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Short Communication

Difference in blood tacrolimus concentration between ACMIA and MEIA in samples with low haematocrit values

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Abstract

Objectives The aim was to compare blood tacrolimus concentrations in anaemic patients between affinity column-mediated immunoassay (ACMIA) and microparticle enzyme immunoassay (MEIA).

Methods Blood concentrations of tacrolimus in 235 whole-blood samples from 64 patients treated with tacrolimus were determined by the two assay methods. Fifty-three samples had low haematocrit (Ht) values (<25%), whereas the other samples had normal Ht values.

Key findings Measured tacrolimus concentrations in samples with normal Ht values did not differ between ACMIA and MEIA (median, range; 6.6, 0–29.1 vs 7.3, 0–27.4 ng/ml). On the other hand, MEIA determined significantly higher tacrolimus concentrations in samples with lower Ht values compared with ACMIA (14.0, 2.4–25.7 vs 11.5, 0–21.3 ng/ml; $P < 0.05$). This difference was caused by overestimated blood concentrations in MEIA derived from lower Ht values, which could be corrected using the Ht value for each sample (calculated MEIA (MEIAcalc)). The corrected concentrations (MEIAcalc; 10.8, 0–21.3 ng/ml) were comparable with those of ACMIA. It was confirmed that the difference in concentrations between ACMIA and MEIA was remarkable in routine monitoring of blood tacrolimus for a liver transplant recipient with anaemia.

Conclusions ACMIA can be applied to routine therapeutic drug monitoring of tacrolimus therapy in anaemic patients.

Keywords ACMIA; haematocrit value; MEIA; tacrolimus; therapeutic drug monitoring

Introduction

Tacrolimus, a potent immunosuppressive agent, is used for the prevention of rejection after organ transplantation and for the treatment of autoimmune diseases.[1] Monitoring of blood tacrolimus concentration is required for adjusting the optimal dose in each patient, due to the narrow therapeutic window and large individual variability in tacrolimus pharmacokinetics.[2,3] Immunoassay is used for determining the concentration of tacrolimus in whole blood in the clinical setting. The most common assay method for determining blood tacrolimus concentration is microparticle enzyme immunoassay (MEIA), which provides overestimated concentrations of tacrolimus in samples with lower haematocrit (Ht) values (<25%).[4–7] Therefore, the quality of MEIA for blood tacrolimus is not guaranteed in samples obtained from anaemic patients.[8] Tacrolimus concentrations determined by MEIA have not always been correct,[7,9] because 29.3% and 61.5% of samples had lower Ht values in liver and bone marrow transplant recipients, respectively.[7]

Affinity column-mediated immunoassay (ACMIA), an alternative assay method, was developed for measuring blood tacrolimus concentration. ACMIA may be used even in samples obtained from anaemic patients, because ACMIA for blood tacrolimus concentration can accept samples with a wide range of Ht values (19.5–52.7%).[10] To confirm this hypothesis, we compared blood tacrolimus concentrations between ACMIA and MEIA in

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Table 1 Patients' characteristics

	All samples	Haematocrit value	
		<25%	≥25%
No. patients	64	10	61
Sex (male/female)	21/43	7/3	19/42
Age (years)	39 (1–86)	31 (1–62)	41 (1–86)
No. samples	235	53	182
Haematocrit value (%)	31.2 (17.8–51.9)	21.4 (17.8–24.9)	34.0 (25.1–51.9)
Patient cohort (sample/patient)			
Liver transplantation	90/17	25/3	65/17
Bone marrow transplantation	90/10	28/7	62/7
Kidney transplantation	5/4	0	5/4
Rheumatoid arthritis	50/33	0	50/33

Data are presented as number or median (range).

samples with Ht values of <25% and ≥25%. We also report two paediatric liver transplantation recipients with unstable Ht values in the early postoperative period.

Materials and Methods

Blood samples and tacrolimus assay

We collected 235 whole-blood samples (90 liver, 90 bone marrow, 5 kidney transplantation and 50 rheumatoid arthritis) from 64 patients (17 liver, 10 bone marrow, 4 kidney transplant recipients, and 33 rheumatoid arthritis) for this study (Table 1). This study was approved by the Ethical Committee of Tsukuba University Hospital. Informed consent was obtained from the patients.

Blood tacrolimus concentrations were determined by ACMIA (TACR Flex; Siemens Healthcare Diagnostics, Tokyo, Japan), using a Dimension Xpand Plus analyzer (Siemens Healthcare Diagnostics), and MEIA (MEIA-II Tacrolimus; Abbott, Chicago, USA) using an IMx analyzer (Abbott). These two assay methods were used in accordance with the manufacturers' instructions. MEIA requires pretreatment of whole blood with methanol for precipitation but ACMIA does not. The between-days coefficients of variation for the assays were 6.3–9.3% and 6.7–9.0% for ACMIA and MEIA, respectively. The overestimated blood concentrations in MEIA were corrected by calculation using the Ht values of each sample, as previously described (MEIAcalc).[9] This study was performed from May 2008 to September 2009.

Statistical analysis

Data are expressed as numbers, percentages, or median (range). Multiple comparisons of blood tacrolimus concentrations among ACMIA, MEIA and MEIAcalc were performed using the Mann–Whitney *U*-test with Bonferroni's correction following the Kruskal–Wallis test. $P < 0.05$ was considered statistically significant.

Results

Bland–Altman analysis for determining the difference in blood tacrolimus concentrations between MEIA and ACMIA is shown in Figure 1. The mean bias was 1.2 ng/ml, which remained unchanged in the range of 0–28 ng/ml. However, the bias seemed to be different between lower and higher Ht values of the assay samples (data not shown).

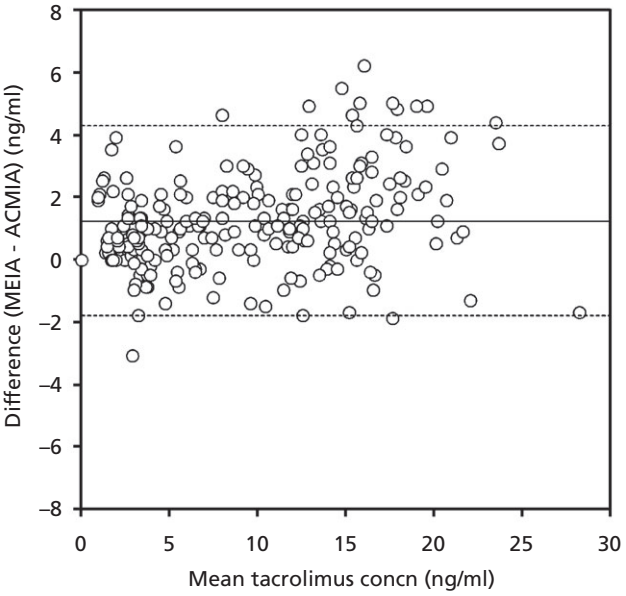

Figure 1 Bland–Altman analysis of blood tacrolimus concentrations determined by MEIA and ACMIA in 235 samples. Solid line, mean bias between the MEIA and ACMIA; dashed lines, the 95% limits of agreement for the bias. The mean tacrolimus concentrations were calculated by following equation: (MEIA + ACMIA)/2.

Of the 235 whole-blood samples, 53 had a low Ht value (<25%), which were out of range for assay quality guaranteed by MEIA. These samples were from liver (25; 47.2%) and bone marrow (28; 52.8%) transplant recipients (Table 1).

MEIA provided a higher concentration than ACMIA and MEIAcalc in all samples (median, range; 9.8, 0–27.4 vs 8.5, 0–29.1 and 8.5, 0–25.7 ng/ml, respectively; Table 2). A significant difference was observed in the values between MEIA and MEIAcalc ($P < 0.05$; Table 2); however, there was no

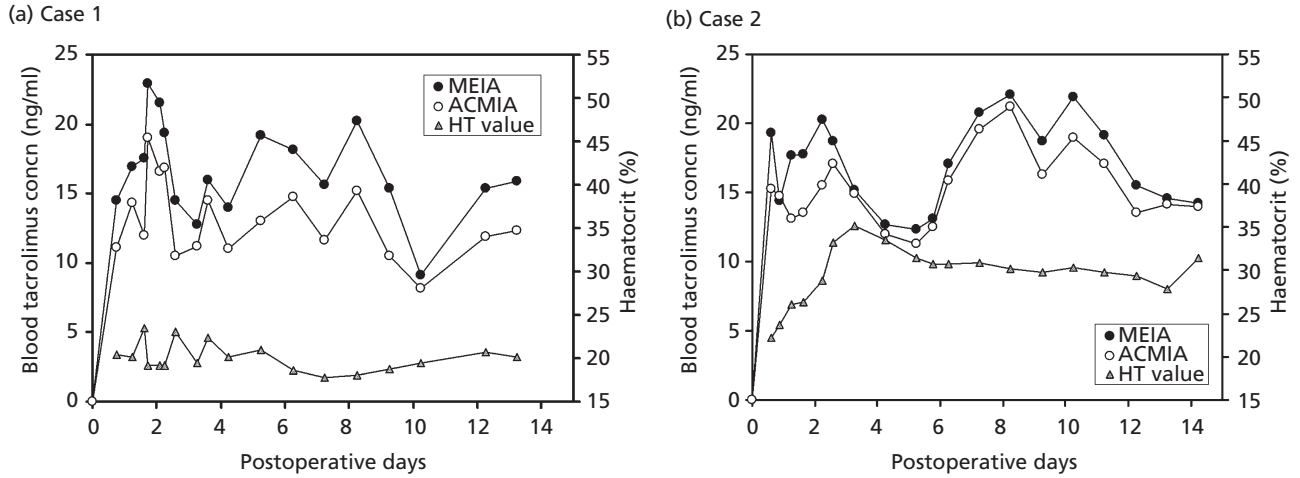

Figure 2 Profiles of blood tacrolimus concentrations determined by MEIA and ACMIA, and Ht value in two paediatric cases who received liver transplantation.

Table 2 Difference in blood tacrolimus concentrations among MEIA, ACMIA and MEIAcalc

	Blood tacrolimus concn (ng/ml)		
	MEIA	ACMIA	MEIAcalc
All samples ($n = 235$)	9.8 (0–27.4)	8.5 (0–29.1)	8.5 (0–25.7)[a]
Haematocrit <25% ($n = 53$)	14.0 (2.4–25.7)	11.5 (0–21.3)[a]	10.8 (0–21.3)[a]
Haematocrit ≥25% ($n = 182$)	7.3 (0–27.4)	6.6 (0–29.1)	6.7 (0–25.7)

MEIA, microparticle enzyme immunoassay; ACMIA, affinity column-mediated immunoassay; MEIAcalc, corrected MEIA by calculation, using the haematocrit value of each sample. Data are presented as median (range). [a]$P < 0.05$ compared with MEIA.

difference between ACMIA and MEIAcalc (Table 2). In samples with low Ht value, MEIA provided a significantly higher concentration than ACMIA (14.0, 2.4–25.7 vs 11.5, 0–21.3 ng/ml; $P < 0.05$; Table 2). In contrast, MEIAcalc (10.8, 0–21.3 ng/ml) was in close agreement with ACMIA (Table 2). There was no difference in blood tacrolimus concentrations among MEIA, ACMIA and MEIAcalc (7.3, 0–27.4; 6.6, 0–29.1; and 6.7, 0–25.7 ng/ml; Table 2) in samples with Ht values of ≥25%.

In all samples, the slope of the regression lines between MEIA and ACMIA was smaller than that of MEIAcalc and ACMIA (0.90 vs 1.01; Table 3). In samples with low Ht values, the slope of the regression lines between MEIAcalc and MEIA was greater than that of MEIAcalc and ACMIA (1.14 vs 0.96; Table 3). The slopes of the regression lines between MEIAcalc and MEIA or ACMIA were almost identical in samples with Ht values of ≥25% (1.09 vs 1.01; Table 3). The intercepts of the regression lines in samples with lower Ht values were greater than those with Ht values of ≥25%, though no difference was observed in the value between MEIAcalc and MEIA or ACMIA (Table 3).

Tacrolimus concentrations were compared among MEIA, ACMIA and MEIAcalc in two paediatric liver transplant recipients. Case 1 maintained low Ht values (19.8, 17.8–23.5%) to prevent hepatic artery thrombosis. Blood tacrolimus in MEIA was constantly higher than those in ACMIA (16.0, 9.1–22.9 vs 12.2, 8.2–19.0 ng/ml). There was no difference between ACMIA and MEIAcalc (12.6, 6.5–17.9 ng/ml). On the other hand, case 2 had Ht values higher than 25%, except for postoperative day 1 (30.2, 22.2–35.1%). There were no differences in blood tacrolimus concentrations among MEIA, ACMIA and MEIAcalc (Figure 2b).

Table 3 Correlation of blood tacrolimus concentrations determined by MEIA, ACMIA and MEIAcalc

x vs y	Slope	Intercept	Correlation
All samples ($n = 235$)			
MEIA vs ACMIA	0.90	–0.18	0.971
MEIAcalc vs ACMIA	1.01	0.25	0.973
Haematocrit <25% ($n = 53$)			
MEIAcalc vs MEIA	1.14	1.62	0.997
MEIAcalc vs ACMIA	0.96	1.23	0.961
Haematocrit ≥25% ($n = 182$)			
MEIAcalc vs MEIA	1.09	0.46	0.995
MEIAcalc vs ACMIA	1.01	0.12	0.974

MEIA, microparticle enzyme immunoassay; ACMIA, affinity column-mediated immunoassay; MEIAcalc, corrected MEIA by calculation, using the haematocrit value of each sample.

Discussion

It has been reported that a good correlation was found in determining blood tacrolimus concentration between ACMIA and MEIA, while the studies scarcely included whole blood

samples with lower Ht values.[11-14] In this study, we found that MEIA determined significantly higher tacrolimus concentrations compared with ACMIA in samples with lower Ht values (Table 2 and 3). This observation is a result of overestimated blood tacrolimus concentrations in MEIA derived from lower Ht values, because overestimated concentrations are corrected using the Ht value for each sample (MEIAcalc). Corrected concentrations determined by MEIAcalc were comparable with those determined by ACMIA (Table 2 and 3). These results showed that ACMIA accurately determined tacrolimus concentrations, even in samples with lower Ht values. ACMIA is considered to be a suitable assay method for determining blood tacrolimus concentration in liver and bone marrow transplant recipients, because such patients occasionally developed anaemia with Ht values of <25% (Table 1).

We confirmed the advantage of ACMIA in therapeutic drug monitoring of tacrolimus concentrations for two paediatric cases of liver transplantation (Figure 2). The difference in blood tacrolimus concentrations between ACMIA and MEIA was remarkable in case 1 with anaemia compared with the non-anaemic case 2. Overestimation may be derived from lower Ht values, because overestimated blood concentrations in MEIA could be corrected by MEIAcalc. Thus, the information of Ht value, which is not a routine measurement for therapeutic drug monitoring of tacrolimus, is required for the correction of whole blood tacrolimus determined by MEIA. ACMIA, therefore, is superior to MEIA in the issue of Ht correction as well as time-consuming sample pre-treatment.

Another possibility for the differences in measured tacrolimus concentrations between ACMIA and MEIA is cross-reactivity of the anti-tacrolimus antibody against tacrolimus metabolites.[11] This remains to be examined in future work, using HPLC-tandem mass spectrometry in samples with lower Ht values.

Conclusions

ACMIA provides relatively accurate blood tacrolimus concentration in samples with low Ht value, compared with MEIA. Therefore, it was considered that ACMIA could be applied to routine therapeutic drug monitoring for blood tacrolimus concentrations, even in anaemic patients.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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