AACC Annual Meeting, July 31, 2013, Houston, TX, poster B-234

Monoclonal antibodies for detection of retinol-binding protein 4 in human urine samples

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Introduction

Acute kidney injury (AKI) is increasingly recognized as life-threatening pathology closely associated with metabolic syndrome and cardiovascular diseases. Current kidney biomarkers such as creatinine and blood urea nitrogen were proved to be not satisfactory in clinical setting for AKI diagnosis due to the late appearance in serum and lack of specificity. New generation of biomarkers has begun to be introduced into clinical practice. Among those new biomarkers is retinol-binding protein 4 (RBP4)[1].

Cardiorenal syndrome is acknowledged as a pathology that includes both heart failure and worsening of renal function. Cardiorenal syndromes types 1 and 2 are pathology subtypes where primary disease is a heart failure and kidney dysfunction is the following. Those subtypes of cardiorenal syndromes are quite common complications of heart failure therefore timely identification of patients' kidney injury is of utmost importance for successful treatment[2].

Retinol-binding protein 4 is a low-molecular weight protein which participates in transport of retinol in the bloodstream. Serum RBP4 is known to be complexed (at least partly) with transthyretin. Recently it was shown that RBP may serve as a biomarker of loss of kidney function in acute kidney injury[1]. RBP4 appears quite early both in serum and urine upon kidney injury therefore serving as early biomarker of kidney damage.

The objective of the study was to develop human RBP4-specific monoclonal antibodies capable of detecting RBP4 in urine samples of patients with cardiorenal syndromes types 1 and 2.

Materials and methods

Native free RBP4 was obtained from HyTest Ltd.

Monoclonal antibodies

Recombinant RBP4 was used as an antigen for Balb/c mice immunization. Splenocytes of immune animals were fused with sp 2/0 myeloma cells. Antibody-producing hybridoma cells were subjected to positive selection using purified RBP4. Selected MAbs were labeled with stable Eu^{3+} chelate and used as detection antibodies in two-site combinations with unlabeled MAbs, to find best combinations for RBP4 sandwich immunofluorescent assay.

Sandwich immunofluorescent assay (IFA)

Capture antibodies, 1 µg per well in 100 µL of PBS, were incubated in immunoassay plates for 30 min at room temperature. After washing, 50 µL of tested sample or calibrator and 50 µL of detection antibodies labeled with stable europium (Eu^{3+}) chelate in assay buffer were added. After 30 min incubation the plates were washed, then enhancement solution was added, and fluorescence was measured.

Urine samples

First morning urine samples from healthy volunteers (n=7) and patients with cardiorenal syndromes types 1 and 2 (n=25) were collected into sterile tubes immediately on rising and were stored -20ºC until use. Before analyte measurement samples were centrifuged at 10000g for 10 minutes and diluted 1:2 with assay buffer. Cardiorenal syndrome clinical criteria included acute decompensated heart failure or chronic heart failure with serum creatinine increase >0.5 mg/dl.

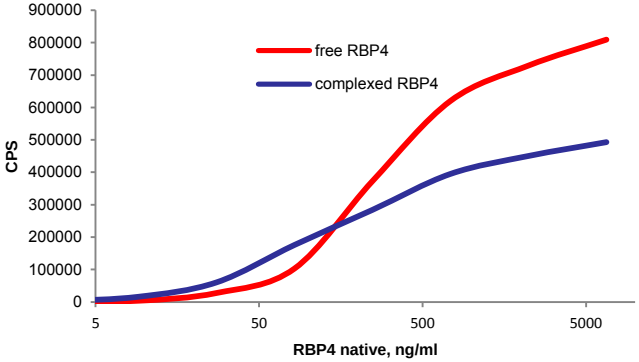

Fig. 1 Calibration curve for sandwich IFA, MAb pair RB48-RB49.
MAb RB48 was used as a capture (1 µg/well), whereas MAb RB49 was labeled with stable Eu^{3+} chelate and was used as a detection antibody (0.2 µg/well). Native RBP4 isolated from normal human plasma (either free or complexed with transthyretin) was used as a calibrator.

Results and discussion

Using standard hybridoma technology and recombinant human RBP4 as an immunogen we raised 6 MAbs specific to human RBP4. All MAbs were tested in sandwich IFA as capture and detection (labeled with stable Eu^{3+} chelate) antibodies. Several of MAb combinations showed good linearity when probed with purified native human RBP4 both in free form and complexed with transthyretin (Fig. 1). Different slope of the curves in linear region might be explained by conformational changes of RBP4 upon interaction with transthyretin leading to different affinity to antibodies. Two-site MAb combination RB48-RB49 (capture-detection, respectively) was selected for further clinical study based on sensitivity data.

It is well established fact that RBP4 in the bloodstream exists in the complex with transthyretin. Raised antibodies were tested for their ability to interact with free and transthyretin-bound RBP4 isolated from normal human serum. All 6 MAbs were shown to react with both types of native RBP4 isolated from serum (data not shown). To our knowledge, our study aimed is the first attempt to measure urine RBP4 levels in patients with cardiorenal syndromes types 1 and 2. Urine RBP4 levels in healthy persons were shown to be 5.2±3.1 ng/ml, which is in good accordance with previously published results[3] (Fig. 2), whereas urine RBP4 levels in patients with cardiorenal syndromes were shown to be 66.3±123.1 ng/ml. RBP4 levels in urine of patients with cardiorenal syndromes types 1 and 2 varied considerably – from 0.3 to 378 ng/ml. Reasons for those discrepancies are unclear and could be explained, at least in part, by co-morbidities of patients which may influence kidney function. Further studies are certainly needed to investigate interconnections between urine RBP4 levels and cardiorenal syndrome, as well as clinical value of RBP4 measurements in this patient group.

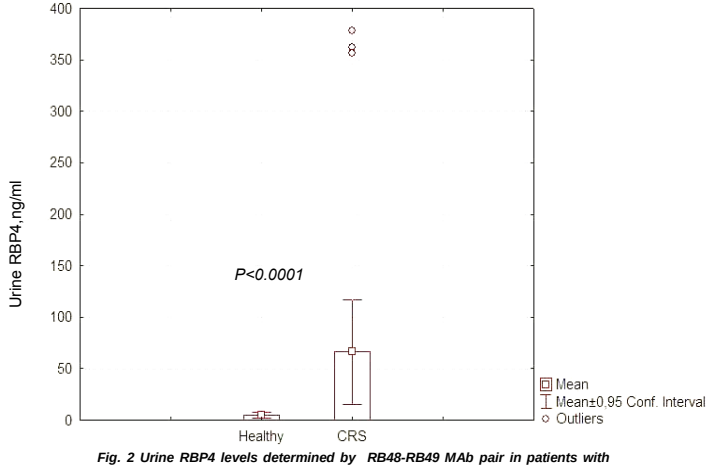

Fig. 2 Urine RBP4 levels determined by RB48-RB49 MAb pair in patients with cardiorenal syndromes (CRS) and healthy donors (healthy).
MAb RB48 was used as a capture (1 µg/well), whereas MAb RB49 was labeled with stable Eu^{3+} chelate and was used as a detection antibody (0.2 µg/well). Native RBP4 isolated from normal human serum was used as a calibrator.

Conclusions

- Sandwich immunoassay utilizing MAb combination RB48-RB49 could be used for RBP4 measurements in both human serum and urine.
- Small scale clinical study demonstrated that patients with cardiorenal syndromes types 1 and 2 have increased levels of urine RBP4.

References

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