA/ADAMTS13) and 45 without the decrease (TMA/other). The plasma levels of TM, VWF, and VWFpp values were significantly high in patients with TMA, especially TMA/other group. The plasma levels of TM and VWFpp were significantly high in non-survivor with TMA. In the TMA/other group, the plasma levels of VWFpp were negatively correlated with ADAMTS13 activity. The plasma levels of TM correlated with the renal function, but the plasma levels of VWFpp did not. A ROC analysis indicated that VWFpp and TM were useful markers for the prediction of a poor outcome. These findings suggest that VWFpp is an useful marker for the diagnosis of TMA and for the prediction of poor outcome.

**Keywords** VWFpp · TM · ADAMTS13 · TMA · Vascular endothelial cell injury

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## 1 Introduction

Thrombotic microangiopathies (TMAs) are defined by acute mechanical hemolytic anemia, thrombocytopenia, and visceral ischemic manifestations related to the formation of platelet thrombi in the microcirculation [1]. TMA includes thrombotic thrombocytopenic purpura (TTP), hemolytic ureic syndrome (HUS), hemolysis, elevated liver enzyme levels, low platelet (HELLP) syndrome, and complications after bone marrow transplantation. In addition, these symptoms show fluctuating bizarre neurologic symptoms, in addition to renal failure and fever [2, 3].

ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type I domain 13), which was identified in 2001 [4–6], is a zinc metalloprotease that specifically cleaves unusually large Von Willebrand factor multimers (UL-VWFM) at the Tyr (1605)-Met(1606) boundary located in the A2 region of VWF [7, 8], suggesting that UL-VWFM cause multiple platelet thrombi due to TMA. Although the diagnosis of TMA has been improved remarkably by the development of a method for measuring ADAMTS13 [9, 10], some problems remain in the diagnosis of TMA without marked decrease of ADAMTS13.

The pre-pro VWF, which is synthesized in endothelial cells and megakaryocytes, undergoes intracellular modifications including signal peptide cleavage, C-terminal

dimerization, glycosylation, sulfation, and N-terminal multimerization [11]. Then proteolysis occurs in the trans-Golgi, where the VWF propeptide (VWFpp) is cleaved, but remains stored together with mature VWF in alpha-granules (megakaryocytes) and Weibel–Palade bodies (endothelial cells). After the secretion of VWFpp and VWF into plasma from endothelial cells due to several physiological or pathological stimuli, VWFpp dissociates from VWF [12, 13].

Vascular endothelial cell injury is one of the main causes of and/or results of TMA and it has been reported that elevated thrombomodulin (TM) and VWF levels can be used as vascular endothelial cell injury markers in the patients with thrombotic thrombocytopenic purpura (TTP) and disseminated intravascular coagulation (DIC) [14, 15]. As elevated TM is observed in patients with renal failure, a more specific marker for vascular endothelial cell injury is required for the accurate diagnosis of TMA.

In this study, the plasma levels of TM, VWF, and VWFpp were measured in 140 patients with suspected TMA to evaluate the usefulness of diagnosis for TMA and for the prediction of a poor outcome.

## 2 Materials and methods

A total of 140 patients were suspected to have TMA and consulted us at Mie University Hospital between 1st January 1990 and 30th June 2010. There were 25 patients without underlying disease, 22 patients with autoimmune disease, 15 with malignant tumors, 12 who had undergone liver transplantation, 7 who had received born marrow or kidney transplantation, 14 with severe infection, 3 with O-157 infection, 4 due to pregnancy, 3 due to post-surgical complications, 2 due to drug use, and 33 patients with other diseases. Out of these patients, 75 were diagnosed to have TMA according to the diagnostic criteria of TMA: (1) thrombocytopenia (less than $12 \times 10^4/\mu l$), (2) hemolytic anemia (less than 11.0 g/dl of hemoglobin) due to the microangiopathy (presence of fragmented red cells, elevated total bilirubin, and LDH), (3) neurological dysfunction, (4) renal failure, and (5) fever [16]. The patients with (1) and (2) who had an ADAMTS 13 activity of less than 10%, who had an O-157 infection, and who had clinical symptoms, such as (3) or/and (4), were diagnosed with TMA.

The plasma levels of ADAMTS13 activity, thrombomodulin (TM), VWF, and VWFpp were measured in these patients and 50 healthy volunteers (19 females and 31 males; median age 31 years; range 19–51 years).

The ADAMTS13 activity was measured using a FRETS-VWF73, which was chemically synthesized by the Peptide Institute, Inc. (Osaka, Japan) according to the method described by Kokame et al. [9, 10]. TM was measured with a Thrombomodulin "MKI" EIA kit (Mitsubishi Chemical Medience Corporation, Tokyo, Japan). VWF and VWFpp levels were measured with a VWF&Propeptide assay kit (GTi DIAGNOSTiCs, Waukesha, USA).

These patients were classified into 3 groups; those with ADAMTS13-related TMA (TMA/ADAMTS13), where the ADAMTS13 level was less than 10%; TMA/other, the cause of which was not known; or non-TMA.

The study protocol was approved by the Human Ethics Review Committees of Mie University School of

**Table 1** Characteristics of the TMA and non-TMA patients

| | TMA/ADAMTS13 | TMA/other | All-TMA | Non-TMA | All |
|---|---|---|---|---|---|
| Age; Median (25%tile–75%tile) | 51.0 (34.8–67.3) | 52.0 (33.8–61.8) | 52.0 (34.5–64.5) | 55.5 (38.0–71.0) | 53.0 (36.0–67.0) |
| Sex (F:M) | 17:13 | 30:15 | 47:28 | 33:32 | 80:60 |
| Underlying disease | | | | | |
| Autoimmune disease | 4 | 5 | 9 | 13 | 22 |
| Malignant tumor | 1 | 3 | 4 | 11 | 15 |
| Liver transplantation | 3 | 5 | 8 | 4 | 12 |
| Other transplantation | 0 | 3 | 3 | 4 | 7 |
| Severe infection | 3 | 8 | 11 | 3 | 14 |
| O-157 infection | 0 | 1 | 1 | 2 | 3 |
| Pregnancy | 1 | 3 | 4 | 0 | 4 |
| Post-surgery | 0 | 0 | 0 | 3 | 3 |
| Drug use | 0 | 1 | 1 | 1 | 2 |
| Other | 4 | 5 | 9 | 24 | 33 |
| None | 14 | 11 | 25 | 0 | 25 |
| Non-survivors/all patients | 5/30 | 16/45 | 21/75 | 7/65 | 28/140 |
| Mortality (%) | 16.7 | 35.6 | 28.0 | 10.8 | 20.0 |

Medicine, and signed informed consent was obtained from each patient.

### 2.1 Statistical analysis

The data are expressed as the medians (25% tile–75% tile). Differences between the groups were examined for significance using the Mann–Whitney *U* test for independence. A *p* value of less than 0.05 was considered to indicate a significant difference. Correlations between TM, VWF, VWFpp, and ADAMTS13 were examined using the Spearman's rank correlation coefficient. All statistical analyses were performed using the SPSS II software package (SPSS Japan, Tokyo).

## 3 Results

The patient group included 30 with TMA/ADAMTS13, 45 with TMA/other, and 65 with non-TMA (Table 1). There were more female than male patients in the TMA/other group. Severe infection was the most frequent underlying disease in patients with TMA, while transplantation was the second leading cause, and autoimmune disease was the third leading cause of the disease. Severe infection was also the most frequent underlying disease in the TMA/other group. The mortality was 28.0% in the TMA, but 10.8% in the non-TMA group. The mortality tended to be higher in patients with TMA/other (35.6%) than TMA/ADAMTS13 (16.7%) (Table 1).

The median value (95% CI) of TM, VWF, and VWFpp in healthy volunteers were 15.2 (11.7–22.3) U/ml, 69.5 (33.0–170.0) U/dl, and 85.0 (39.5–160.3) U/dl, respectively (Table 2). The plasma levels of TM, VWF, and VWFpp values were significantly higher in non-TMA and all-TMA (TMA/ADAMTS13 and TMA/other) patients than in healthy volunteers ($p < 0.001$). The plasma levels of TM and VWFpp values were significantly higher in the all-TMA than in non-TMA patients ($p < 0.01$ and <0.001, respectively). Plasma levels of TM and VWFpp were significantly higher in the TMA/other [52.1 (31.8–69.2) U/ml and 279.0 (194.0–431.8) U/dl] than non-TMA patients [24.7 (17.3–35.0) U/ml and 171.0 (132.8–236.3) U/dl, $p < 0.001$, respectively], and in the TMA/other than the TMA/ADAMTS13 group [24.0 (19.4–33.9) U/ml and 196.0 (154.0–246.0) U/dl, $p < 0.001$ and <0.01, respectively, Table 2].

The plasma levels of TM in all patients ($p < 0.001$), all-TMA patients ($p < 0.001$), TMA/ADAMTS13 ($p < 0.05$), and TMA/other patients ($p < 0.05$) were significantly higher in non-survivors than survivors (Fig. 1). In addition, plasma levels of VWF in all patients ($p < 0.05$) and all-TMA ($p < 0.01$) were significantly higher in non-survivors than

**Table 2** Thrombomodulin, Von Willebrand Factor, and Von Willebrand Factor propeptide values in control, non-TMA, and TMA groups

| | Healthy volunteers median (95%CI) | Non-TMA median (25–75%tile) | All-TMA median (25–75%tile) | Non-TMA median (25–75%tile) | TMA/ADAMTS13 median (25–75%tile) | TMA/other median (25–75%tile) |
|---|---|---|---|---|---|---|
| TM (U/ml) | 15.2 (11.7–22.3) | 24.7*** (17.3–35.0) | 35.7***, ## (21.2–59.1) | 24.7* (17.3–35.0) | 24.0*** (19.4–33.9) | 52.1***, ###, +++ (31.8–69.2) |
| VWF (U/dl) | 69.5 (33.0–170.0) | 206.0*** (141.8–260.0) | 191.0*** (142.3–265.8) | 206.0* (141.8–260.0) | 161.5***, # (109.0–206.0) | 217.0***, ++ (149.3–281.3) |
| VWFpp (U/dl) | 85.0 (39.5–160.3) | 171.0*** (132.8–236.3) | 233.0***, ### (166.5–341.3) | 171.0* (132.8–236.3) | 196.0*** (154.0–246.0) | 279.0***, ###, ++ (194.0–431.8) |

Data are expressed as the medians (95%CI) or median (25%–75%tile)

***, **, * $p < 0.001$, <0.01, or <0.05 in comparison to healthy volunteers; ###, ##, # $p < 0.001$, <0.01, or <0.05 in comparison to Non-TMA

+++, ++, + $p < 0.001$, <0.01, or <0.05 in comparison to TMA/ADAMTS13

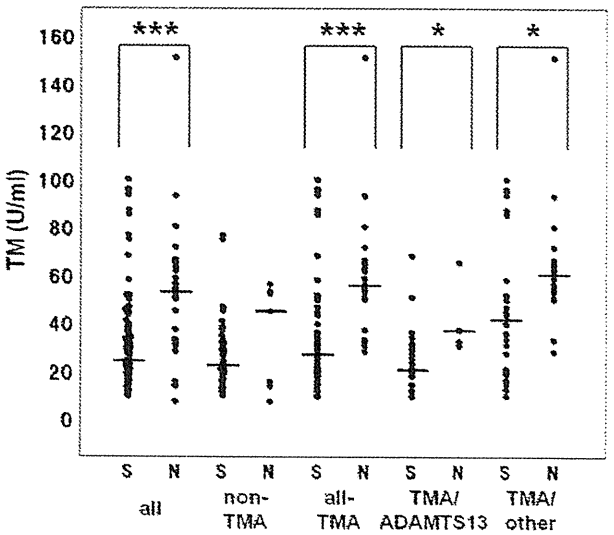


**Fig. 1** Plasma levels of TM in the non-survivor and survivor groups. *S* survivor, *N* non-survivor. ***$p < 0.001$; *$p < 0.05$

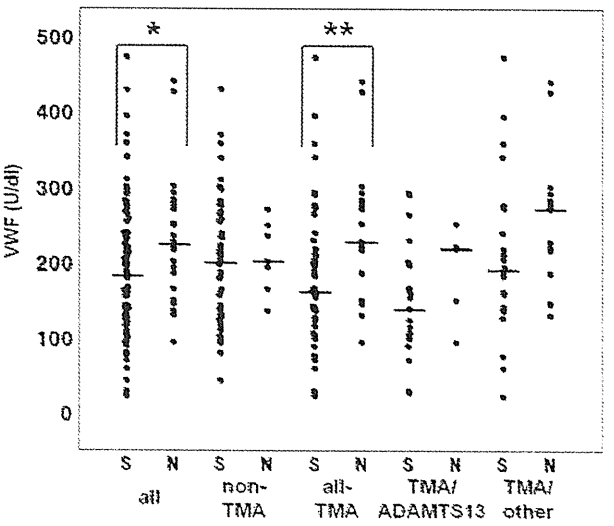


**Fig. 2** Plasma levels of VWF in the non-survivor and survivor groups. *S* survivor, *N* non-survivor. **$p < 0.01$, *$p < 0.05$

survivors (Fig. 2). The plasma level of VWFpp in all patients (302.5 [230.5–457.5] U/dl vs. 182.0 [134.5–237.3] U/dl, $p < 0.001$), all-TMA (343.0 [278.5–510.0] U/dl vs. 201.0 [151.8–276.0] U/dl, $p < 0.001$), and TMA/other patients (436.5 [305.0–585.5] U/dl vs. 228.0 [152.0–308.0] U/dl, $p < 0.001$) were significantly higher in non-survivors than survivors (Fig. 3).

In the TMA/other group, the Spearman's rank correlation coefficient ($r_S$) with ADAMTS13 was −0.389 in TM, −0.298 in VWF, and −0.474 in VWFpp ($p < 0.01$, <0.05, and <0.01, respectively). There was a very low correlation of VWF and VWFpp with ADAMTS 13 activity in the all-TMA, TMA/ADAMTS13, and non-TMA groups. Plasma

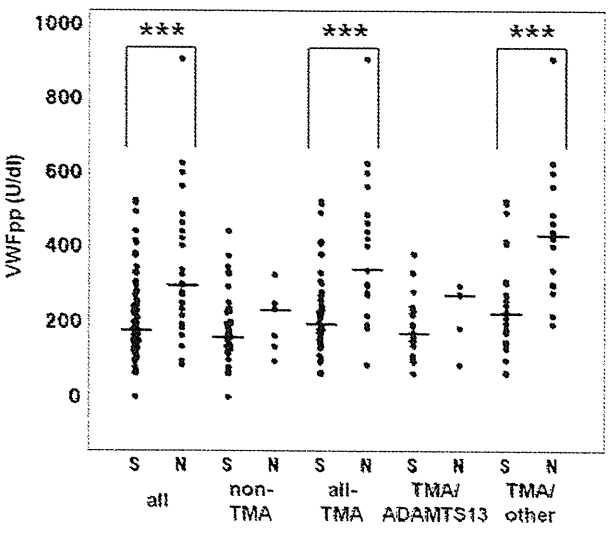


**Fig. 3** Plasma levels of VWFpp in the non-survivor and survivor groups. *S* survivor, *N* non-survivor. ***$p < 0.001$

levels of TM in all patients were well correlated with E-glomerular filtration rate ($r_S = -0.734$, $p < 0.001$), but those of VWF ($r_S = -0.219$, NS), and VWFpp ($r_S = -0.261$, NS) were not.

In an ROC analysis of TM, VWF, and VWFpp for prediction of poor outcome, the area under the curve (AUC) was 0.783 for TM, 0.706 for VWF, and 0.796 for VWFpp in the all-TMA group, and the AUC was 0.716 for TM, 0.681 for VWF, and 0.825 for VWFpp in the TMA/other group (Fig. 4).

## 4 Discussion

In this study, the frequency of TMA/ADAMTS13 was 40% and the patients with an ADAMTS13 activity of less than 5% had an inhibitor for ADAMTS13. It was reported that a high titer of ADAMTS13 inhibitor has been reported to be related to a poor outcome [17]. In our cases, almost all patients with TMA/ADAMTS13 had a low titer of inhibitor demonstrated no relapse. The frequency of TMA/other was markedly high compared with national questionnaire survey done by the Japanese Ministry of Health, Labor and Welfare [18, 19] and other report [20]. As there are many reports of TMA due to abnormalities of ADAMTS13, most physicians look for a decrease in ADAMTS13 activity in patients with TMA. The high frequency of TMA/ADAMTS13 in the national questionnaire survey might have thus been caused by physician's bias. With regard to the diseases underlying the development of TMA, severe infection and transplantation were the most frequent in this study, but O-157 infection and autoimmune disease were the most frequent in the national questionnaire survey

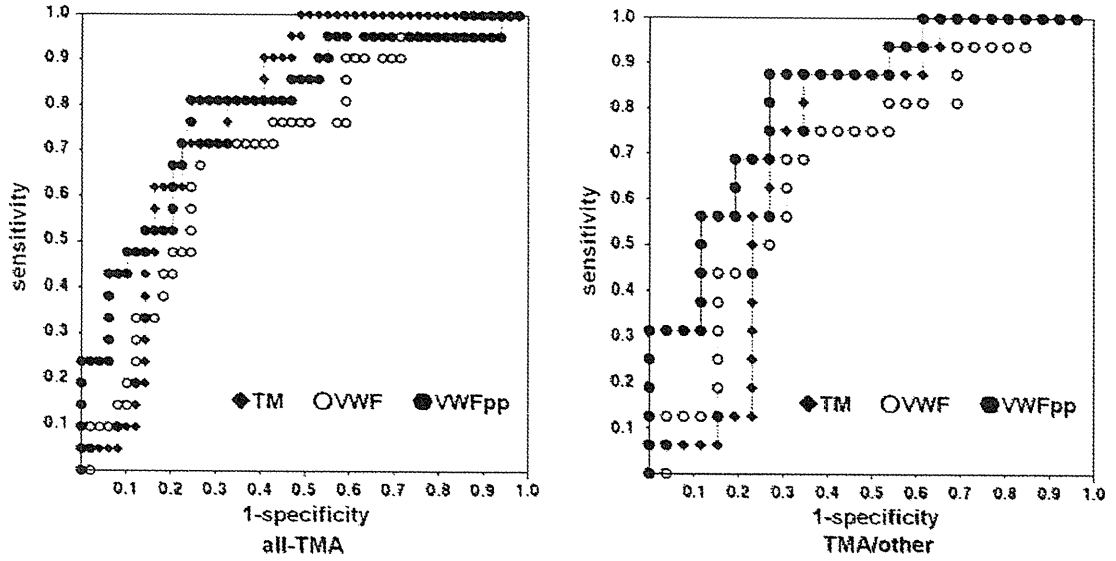


**Fig. 4** Analysis of ROC for TM, VWF, and VWFpp in the prediction of poor outcome. The left side is all-TMA (TMA/ADAMTS13 + TMA/other) and right side is TMA/other. *Filled diamond* TM, *open circle* VWF, and *filled circle* VWFpp

[18, 19]. The deficiency of ADAMTS13 is a known cause of TMA, but the over-release of UL-VWFM from vascular endothelial cell may also be important. Severe infection often is associated with vascular endothelial cell injury and multiple organ failure. Auto-antibodies against ADAMTS13 were rarely detected in patients with malignant diseases or infections, and in those that were post-surgery or post-transplantation, all of which may cause TMA via vascular endothelial injuries and inflammation [21, 22].

The mortality of TMA in this study was 28.0%, which was slightly higher than in the national questionnaire survey [18, 19]. The mortality tended to be higher in TMA/other than in TMA/ADAMTS13 patients both in this study and in the national questionnaire survey. The high frequency of TMA/other in this study may have increased the mortality in comparison to the national questionnaire survey. These findings suggest that vascular endothelial cell injury may be related to poor outcome. Another study showed that a high titer of ADAMTS13 inhibitor may be related to poor outcome in Oklahoma study [17]. This finding suggests that a high titer of the inhibitor for ADAMTS13 may be related to the relapse of TMA. This discrepancy may be caused by the differences in the background of TMA. In analysis of TMA/other, VWFpp might be more useful for the prediction of poor outcome than TM.

The plasma levels of TM and VWFpp were significantly higher in the patients with TMA, especially TMA/other, thus suggesting that TMA might be associated with vascular endothelial cell injury and that elevated TM and VWFpp might be useful for the diagnosis of TMA/other. A contribution of acute endothelial dysfunction to renal impairment in sepsis is suggested by the significantly higher VWFpp and soluble TM levels in patients with increased creatinine values as well as by their strong positive correlations [23]. In contrast, the plasma levels of TM correlated with renal function, but those of VWFpp did not. In TMA/other patients, the VWFpp and TM levels were negatively correlated with ADAMTS13 activity, suggesting that vascular endothelial cell injury or the causes of vascular endothelial cell injury reduce the ADAMTS13 activity. In the event of severe sepsis, elastase derived from activated granulocyte might reduce the activity of ADAMTS13 [24].

In summary, there are many patients with TMA not due to markedly reduced ADAMTS13, and VWFpp may be useful for the diagnosis of this type of TMA.

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**Conflict of interest** All authors disclose no financial or personal relationship with other people or organizations that could inappropriately influence their work.

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ORIGINAL ARTICLE

# Monitoring for anti-Xa activity for prophylactic administration of Fondaparinux in patients with artificial joint replacement

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**Abstract** The efficacy of measuring anti-Xa activity was evaluated in major orthopedic surgery patients receiving thrombo-prophylaxis with Fondaparinux. Although 98 orthopedic patients including those receiving total hip replacement (THR) and total knee replacement (TKR) were treated with 1.5 mg of Fondaparinux for prophylaxis of deep vein thrombosis (DVT). Sixteen patients developed DVT, but none was associated with a fatal pulmonary embolism. There was a wide range of anti-Xa activity, but there were no patients with less than 0.15 mg/l or more than 0.90 mg/l. Anti-Xa activity gradually increased from days 1 to 8 and showed no significant difference between patients with and without DVT. Anti-Xa activity was correlated with weight, height, body mass index, and antithrombin activity. Postoperative plasma levels of D-dimer and soluble fibrin (SF) were markedly high, and those were significantly reduced at days 1 and 4 of treatment with Fondaparinux. Plasma levels of SF were significantly reduced at days 8 and 15, but D-dimer was not. These findings suggested that there was continued thrombin generation after the injection of Fondaparinux until day 8 and secondary fibrinolysis occurred on day 8. In conclusion, 1.5 mg of Fondaparinux may not be sufficient for the prophylaxis of silent DVT, but it was found to be useful for that of fatal pulmonary embolism. Consequently, monitoring anti-Xa activity may be unnecessary for the administration of Fondaparinux at such doses.

**Keywords** Deep vein thrombosis (DVT) · Total hip replacement (THR) · Total knee replacement (TKR) · Anti-Xa activity · Fondaparinux

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## 1 Introduction

Orthopedic surgery is associated with a very high rate of postoperative venous thromboembolism (VTE) [1, 2], the incidence of venographically proven VTE ranges from 45 to 57% after total hip replacement (THR) surgery in the absence of thrombo-prophylaxis, and 40–84% after total knee replacement (TKR) surgery [1]. Multiple studies [3–7] have established the superior efficacy of low-molecular-weight heparin (LMWH) over unfractionated heparin (UFH) or warfarin for VTE prophylaxis in orthopedic surgery patients, with relative risk reductions ranging from 44 to 70%, depending on the type of surgery. The incidence of symptomatic postoperative breakthrough VTE is considerably lower (1–4%) [3–6] and studies have demonstrated that 40–90% of such episodes manifest as proximal deep

vein thrombosis (DVT) [3, 4], which is associated with a high risk of pulmonary embolism (PE) [7].

Fondaparinux is the first selective factor Xa inhibitor approved for use in thrombo-prophylaxis after orthopedic surgery [8–10] and studies comparing Fondaparinux with LMWH showed it to be very efficient in thrombo-prophylaxis in patients after orthopedic surgery [9, 10]. In Japan, Fondaparinux is frequently administered at a dose of 1.5 mg instead of 2.5 mg to avoid serious bleeding. No method has so far been clinically established to monitor drugs because a sufficient prolongation of activated partial thromboplastin time (APTT) cannot be observed in patients treated with Fondaparinux or LMWH. Anti-Xa activity has been measured as UFH or LMWH activity [11, 12].

This study measured the anti-Xa activity of Fondaparinux in 98 orthopedic patients after THR or TKR and examined the relationship between anti-Xa activity and various factors.

## 2 Materials and methods

Ninety-eight orthopedic patients treated with 1.5 mg of Fondaparinux (GlaxoSmithKline, Tokyo, Japan) and intermittent pneumatic compression for prophylaxis of DVT from 1 February 2010 to 31 December 2010 were registered in this study (Table 1). Anti-Xa activity, fibrin and fibrinogen degradation products (FDP), D-dimer, soluble fibrin (SF) and antithrombin (AT) activity were measured in 73 patients after THR and 23 patients after TKR on days 1, 4, 8, and 15 of the administration of Fondaparinux. The patients received 1.5 mg of Fondaparinux by hypodermic injection once a day during 14 days, beginning 24 h after extubation of epidural anesthesia. The anti-Xa activity was monitored 3 h after injection of Fondaparinux. The study protocol was approved by the Human Ethics Review Committee of the Mie University School of Medicine and a signed consent form was obtained from each subject. This study was faithfully carried out in accordance with the Declaration of Helsinki.

**Table 1** Patients' characteristics

| | Median (25–75%) |
|---|---|
| Age (years old) | 68.0 (61.0–75.0) |
| Female:male | 75:23 |
| THA:TKA | 73:25 |
| Weight (kg) | 57.1 (50.1–66.9) |
| Height (cm) | 153.0 (147.5–158.5) |
| Body mass index (kg/m$^2$) | 24.2 (21.9–27.0) |
| Body surface area (cm$^2$) | 1.52 (1.44–1.68) |
| Creatinine (mg/ml) | 0.67 (0.56–0.80) |
| eGFR | 73.0 (59.2–85.2) |
| Hemoglobin (Hb, pre; g/dl) | 12.2 (11.4–12.8) |
| Reduction of Hb from the beginning of Fondaparinux injection to day 15 (g/dl) | 1.20 (0.68–1.60) |
| Antithrombin (pre; %) | 81.5 (72.5–88.8) |

The anti-Xa activity of Fondaparinux was measured using Testzym®Heparin S (Sekisui Medical Co. Ltd., Japan) and a Coagrex®800 (an instrument from Sysmex Co. Ltd.). Testzym® Heparin S consists of bovine Xa (71 nkat/vial), AT (10 IU/vial), Chromogenic substrate (S-2222: Benz-Ile-Glu-Gly-Arg-pNA·HCl 25 mg), pooled lyophilized normal plasma, and buffer (pH 8.4) [11, 12]. A standard curve was made up for the lyophilized normal plasma using various concentrations of Fondaparinux.

The reagents and objects were loaded into the Coagrex 800, and the anti-Xa activity of Fondaparinux was automatically measured.A 135 μL aliquot of Xa was added to 8 μL of plasma (with diluent solution added in advance), and 75 μL of substrate was added.The rate at which the p-NA was released was measured photometrically at 405 nm.The anti-Xa activity of Fondaparinux was then calculated using the standard curve.

Plasma levels of FDP, D-dimer and SF were measured by the latex agglutination method using Nanopia FDP, Nanopia D-dimer and Nanopia SF (Sekisui Medical), respectively [13]. The plasma levels of AT were measured by chromogenic substrate using a Testzym S ATIII kit (Sekisui Medical).

The diagnosis of DVT was carried out by echography before the operation, on days 4 and 14.

### 2.1 Statistical analysis

The data are expressed as the medians (25–75 percentile) or (95% CI). The differences between the groups were examined using the Mann–Whitney $U$ test. A $p$ value of less than 0.05 was considered to be statistically significant. The correlations between 2 variables were tested by Pearson's correlation analysis. All statistical analyses were performed using the SPSS II software package (SPSS Japan, Tokyo).

## 3 Results

The median (95% CI) of anti-Xa activity was 0.02 (0.0–0.16) mg/l, 0.30 (0.19–0.54) mg/l, 0.40 (0.23–0.70) mg/l, 0.47 (0.26–0.73) mg/l and 0.22 (0.02–0.51) mg/l in before, and on days 1, 4, 8 and 15 of the administration of Fondaparinux, respectively (Fig. 1). There was a wide range of anti-Xa activity but there were no patients with less than 0.15 mg/l or more than 0.90 mg/l. The anti-Xa activity from days 1 to 8 was significantly high in

comparison to day 0 (before treatment) ($p < 0.001$), and gradually increased during this period.

Table 2 shows the relationships between anti-Xa activity and various factors. The correlation coefficient ($r$ value) was high with AT before the injection, with weight, height, body surface area (SBA), AT, creatinine and estimated glomerular filtration rate (eGFR) at day 1, with weight, SBA and AT at day 4, and with weight, height, body mass index (BMI), SBA and AT at day 8. There were 18 patients with a reduction of more than 2 g/dl of hemoglobin from the beginning of Fondaparinux injection to day 15, but no fatal bleeding. There was no significant difference in anti-Xa activity between the patients with and without a reduction of more than 2 g/dl of hemoglobin during the above period.

Sixteen patients developed DVT, despite prophylaxis with Fondaparinux, but there was no incidence of fatal PE. Only one case developed proximal DVT. Figure 2 shows that there was no significant difference in anti-Xa activity between patients with and without DVT.

The plasma levels of FDP, D-dimer and SF were markedly high before the injection of Fondaparinux, and those were significantly lower at days 1 and 4 in comparison to those before the injection (Fig. 3; Table 3). The plasma levels of SF were significantly lower at days 8 and 15 in comparison to those before the injection but the levels of FDP and D-dimer were not lower. Plasma SF levels were also high at days 1, 4 and 8 in comparison to those in healthy volunteers (<5.5 μg/ml).

## 4 Discussion

In the artificial joint replacement of our hospital, there were more than 15% of patients with reduction of more

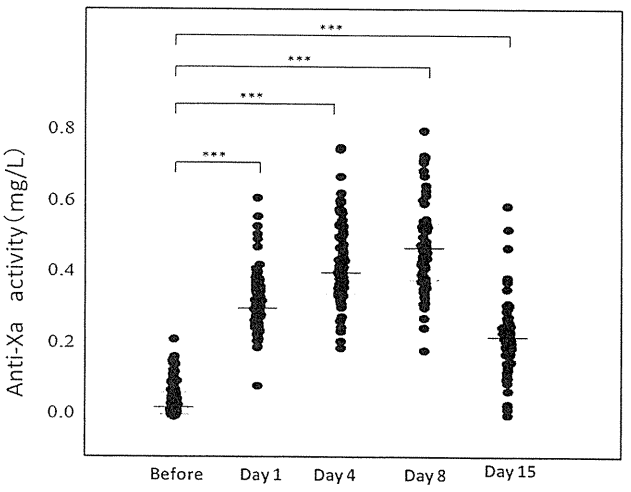


**Fig. 1** Anti-Xa activity in patients treated with Fondaparinux. The blood was sampled 3 h after injection of Fondaparinux. ***$p < 0.001$

**Table 2** Relationships between the anti-Xa activity and various factors

| | Age | Weight | Height | BMI | SBA | FDP | D-dimer | SF | AT | Creatinine | eGFR | Hb |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Before | −0.088 (NS) | −0.040 (NS) | 0.046 (NS) | −0.090 (NS) | −0.008 (NS) | 0.099 (NS) | 0.150 (NS) | −0.077 (NS) | 0.247 ($p < 0.05$) | −0.186 (NS) | 0.185 (NS) | 0.083 (NS) |
| Day 1 | −0.175 (NS) | −0.275 ($p < 0.01$) | −0.223 ($p < 0.05$) | −0.166 (NS) | −0.284 ($p < 0.01$) | 0.068 (NS) | 0.057 (NS) | 0.005 (NS) | 0.342 ($p < 0.01$) | −0.222 ($p < 0.05$) | 0.252 ($p < 0.05$) | −0.107 (NS) |
| Day 4 | −0.019 (NS) | −0.237 ($p < 0.05$) | −0.142 (NS) | −0.168 (NS) | −0.223 ($p < 0.05$) | −0.017 (NS) | −0.014 (NS) | −0.726 (NS) | 0.350 ($p < 0.01$) | 0.163 (NS) | −0.247 (NS) | −0.421 (NS) |
| Day 8 | −0.074 (NS) | −0.524 ($p < 0.001$) | −0.237 ($p < 0.05$) | −0.429 ($p < 0.001$) | −0.477 ($p < 0.001$) | 0.005 (NS) | −0.042 (NS) | 0.064 (NS) | 0.446 ($p < 0.001$) | 0.076 (NS) | −0.116 (NS) | 0.083 (NS) |

Data show the correlation coefficient

*BMI* body mass index, *SBA* body surface area, *FDP* fibrin and fibrinogen degradation products, *SF* soluble fibrin, *AT* antithrombin, *eGFR* estimated glomerular filtration rate, *Hb* hemoglobin

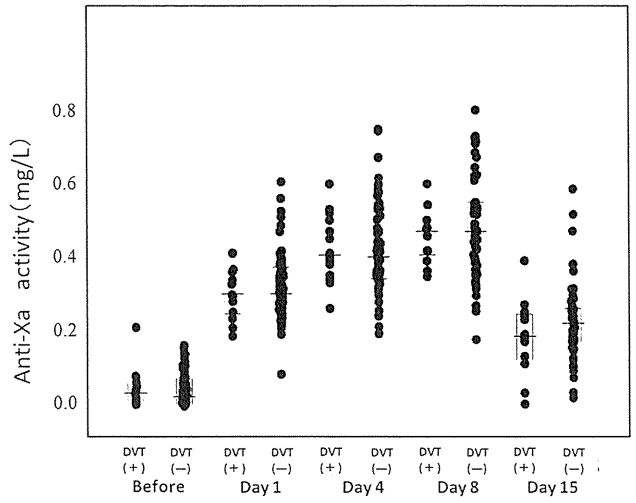


**Fig. 2** Anti-Xa activity in patients treated with Fondaparinux with or without DVT. The blood was sampled 3 h after injection of Fondaparinux

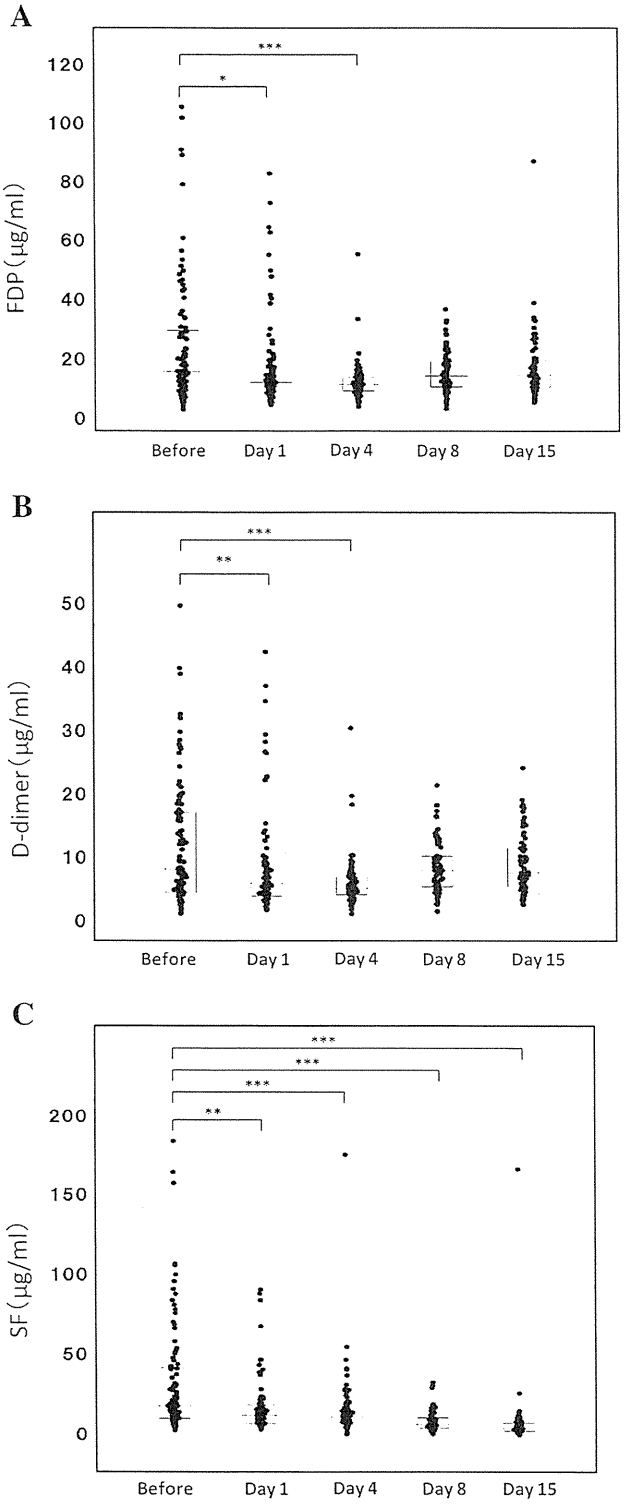


**Fig. 3** **a** FDP levels in patients treated with Fondaparinux. $***p < 0.001$, $*p < 0.05$. **b** D-dimer levels in patients treated with Fondaparinux. $***p < 0.001$, $**p < 0.01$. **c** SF levels in patients treated with Fondaparinux. $***p < 0.001$, $**p < 0.01$

than 2 g/dl of hemoglobin from the beginning of the 2.5 mg Fondaparinux injection until day 15. Therefore, the patients with artificial joint replacement were treated with 1.5 mg instead of 2.5 mg Fondaparinux in this hospital. Indeed, 1.5 mg Fondaparinux is often used in Japanese patients with a low weight, renal failure or who pose a high risk. Table 1 shows that most of those patients were females, old and low weight individuals.

Sixteen of 98 cases receiving prophylaxis with 1.5 mg Fondaparinux developed DVT, but 15 cases were distal DVT, which has a low risk for PE. Several previous studies [3–6] of orthopedic surgery patients found that the rates of symptomatic VTE after similar durations of LMWH prophylaxis ranged from 1 to 4%. There was only one case of proximal DVT in the current cohort, thus suggesting that the injection of 1.5 mg Fondaparinux is useful for the prophylaxis of proximal DVT following THR or TKR. Clinical examination for DVT in the context of orthopedic surgery has poor predictive value [14]; therefore, patients with symptoms caused by the surgery itself (e.g., pain, lower leg swelling) may have DVT diagnosed by venous ultrasound, and thereby the DVT is misclassified as symptomatic.

There was a wide range of anti-Xa activity in the patients treated with Fondaparinux, suggesting that high dose administration of Fondaparinux should be monitored by the anti-Xa activity. However, it might not be necessary to monitor anti-Xa activity following the injection of 1.5 mg Fondaparinux. Indeed, there was no significant difference in the anti-Xa activity between patients with and without DVT, and the highest anti-Xa activity was less than 1 mg/l. The plasma levels of FDP, D-dimer and SF were markedly high from days 1 to 8. D-dimer remained elevated long after the onset of DVT but that of SF was short [15]. These finding suggested that the generation of thrombin continued until day 8. While, a re-elevation of FDP and

**Table 3** Effects of Fondaparinux on the fibrin-related markers and AT activity

| | FDP (μg/ml) | D-dimer (μg/ml) | SF (μg/ml) | AT (%) |
|---|---|---|---|---|
| Before | 16.3 (10.1–30.3) | 8.3 (4.6–17.3) | 18.4 (10.7–42.2) | 81.5 (72.5–88.8) |
| Day 1 | 12.7 (9.5–17.9)* | 6.1 (4.1–9.4)** | 12.5 (7.7–19.2)** | 83.0 (75.3–90.7) |
| Day 4 | 12.0 (9.7–14.4)*** | 5.4 (4.3–7.2)*** | 11.8 (8.2–17.1)*** | 92.8 (84.2–103.7)*** |
| Day 8 | 14.9 (11.2–19.7) | 8.2 (5.6–10.4) | 7.0 (4.6–11.2)*** | 99.9 (92.1–113.2)*** |
| Day 15 | 15.3 (11.0–20.3) | 7.8 (5.6–11.6) | 4.500 (2.9–8.0)*** | 94.5 (88.1–104.6)*** |

Data express the median (25–75 percentile)

* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ in comparison to "before"

D-dimer indicated that secondary fibrinolysis may occur from days 8 to 15. The dose of Fondaparinux might be not sufficient (Table 3).

The anti-Xa activity was significantly correlated with weight, height, BMI, SBA and AT. Obesity with BMI > 25 also increases the risk of postoperative symptomatic VTE, which is likely related to an insufficient dosage of LMWH and the ineffectiveness of mechanical prophylaxis such as pneumatic compression [16]. The current study did not find independent associations between traditional VTE risk factors and breakthrough VTE. There is a relationship between the concentration of Fondaparinux and renal function, but only a slight correlation was observed in this study. The AT activity was low after the operation ("before" the injection and on "day 1" of the injection) and it significantly correlated with the anti-Xa activity, thus suggesting that the patients with a reduced AT activity may, therefore, have a low anti-Xa activity.

Studies [17] of symptomatic DVT after orthopedic surgery show that the proportion proximal DVTs ranges from 50 to 90% in THR patients and from 40 to 50% in TKR patients; however, only about 1.0% of the DVTs diagnosed in the current patients were proximal, and there were no pulmonary emboli. Most postoperative DVTs begin in the deep veins of the calf. Isolated distal DVT has a negligible rate of PE; however, one in six asymptomatic distal DVTs, and up to one in three symptomatic distal DVTs will extend to involve the proximal veins without treatment [7].

In conclusion, the administration of 1.5 mg Fondaparinux was useful for the prevention of fatal PE, but this amount of Fondaparinux might not be sufficient for the prophylaxis of the silent DVT. The monitoring of the anti-Xa activity may not be necessary for this amount of Fondaparinux.

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