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Letter to the Editors-in-Chief

Higher FVIII:C measured by chromogenic substrate assay than by one-stage assay is associated with silent hemophilic arthropathy

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1. Introduction

The Joint Outcome Study demonstrated that prophylactic therapy reduced joint bleeding and improved joint status. However, magnetic resonance imaging (MRI) revealed that the joints of patients who had no clinically evident joint bleeding (CEJB) displayed some abnormalities indicative of hemophilic arthropathy [1]. One explanation might be the presence of subclinical joint bleeding that passed unrecognized by patients. However, although many reports have implied their existence [1,2], such bleeds have remained unproven. If subclinical bleeds are really present, annualized joint bleeding rate (AJBR) would be of limited use for monitoring hemophilic arthropathy. Factors more closely related to development of arthropathy should thus be elucidated.

Here, we performed a fact-finding survey of 15 adult patients with hemophilia A who had never experienced CEJB throughout their lives. The aim was to confirm whether patients who had never experienced CEJB did not develop hemophilic arthropathy, and also whether patients can develop hemophilic arthropathy without CEJB, to identify the characteristics of such patients.

2. Methods

2.1. Study participants and investigation items

This study included adult hemophilia A patients (age > 20 years) who had never experienced CEJB. To investigate the history of CEJB, patients were specifically interviewed and their medical records were reviewed as far back as possible. Exclusion criteria comprised the presence or history of FVIII inhibitor. Investigation items comprised: age, body mass index (BMI), FVIII:C, FVIII antigen (FVIII:Ag), causative mutation, and Pettersson score (PS). All patients provided written, informed consent to participate in this study, which then proceeded according to the Declaration of Helsinki under the approval of the Nagoya University Hospital Ethics Committee (approval number: 2015-0391).

2.2. PS

Hemophilic arthropathy was assessed in bilateral knees, ankles and elbows using Pettersson radiological scores [3]. Having a joint replaced with an artificial prosthesis was considered a full score (13 points). Only orthopedists estimated joint scores. The PS employed was the most recent one available. Any positive score (i.e., ≥ 1) for the joint was judged as representing a positive PS in this study. As a positive PS represents radiologic changes, such a score indicates the development of arthropathy.

2.3. FVIII activity (FVIII:C) and FVIII antigen (FVIII:Ag)

We measured FVIII activity (FVIII:C) using both chromogenic substrate assay (CSA) and one-stage assay (OSA). CSA measures FVIII activity using Biophen FVIII:C (Hyphen BioMed, Neuville-sur-Oise, France). The OSA is based on the activated partial thromboplastin time (APTT) of FVIII immune-depleted plasma using an APTT reagent provided with an analyzer. The OSA proceeded using Thrombocheck APTT with ellagic acid activator, Thrombocheck FVIII and a CS-5100 automated coagulation analyzer (all from Sysmex, Kobe, Japan) [4].

Factor VIII antigen (FVIII:Ag) was quantified using the VisuLize™ FVIII Antigen Kit (Affinity Biologicals, Ancaster, Canada), an enzyme-linked immunosorbent assay, in accordance with the instructions from the manufacturer.

3. Results

3.1. Background and clinical stratification of patients

We enrolled 15 male adult patients with hemophilia A (mean age ± standard deviation (SD), 47.3 ± 17.1 years; median age, 37 years). Mean and median BMI were 23.3 ± 3.9 kg/m^2 and 21 kg/m^2, respectively (Supplemental Table 1). Disease severity was classified as severe in 1 patient, differed according to determination by CSA or OSA in 3 patients (moderate by CSA and mild by OSA in 1 patient, mild by CSA and moderate by OSA in 2 patients), moderate in 4 patients, and mild in 7 patients.

3.2. Patients with hemophilic arthropathy displayed higher FVIII:C measured by CSA than that measured by OSA

Relationships between baseline FVIII:C and PS in the 15 patients are

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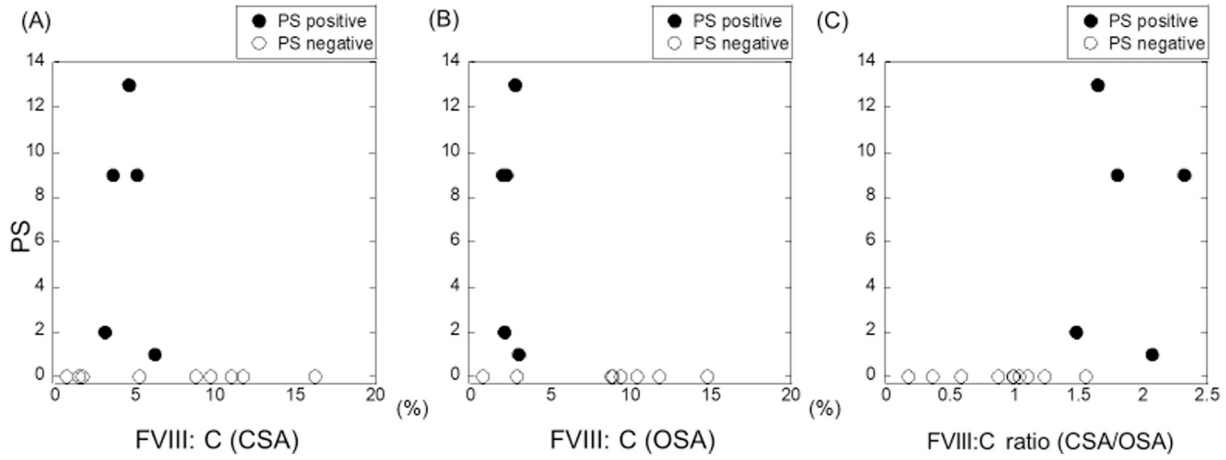

Fig. 1. Five of 15 patients were PS-positive.
PS of all enrolled patients by baseline FVIII:C by CSA (A) or OSA (B). Five patients were PS-positive. They all displayed a discrepancy in FVIII:C between CSA and OSA, with higher FVIII:C by CSA, Their FVIII:C ratio (CSA/OSA) was thus also higher than that of the others (C).
PS: Pettersson score, CSA: chromogenic substrate assay, OSA: one-stage assay.

Table 1
Comparison of the two groups.

	PS negative			PS positive			*P*-value
	n	Mean ± SD	Median [IQR]	n	Mean ± SD	Median [IQR]	
Age (years)	10	42.2 ± 16.1	34 [31.8, 56.8]	5	57.4 ± 15.7	67 [40.5, 69.5]	0.342
FVIII:C (CSA) (%)	10	7.6 ± 5.1	9.3 [1.7, 11.2]	5	4.5 ± 1.2	4.6 [3.4, 5.7]	> 0.999
FVIII:C (OSA) (%)	10	8.6 ± 4.0	8.9 [7.3, 10.8]	5	2.4 ± 0.4	2.2 [2.1, 2.9]	0.118
FVIII:C ratio (CSA/OSA)	10	0.9 ± 0.4	1.0 [0.5, 1.1]	5	1.9 ± 0.3	1.8 [1.6, 2.2]	**0.020***
FVIII:Ag (%)	10	19.3 ± 20.5	10.8 [6.9, 25.2]	5	5.2 ± 2.9	4.9 [2.4, 8.2]	0.120
PS	10	0.0	0.0 [0.0, 0.0]	5	6.8 ± 5.1	9.0 [1.5, 11.0]	–

Mann-Whitney *U* test (Bonferroni correction).
PS: Pettersson score. *:P < 0.05

shown in Fig. 1A and B. Five of these patients showed positive PS. No patients with baseline FVIII:C > 6.2% as measured with CSA showed positive PS, nor did any patients with baseline FVIII:C > 3% as measured with OSA. All patients who showed a positive PS displayed higher FVIII:C ratio (CSA/OSA) (Fig. 1C). The 15 patients were divided into two groups: one comprising patients with positive PS; and the other comprising patients with negative PS. The results were analyzed by non-parametric test (Table 1). Mean and median FVIII:C ratio (CSA/OSA) were significantly higher for those patients who were PS-positive than for patients who were PS-negative. In other words, a discrepancy existed in baseline FVIII:C between CSA and OSA, with higher baseline FVIII:C as measured from CSA.

3.3. Causative mutation in PS-positive patients

The causative mutations for hemophilia A were identified in all patients, and the five patients who were PS-positive displayed missense mutations, as p. Asp182Tyr, p. Thr314Ile, p. Arg1800His and p. Arg2169His (Supplemental Table 2). These mutations were not located near thrombin cleavage or factor IXa-binding sites that have been reported to be associated with discrepancies between the two methods with higher FVIII:C as measured by CSA, not only from the perspective of gene sequences, but also in terms of spatial location (Supplemental Fig. 1).

4. Discussion

PS is an additive radiographic scoring system for the severity of hemophilic arthropathy, and has been adopted by the Orthopedic Advisory Committee of the World Federation of Hemophilia. However, the score has been reported to vary according to the evaluator [5]. To reduce variation due to the evaluator, the same expert orthopedist (X-rays of joints are evaluated by orthopedists in Japan, not by radiologists) performed all evaluations in this study. As this scoring system is based on radiologic changes, early-phase hemophilic arthropathy cannot be detected. These issues represent limitations in the present study. Five of the 15 patients who were free of CEJB showed a positive PS in the study, providing evidence for the development of hemophilic arthropathy. This result indicated that lack of CEJB does not represent a necessary condition for a lack of hemophilic arthropathy. Interestingly, these patients displayed discrepancies in FVIII:C as measured by CSA and OSA, with higher FVIII:C by CSA than by OSA. The discrepancy in FVIII:C between CSA and OSA has been discussed for years [6,7]. Trossaert et al. reported that FVIII:C as measured by CSA better reflected the clinical bleeding tendency compared with the FVIII:C by OSA [8]. Postgieter et al. also reported that patients who had higher FVIII:C by CSA than by OSA displayed few or no bleeding symptoms [9]. Considering these reports, these patients might escape CEJB, but not subclinical bleeding or subsequent hemophilic arthropathy. As the mechanism underlying such discrepancy, they hypothesized that the genetic mutations causing the discrepancy clustered around thrombin cleavage or factor IXa binding sites. These mutations would impair FVIII activation by thrombin or binding of FVIII to factor IX (FIXa). However, such effects were overcome in CSA by supraphysiological concentrations of thrombin and FIX, as well as the longer reaction time compared to OSA [9]. Considering these findings, FVIII that is higher by CSA than by OSA can prevent CEJB, but not subclinical joint bleeding, because the hemostatic effects are slow. However, this theory does not

explain all cases. We actually identified four kinds of missense mutations in these patients. These mutations were not located near thrombin cleavage or factor IXa-binding sites, not only from the viewpoint of gene sequence, but also in terms of spatial location. As for p. Arg1800His, Yada et al. reported that the mild clinical phenotypes of patients with p. Arg1800His were associated with enhanced binding affinity of FVIII for FX [10]. However, the mechanisms underlying these discrepancies are far from being completely elucidated, and further investigations are required.

When we estimated hemophilic arthropathy, variations in activity should have been considered in greater detail. Unfortunately, physical loading by lifestyle (such as participation in sports) was not investigated in the study. Such differences might influence the development and progression of hemophilic arthropathy. Two of the PS-positive patients showed the p. Arg2169His mutation. These patients were almost the same age, but PS status differed. One possibility is that differences in the physical load according to lifestyle among patients with the same causative mutation might cause variations in PS.

5. Conclusions

Our study indicated that even hemophilia A patients who had never experienced CEJB risked developing hemophilic arthropathy. A distinct trend was seen for these patients to show higher FVIII:C by CSA than by OSA. Although patients who show higher FVIII:C by CSA than by OSA are generally known to display mild bleeding tendency, subclinical bleeding may still occur and lead to the development of silent hemophilic arthropathy.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.thromres.2020.01.003.

Declaration of competing interest

The authors have no interests that might be perceived as posing a conflict of interest or bias.

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Author contribution

MO, AS and TK performed the research. NS analyzed the data and wrote the paper. NT estimated the Pettersson score. MO, ST, SS, YH, MK, FH and TK performed the genetic analyses. TK, NI, AK, HK and TM designed the research study and reviewed the paper.

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