Hycult Scope

Complement markers of renal disease and injury

Complement is a major mediator system in pathogenesis of various kidney diseases. The presence and localization of complement components in glomerulus and/or the tubulo-interstitial area provides diagnostic tools for several human renal diseases (Table).

Determination of complement deposition also serves a valuable supplementary role in diagnosis of antibody mediated rejection in kidney transplantation. Particularly important has been the inclusion of C4d staining in the Banff scoring criteria for assessment of renal transplant pathology, in 2003.

In addition, the application of anti-complement antibodies is of utmost importance for studying experimental models of kidney disease in rat and mouse. A specific example is the mouse model where antibodies directed against components of the globular basement membrane (GBM) cause renal injury (Figure 1b). Deposition of C1q along the GBM demonstrates an activated innate immune response.

Furthermore, localization studies of complement proteins provide insight into the underlying pathophysiology of acute kidney injury. Staining for complement proteins has therefore become a routine part of renal biopsy analysis. However, early diagnosis of renal tissue damage requires non-invasive biomarkers, like NGAL (neutrophil-gelatinase-associated lipocalin), nitrotyrosine and FABPs (fatty acid binding proteins) that can easily and timely be measured in serum or urinary samples.

DETERMINANTS OF HUMAN RENAL DISEASE AND INJURY

Disease	Complement deposition / Biomarkers
Poststreptococcal glomerulonephritis	$C3^a$
Membranous glomerulonephritis	$C3^a$
Membranoproliferative glomerulonephritis I	$C1q^a$, $C4^a$, $C3^a$
Membranoproliferative glomerulonephritis II	$C3^a$
IgA nephropathy	$C3^a$, {$C4d^a$, MBL^a, $MASP^a$, L-ficolin^a}†
Lupus nephritis	$C1q^a$, $C4^a$, $C3^a$
Antibody-mediated rejection	$C4d^b$
Acute kidney injury (AKI)	$C3d^c$, $C8^d$, $MBL^{b,c}$ / NGAL, Nitrotyrosine, FABP

a = in glomeruli; b = in peritubular capillaries; c = in renal tubules; d = in tubulointerstitium; † = in subpopulation of patients

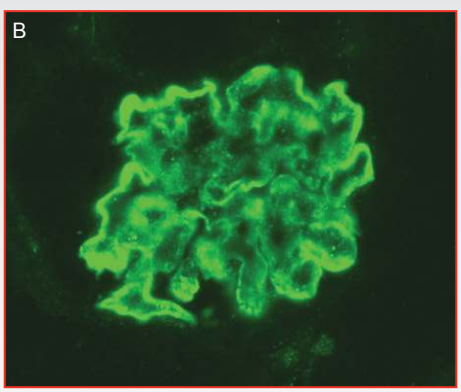

Figure 1 A) Glomerulus. B) Deposition of mouse C1q in an anti-GBM model of glomerulonephritis. Glomerulonephritis was induced in mice by injection of Rabbit anti-Mouse Glomerular Basement Membrane (GBM) IgG. Activation of complement is shown by C1q deposition along the GBM (Cat.# HM1096).

References:

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Molitoris et al., Nat Clin Pract Nephrol (2008), *4*:154-165
Portilla et al., Kidney Intern (2008), *73*: 465-472
Roos et al., Nephrol Dial Transplant (2007), *22*: 3370-3377
Thurman et al., Kidney Intern. (2005), *67*: 524-530
Thurman et al., Kidney Intern. (2008), *73*: 379-381
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Kidney diseases

In this Issue

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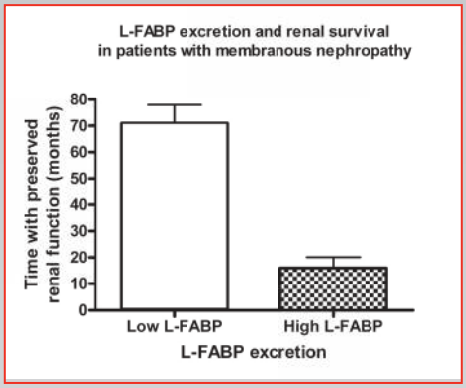


Urinary FABPs as biomarkers in renal disease

J.M.Hofstra MD, Dept. of Nephrology, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands

Renal disease is a common health problem. Progression of renal injury leads to end stage renal failure, which requires renal replacement therapy (hemodialysis, kidney transplantation). The recognition that tubular injury is important in progressive renal injury has stimulated the interest in urinary markers of tubular cell injury. Several tubular markers, such as β-2-microglobulin, α-1- microglobulin, Alkaline Phosphatase or β-NAG can predict prognosis in patients with renal diseases. The above-mentioned proteins are rather non-specific markers of proximal tubular cell injury. Little is known on the role of distal tubular injury in progressive renal disease. In addition, more specific or pathogenetically relevant markers may offer advantages in evaluating various patient groups.

Fatty-acid binding proteins (FABPs) are intracellular carrier proteins. Two types of FABPs are localized in human renal tubular cells. L-FABP is found in the cytoplasm of proximal tubules, whereas H-FABP is localized in the distal tubules. An increased urinary excretion of FABPs may simply result from release by structurally damaged tubular cells. However, it is suggested that tubular L-FABP expression is upregulated by hypoxia and increased excretion may thus occur before the occurrence of the actual structural damage, making it an early biomarker. In our patients with membranous nephropathy both L-FABP and H-FABP predicted prognosis with rather high sensitivity and specificity. Further investigation in differences between proximal and distal tubular markers like L-FABP and H-FABP may bring new insights to the pathogenesis and process of tubulo-interstitial damage.

HOT NEWS IN KIDNEY RESEARCH:

Akirin

Akirin (Japanese for “making things clear”) plays an important role in triggering the innate immune response of the fruit fly Drosophila, mice and even humans. Suppressing Akirin production in immune cells of the fly leads to a significantly enhanced susceptibility to bacterial infections.

Goto et al Nature Immunology 2008, *9*: 97-104

KIM-1

Kidney injury molecule-1 (KIM-1) staining identifies sensitively and specifically proximal tubular injury and correlates with degree of renal dysfunction.

Zhang et al. Kidney Int 2008, *73*: 608-14

Get “stoned” from fructose

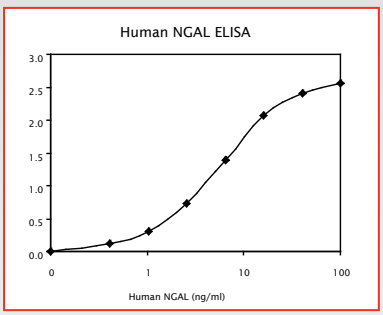
High fructose intake is associated with increased risk of kidney stones. Although patients with nephrolithiasis require increased fluid consumption, the authors advise them to avoid intake of fructose-rich drinks like sugar sweetened soft drinks, diet soft drinks, and fruit juice.

Taylor et al. Kidney Int. 2008, *73*: 207-12

NGAL: biomarker for renal injury and neoplasia

A sensitive, non-invasive tool for monitoring epithelial injury and carcinogenesis (Cat. # HK330)

- human neutrophil gelatinase-associated lipocalin (NGAL)
- induced in most tissues exposed to microorganisms, and in epithelial cells during inflammation
- antimicrobial properties and plays a role in regulating inflammation and cellular growth
- detectable in several body fluids upon epithelial injury
- important and informative biomarker of acute ischemic injury
- biomarker for early detection of renal failure
- NGAL activates nephron formation in the embryonic kidney, is rapidly and massively induced in renal failure and possesses kidney-protective activity
- blood, urine, and kidney NGAL levels are the real-time indicators of active kidney damage, rather than one of many markers of functional nephron number
- biomarker in ovarian cancer, colorectal cancer, adenocarcinomas, urothelial carcinomas, ischemic cerebrovascular disease



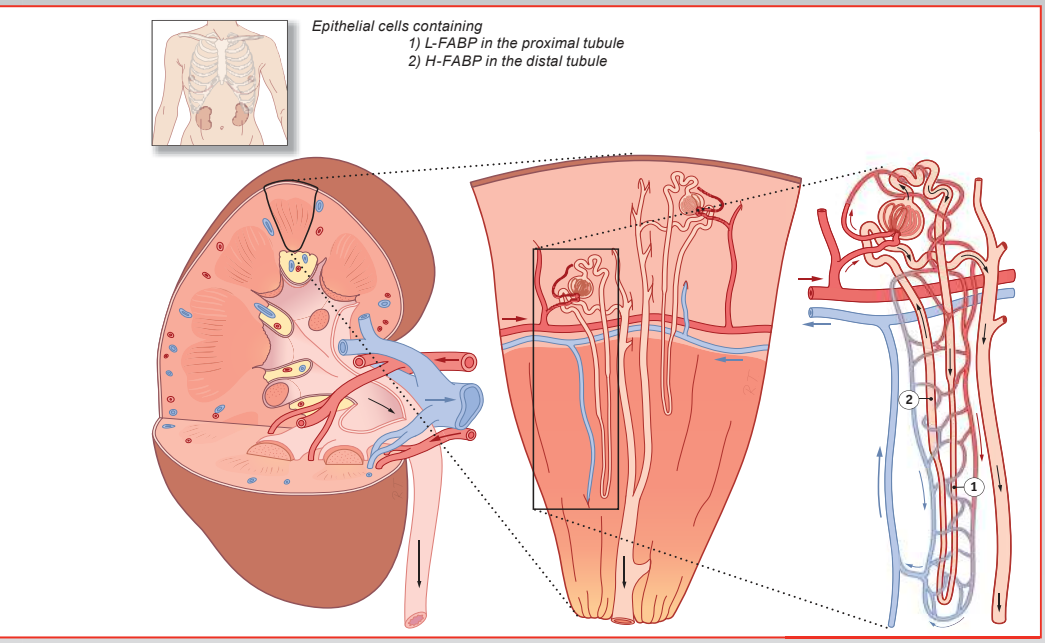
Kidney damage

ASSAYS

SPECIFITY		CAT. #
H-FABP, Human		HK401
H-FABP, Mouse, Rat	*Unique*	HK403
NGAL, Human		HK330
Nitrotyrosine	*Unique*	HK501
Lectin NPn early apoptosis detection kit	*Unique*	HIT303
α-Defensin 1-3, Human	*Unique*	HK317
Annexin V-FITC		HIT304
Calprotectin, Human		HK325
Elafin/SKALP, Human	*Unique*	HK318
Elastase, Human		HK319
LL-37, Human	*Unique*	HK321

MONOCLONAL ANTIBODIES

SPECIFITY	CAT. #
H-FABP, Human, mAb 66E2	HM2016
H-FABP, Human, mAb 67D3	HM2018
L-FABP, Human, mAb K5A6	HM2051
L-FABP, Human, mAb L2B10	HM2049
NGAL, Human, mAb 697	HM2193
Nitrotyrosine, mAb HM11	HM5001



The role of complement in ANCA related necrotizing small vessel vasculitis

C5 depletion with mAb BB5.1

P. Heeringa, PhD, Department of Pathology and Laboratory Medicine, Medical Biology Section, University Medical Center Groningen, Groningen, The Netherlands

Anti-neutrophil cytoplasmic autoantibodies (ANCA) are specific for enzymes in primary granules of neutrophils and peroxidase-positive lysosomes of monocytes. Approximately 90% of ANCA detected in patients with glomerulonephritis and/or vasculitis are specific for proteinase 3 (Pr3) and myeloperoxidase (MPO). ANCA are strongly associated with pauci-immune necrotizing crescentic glomerulonephritis and a spectrum of necrotizing small vessel vasculitis (1).

The pathogenic potential of anti-MPO antibodies has been confirmed in mouse studies (2). These experiments demonstrated that passive administration of murine anti-MPO IgG induces glomerulonephritis similar to that observed in patients.

The effect of pretreatment or intervention with the C5-inhibiting monoclonal antibody BB5.1 was investigated (3). Mice received BB5.1 either 8 hours before or 1 day after disease induction with anti-MPO IgG and lipopolysaccharide. Anti-MPO treated mice developed hematuria, leukocyturia, albuminuria, and crescentic glomerulonephritis. In contrast, BB5.1 pretreatment completely prevented disease development, as evidenced by the absence of urinary abnormalities, a marked reduction in glomerular neutrophil influx and normal renal morphology. Importantly, BB5.1 administration at 1 day after disease induction also resulted in a marked attenuation of urinary abnormalities and a more than 80% reduction in glomerular crescent formation. From these experiments we conclude that inhibition of C5 activation attenuates disease development in the mouse model of anti-MPO IgG-induced glomerulonephritis. Furthermore, these results favor further investigations into the role of complement activation in human MPO-anti-neutrophil cytoplasmic autoantibody-mediated glomerulonephritis, and indicate that inhibition of C5 activation is a potential therapeutic approach in this disease.

1. Jennette JC, Xiao H, and Falk RJ: Pathogenesis of vascular inflammation by anti-neutrophil cytoplasmic antibodies. J Am Soc Nephrol 2006, *17*: 1235-12422
2. Xiao H, Heeringa P, Hu P, Liu Z, Zhao M, Aratani Y, Maeda N, Falk RJ, and Jennette JC: Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. J Clin Invest 2002, *110*: 955-963
3. Huugen D, van Esch A, Xiao H, Peutz-Kootstra CJ, Buurman WA, Cohen Tervaert JW, Jennette JC and Heeringa P: Inhibition of complement factor C5 protects against anti-myeloperoxidase antibody-mediated glomerulonephritis in mice. Kidney Int 2007, *71*: 646-654

Antibodies for Kidney Research

MONOCLONAL ANTIBODIES

SPECIFICITY	CAT. #
Activated C3, Human, mAb bH6	HM2168
Activated Protein C, Human, mAb PC107	HM2151
α-Defensin 1-3, Human, mAb D21	HM2058
α-V-beta3 Integrin, Human, mAb BV3	HM2034
Arginase type-1, Human, mAb 6G3	HM2162
Arginase type-1, Human, mAb 9C5	HM2163
Barmotin/7H6 antigen, Human, mAb 7H6	HM2102
β Catenin, Human, mAb 9F2	HM2112
β Integrin, Human, mAb BV7	HM2033
C1q, Mouse, mAb 7H8	HM1044
C1q, Mouse, mAb JL-1	HM1096
C3, Mouse, mAb 11H9	HM1045
C3/C3a, C-terminus, Human, mAb 2898	HM2075
C3/C3a, Human, mAb 474	HM2073
C3/C3b, Human, mAb 755	HM2072
C3/C3b, Rat, mAb 2B10B9B2	HM3031
C3a, Mouse mAb 3/11	HM1072
C3a/C3a des-Arg, Human, mAb 2991	HM2074
C3aR, Human, mAb 17	HM2195
C3aR, Rat, mAb 74	HM3028
C3b/iC3b/C3c, Mouse mAb 2/11	HM1065
C3b/iC3b/C3c, Mouse, mAb 3/26	HM1078
C3c, Human, mAb 4	HM2200
C3d, Human, mAb 3	HM2198
C3g, Human, mAb 9	HM2199
C4, Mouse, mAb 16D2	HM1046
C4d, Human, mAb 11D12	HM2229
C4d, Human, mAb 7H4	HM2230
C5/C5a, Human, mAb 557	HM2077
C5/C5a, N-terminus, Human, mAb 561	HM2076
C5/C5b, Human, mAb 568	HM2080
C5b-9, Rat, mAb 2A1	HM3033
C6, Rat, mAb 3G11	HM3034
Calprotectin, Human, mAb 27E10	HM2156
Caveolin-1, Rat, mAb 7C8	HM3014
CD13, Mouse, ER-BMDM1	HM1083
CD21 (CR2), Human, mAb 21B9	HM2139
CD34, Mouse, mAb MEC14.7	HM1015
CD35 (CR1), Human, mAb 31R	HM2107
CD44, Human, mAb NKI-P2	HM2127
CD73, Human, mAb 4G4	HM2215
Crry, Rat, mAb TLD-1C11	HM3032
DPP IV (CD26), Rat, mAb 5E8	HM3021
Elafin/SKALP, Human, mAb TRAB2F	HM2063
Elafin/SKALP, Human, mAb TRAB2O	HM2062
Elastase, Human, mAb 265-3K1	HM2174
Endostatin, Human, mAb 1837-46	HM2188
G-Protein beta 1&2, Human, mAb Raft.1	HM2166
H-FABP, Human, mAb 66E2	HM2016
H-FABP, Human, mAb 67D3	HM2018
JAM-C, Mouse, mAb CRAM-19 H36	HM1056
L-FABP, Human, mAb K5A6	HM2051
L-FABP, Human, mAb L2B10	HM2049
L-Ficolin, Human, mAb GN4	HM2090
L-Ficolin, Human, mAb GN5	HM2091
LL37/CAP18, Human, mAb 1-1C12	HM2071
LL37/CAP18, Human, mAb 3D11	HM2070
MBL, Human, mAb 3E7	HM2061
MBL, Human, mAb D8.18	HM2081
MRP-8, Human, mAb 7C12/4	HM2175
MRP-14, Human, mAb 1H9	HM2176
Nectin-2, Mouse, mAb 502-57	HM1052
Nectin-3, Mouse, mAb 103-A1	HM1053
NGAL, Human, mAb 697	HM2193
Nitrotyrosine, mAb HM11	HM5001
PLVAP, Human, mAb 174/2	HM2214
Regucalcin, Human, mAb Regucalcin M	HM3018
SAA-1, Human, mAb Reu 86.1	HM2100
SAA-1, Human, mAb Reu 86.5	HM2101

POLYCLONAL ANTIBODIES

SPECIFICITY	CAT. #
B-FABP, Human	HP9029
C1q, Rat	HP8021
C3, Mouse	HP8012
C3, Rat	HP8022
C4, Rat	HP8023
C5, Mouse	HP8013
C5L2, Human	HP9036
C5L2, Mouse	HP8015
C5L2, Rat	HP8018
Elafin/SKALP, Human	HP9025
Elastase, Human	HP9027
L-FABP, Human	HP9021
L-FABP, Rat	HP8010
Occludin, Human	HP9047

New assay for L-Ficolin quantification

Human L-Ficolin ELISA:

L-Ficolin recognizes distinct danger-associated molecular patterns (DAMP) like GlcNAc-structures in LTA and fungal 1,3-β-D-glucan.
L-Ficolin also recognizes N-acetylated carbohydrates and other non-carbohydrate acetylated compounds such as acetylcholine.
Furthermore, L-Ficolin recognizes apoptotic cells and participates in the removal of host cells. L-ficolin circulates in complex with MASP-2 and can activate the lectin pathway.
Low serum levels of L-Ficolin are reported to be associated with recurrent respiratory infections in children.
Interestingly, L-Ficolin has been implicated in the unique immune challenge during pregnancy. In maternal plasma of normal pregnancies a 4- to 5-fold increase in L-Ficolin was detected compared to healthy non-pregnant persons.
However, significantly lower L-Ficolin levels were associated with preeclamptic pregnancies.
Therefore, assessment of L-Ficolin is warranted to study its regulatory role in the innate immune system.

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ELISA

SPECIFITY	CAT. #
L-Ficolin, Human	***Unique*** HK336
Functional MBL/MASP-2, Human, (C4 deposition)	***Unique*** HK327
MASP-2, Human	***Unique*** HK326
MBL, Human, (lectin activity)	HK323
SP-D, Human	HK335
TCC/sC5b-9/MAC, Human	HK328

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Higher expression of MAdCAM-1 is reflected in elevated levels of the circulating soluble form of MAdCAM-1 (sMAdCAM-1). Since MAdCAM-1 is elevated in inflammatory, infectious and malignant diseases, sMAdCAM-1 serves as a perfect non-invasive biomarker for disease activity.

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- Standard curve: 0.4 to 100 ng/ml
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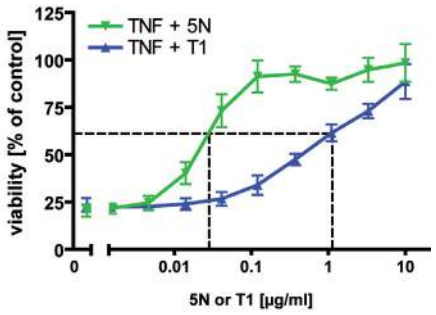

MONOCLONAL ANTIBODIES

SPECIFITY	CAT. #
TNFα, Human, mAb, 5N	HM2218
TNFα, Human, mAb, 5N	HM2218-05
TNFα, Human, Protein	HC2040

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