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Simultaneous LC-MS/MS quantification of creatinine, iohexol and five immunosuppressants in renal transplant recipients using volumetric dried blood spot sampling

Background & aims

- Renal function and immunosuppressant exposure are considered key laboratory markers in the clinical management of renal transplant recipients. These markers are typically quantified from venous whole blood samples during outpatient clinic visits, typically every 3-4 months.
- The dried blood spot (DBS) technique has introduced options for remote self-sampling and allows for more flexible renal transplant recipient monitoring.
- We have previously developed a LC-MS/MS assay, capable of quantifying five immunosuppressants in DBS samples simultaneously, and clinically validated this assay for tacrolimus and mycophenolic acid exposure monitoring in renal transplantation.[1]
- However, the absence of a renal function marker was considered an important limitation of our previous assay, as patients still need to visit the outpatient clinic for renal function monitoring.
- Creatinine has provided a convenient renal function marker for decades but shows limited agreement with measured GFR. In recent years, measured GFR by determination of the plasma clearance of intravenous iohexol, has been advocated for clinical situations which dictate the application of more accurate renal function assessment techniques.
- In this study, we modified our previous LC-MS/MS assay to also enable quantification of creatinine and iohexol, to allow for simultaneous remote renal function monitoring and immunosuppressant exposure assessment in renal transplant recipients.

Methods

- A new multi-analyte LC-MS/MS assay was developed for quantification of tacrolimus, cyclosporine, everolimus, sirolimus, mycophenolic acid, creatinine and iohexol in DBS samples, in accordance with the EMA guidelines for bioanalytical method validation.
- DBS samples comprised standard Whatman 903 protein saver cards, incorporated in the Hemaxis DB 10 volumetric sampling device (DBS system, Gland, Switzerland) for standardized volume blood sampling (10 µL).
- Analytical cross-validation was performed for tacrolimus, cyclosporine, everolimus, mycophenolic acid and creatinine, using manually spotted DBS samples, originating from leftover venous whole blood samples from routine clinical care. No routine venous whole blood samples were available for sirolimus or iohexol, hampering analytical cross-validation for these agents. Agreement between the DBS and venous reference samples was evaluated using Passing-Bablok regression and Bland-Altman analysis.
- Quantification of tacrolimus, everolimus, cyclosporine, and mycophenolic acid from the reference venous whole blood samples was performed using previously validated LC-MS/MS assays at our department, as part of routine clinical care.[1,2] Creatinine was quantified on a Cobas 8000 instrument as part of routine clinical care at the LUMC clinical chemistry department using a standard creatininase-sarcosine enzymatic assay.

Analytical validation

The assay was validated successfully for all analytes

The assay showed adequate overall agreement with venous whole blood / plasma samples during analytical cross-validation

While only moderately accurate for creatinine, the assay showed adequate performance for creatinine trend monitoring

Accuracy and precision

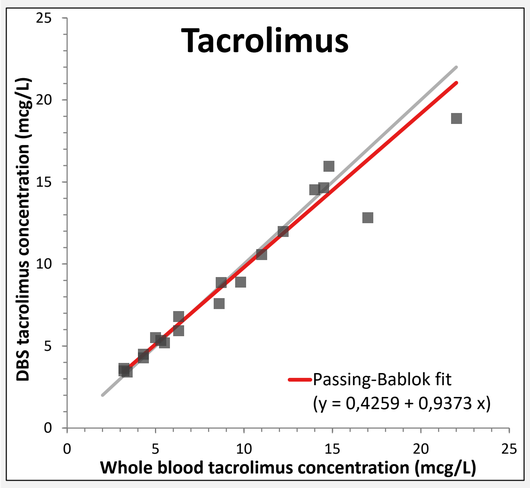
Analyte	Concentration (mcg/L)	Accuracy (%)		Precision (CV%)	
		Within-run	*Between-run*	*Within-run*	*Between-run*
Creatinine	16300	100.9	102.6	2.3	3.8
	31300	103.5	103.9	2.1	3.4
	46300	114.4	115.0	0.4	1.6
Cyclosporine	47.50	103.7	102.6	2.1	5.3
	225.0	108.6	107.3	1.9	5.4
	474.0	103.9	102.5	2.9	6.4
	1202	91.5	89.9	1.8	5.6
Everolimus	2.46	87.3	99.4	3.9	10.7
	4.71	91.6	100.1	4.3	7.6
	9.48	99.7	104.0	7.7	5.9
	33.7	101.6	102.8	2.1	2.2
Iohexol	15000	94.9	95.8	0.6	2.5
	45000	92.4	92.9	2.1	1.7
	75000	94.2	93.6	1.4	1.2
Mycophenolic acid	2500	101.3	98.7	0.6	3.0
	10000	98.0	98.6	4.5	2.8
	15000	99.2	99.0	1.4	3.0
Sirolimus	2.58	116.8	114.4	3.8	7.3
	9.20	103.6	108.2	5.3	4.6
	19.1	104.5	105.7	5.0	3.5
	39.3	96.5	101.6	2.2	4.3
Tacrolimus	2.56	97.7	102.7	1.9	4.1
	7.39	96.5	98.3	4.5	3.3
	15.8	99.6	100.4	1.8	2.4
	34.9	100.2	100.7	2.2	2.2

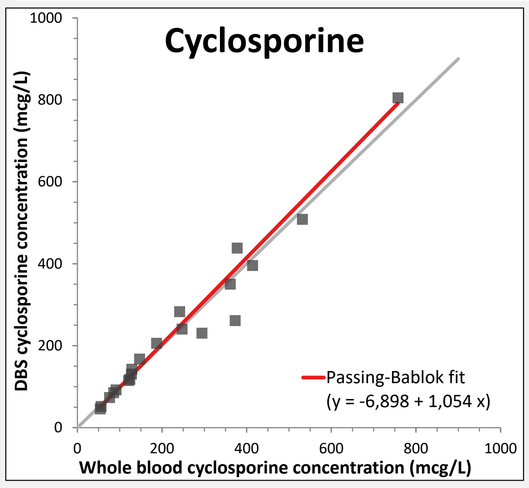
Linearity

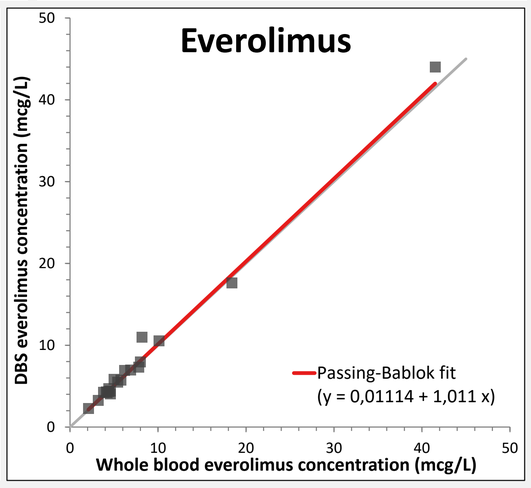
Analyte	LLQ (mcg/L)	ULQ (mcg/L)
Creatinine	3000	45000
Cyclosporine	10.6	1100
Everolimus	1.64	34.0
Iohexol	7500	90000
Mycophenolic acid	300	20000
Sirolimus	2.58	40.0
Tacrolimus	1.14	35.0

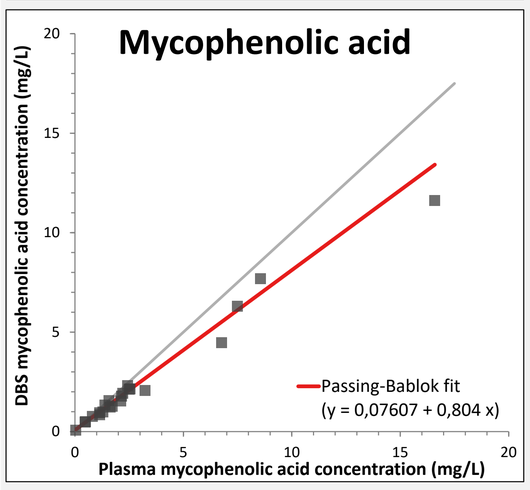
Recovery

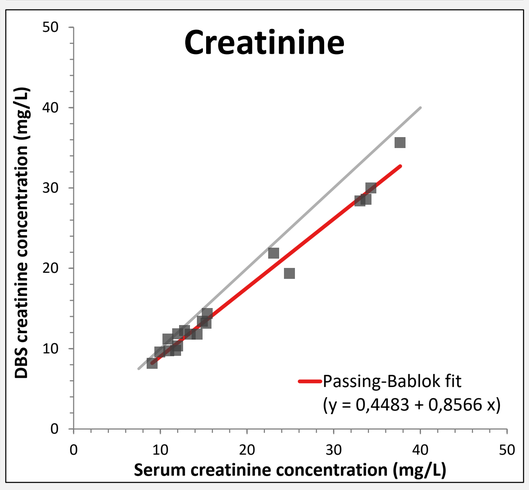
Analyte	Level	Recovery (%CV)
Creatinine-D3C13	Low	89% (9.5%)
	High	109% (9.2%)
Cyclosporine	Low	111% (8.1%)
	High	95% (9.2%)
Everolimus	Low	106% (11%)
	High	89% (10.1%)
Iohexol	Low	104% (16.5%)
	High	104% (1.8%)
Mycophenolic acid	Low	94% (19.2%)
	High	82% (25%)
Sirolimus	Low	114% (14.5%)
	High	97% (8.1%)
Tacrolimus	Low	119% (17.2%)
	High	111% (8.1%)











Cross-validation — Passing-Bablok regression

Cross-validation	N	*Slope [95% CI]*	*Intercept [95%CI]*
Tacrolimus	20	0.93 [0.85,1.00]	0.15 [0.01,0.29]
Everolimus	20	0.99 [0.93,1.07]	0.03 [-0.11,0.15]
Cyclosporine	20	1.04 [0.98,1.14]	-0.21 [-0.71,0.11]
MPA	20	0.80 [0.67,0.90]	0.08 [-0.13,0.19]
Creatinine	19	0.97 [0.87,1.05]	-0.03 [-0.23,0.25]

Cross-validation — Bland-Altman analysis

Cross-validation	N	*Bias [95% CI]*	*Limits of agreement [95% CI]*
Tacrolimus	20	-0.32 [-0.91,0.26]	-2.78 [-3.81,-1.76], 2.14 [1.12,3.16]
Everolimus	20	0.33 [-0.09,0.74]	-1.41 [-2.13,-0.69], 2.06 [1.34,2.78]
Cyclosporine	20	-2.63 [-20.0,14.8]	-75.5 [-106,-45.3], 70.3 [40.0,101]
MPA	20	-0.64 [-1.13,-0.15]	-2.81 [-3.66,-1.96], 1.52 [0.67,2.37]
Creatinine	19	-1.99 [-2.81,-1.16]	-5.34 [-6.77,-3.91], 1.36 [-0.07,2.80]

References, COI, Funding

1. Zwart *et al.* Br J Clin Pharmacol. 2018.
2. Moes *et al*. Eur J Clin Pharmacol. 2016.

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Discussion

- As no reference venous whole blood samples were available for sirolimus and iohexol, no cross-validation could be performed for these analytes.
- To facilitate clinical interpretation, a conversion factor to relate DBS concentrations to their corresponding plasma concentrations should be (re-)determined for mycophenolic acid and creatinine.

Conclusions

- **A promising multi-analyte LC-MS/MS assay to enable simultaneous remote renal function evaluation and immunosuppressant monitoring in renal transplant recipients was developed and validated analytically.**
- **Clinical validation based on paired DBS and venous whole blood / plasma samples is warranted before considering this assay for routine clinical care.**

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