

## Fatty acid-binding protein as marker for renal injury

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### Introduction

End-stage renal failure, ischemia-reperfusion or exposure to heavy metals can lead to tubulointerstitial as well as glomerular injury. Next to applying glomerular filtration rate for monitoring kidney function is the search of rapid biomarkers for detection of kidney injury. One of these promising new biomarkers is the family of 15 kDa cytoplasmic fatty acid-binding proteins (FABPs) which facilitate intracellular long-chain fatty acid transport. So far, nine distinct types have been identified and named after the tissue in which they were first identified, with each type showing a characteristic pattern of tissue distribution (1). In kidney, 2 types of FABP are present, Heart-type FABP (distal tubular cells) and Liver-type FABP (proximal tubular cells) (2).

### Methods

This review is based on an overview of the literature on clinical diagnostics applying plasma and urinary H-FABP as well as L-FABP levels in renal toxicity, kidney transplantation or as clinical parameter in end stage renal failure.

### Results

#### Assays

Tissue contents and serum/plasma as well as urinary concentrations of specific FABP types can be measured with immunobased assays (3). For H-FABP, a sandwich-type ELISA for plasma, serum, kidney perfusates and urine (3) as well as a micro-particle enhanced immunoassay were developed (3). For measuring urinary L-FABP levels, Kamijo et al. developed a specific sandwich-type ELISA with a calibration range of 0-400 ng/ml (4). The specificity of the reported immunochemical assays for FABP generally is very high, because essentially no cross-reactivity of the used poly- or monoclonal antibodies (pAbs and mAbs, respectively) with other FABP types was observed (3). FABP appears a very stable protein as both plasma samples and recombinant protein solutions can be subjected to repeated freezing/thawing at least 8 times without loss of immunoreactivity (3). No data have been reported about possible endogenous and exogenous interfering substances. For H-FABP, a plasma upper reference limit (URL) of 6 µg/L has been proposed (3). However, no URL has been reported for

urinary H-FABP concentrations. Urinary L-FABP reference value was 2.6 µg/g creatinine (range 0.4-17.0 µg/g creatinine; mean ± 2 SD). The URL was set at 17.0 µg/g creatinine (5).

#### Clinical utility of FABP in renal injury

Exposure to toxic heavy metals such as mercury, lead, cadmium or cis-platinum, is well known to lead to renal diseases like acute renal failure. Although the exact mechanisms of metal nephrotoxicity remain unclear, histologically characteristic features are lesions that tend to predominate in specific regions of the nephron within specific cells, leading to functional alterations as changes in renal plasma flow and glomerular filtration rate, and proximal or distal tubular damage. This suggests that certain regions of the nephron are selectively sensitive to specific metals. Renal damage to specific regions of rat kidney was evaluated following the administration of mercuric chloride (HgCl<sub>2</sub>), cyclosporin A or gentamicin (6). LDH, total protein, H-FABP and glutathione-S-transferase (GST) in urine showed that HgCl<sub>2</sub> induced a broad nephrotoxic effect, while cyclosporin induced only mild injury. Especially in gentamicin treated rats, urinary H-FABP concentrations increased markedly, indicating distal tubular injury and expressing the sensitivity of H-FABP compared to currently used markers. Kamijo et al. (7) reported urinary L-FABP correlations with the severity of tubulointerstitial damage and kidney function in either acute kidney injury as well as in chronic renal disease (4,7,8) and showed that urinary L-FABP concentrations were significantly higher in deteriorated kidneys compared to kidneys with stable function (4).

End-Stage Renal Failure (ESRF) often leads to kidney transplantation. Non heart beating donors (NHBDS) are regaining importance to significantly increase the kidney donor pool, with estimates indicating a potential increase by 20 - 40%. However, tissue damage sustained from periods of warm ischemia in these kidneys is often associated with primary non-function and delayed graft function. Perfusion characteristics (flow, pressure, resistance, temperature, weight gain) together with biochemical marker analysis of kidney effluents are investigated to assess viability. After aortic clamping in rats, H-FABP plasma concentrations were elevated significantly earlier than those of the currently used markers GST and LDH (9). L-FABP showed no significant changes. In a human setting, the potential of H-FABP as biochemical marker was compared with alanine aminopeptidase (Ala-AP) and GST, to indicate renal tissue injury in pre-transplantation machine perfusion and to predict long-term renal function (3). GST, Ala-AP and H-FABP plasma

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concentrations showed similar increases in NHBD kidney perfusates over a 4 h machine perfusion period. The activities of each marker were increased in discarded versus transplanted kidneys, with H-FABP concentration having the tendency to be the most sensitive marker (3). Based on these findings, the conclusion was reached that next to donor age, donor medical history, macroscopic appearance, warm ischemic time and *ex vivo* perfusion, biomarkers like H-FABP represent useful pre-transplant indicators of the suitability of NHBD kidneys for transplantation (3). L-FABP as marker for human ischemic injury has only recently been evaluated (10). Urinary L-FABP levels from human renal transplants showed a significant correlation with peritubular capillary blood flow and ischemic time of the transplanted kidney (11). These data showed that L-FABP could be a sensitive and predictive early biomarker of acute ischemic injury.

### Conclusion

H- and L-FABP appear to be useful markers for rapid detection and monitoring of renal injury enabling better monitoring of patient treatment and status of kidney viability. As renal disease also appears to be an independent risk factor for cardiovascular disease (CVD), earlier detection by these biomarkers can stratify treatment and reduce death by CVD. The use of H-FABP as plasma marker for cardiac injury has already been recognized (3) while especially urinary L-FABP indicates to be a promising and useful marker for renal injury.

Ned Tijdschr Klin Chem Labgeneesk 2009; 34: 251-254

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## Vitamine-B12-bepaling: probleem opgelost!

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### Inleiding

Onderzoek naar de totale vitamine-B12-concentratie in bloed (cobalamine) is een veelvuldig voorkomende bepaling in klinisch-chemische laboratoria. De nauwkeurigheid van de vitamine B12-bepaling is al jaren onderwerp van discussie. Met name in het lage, klinisch-relevante gebied, kennen alle beschikbare *in vitro*-diagnostische testen een grote variatie. De variaties in het normale bereik zijn aanzienlijk geringer.

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Ondanks de diagnostische beperkingen blijft de bepaling van vitamine B12 de belangrijkste biochemische screeningsmarker voor vitamine-B12-deficiënties (1). Patiënten met vitamine-B12-concentraties lager dan 150 pmol/l worden in het algemeen geduid als vitamine-B12-deficiënt, terwijl een deficiëntie onwaarschijnlijk wordt geacht bij patiënten met concentraties boven de 221 pmol/l (2, 3). Echter, een vitamine-B12-concentratie van <150 pmol/l in bloed houdt niet altijd in dat er sprake is van een vitamine-B12-deficiëntie op weefselniveau (4) en vitamine-B12-concentraties tussen de 150 en 221 pmol/l zijn lastig te interpreteren (2, 3). Dergelijke concentraties behoeven dan ook een aanvullende methylmalonzuur(MMA)- of totaal-homocysteïne(tHcy)bepaling ter uitsluiting dan wel bevestiging van een vitamine-B12-deficiëntie (1, 3, 5).

Siemens Healthcare Diagnostics (Siemens) heeft door de jaren heen vitamine-B12-assay's op de markt ge-