

23. Fritsche LG, Igl W, Bailey JN, Grassmann F, Sengupta S, Bragg-Gresham JL, Burdon KP, Hebring SJ, Wen C, Gorski M, Kim IK, Cho D, Zack D, Souied E, Scholl HP, Bala E, Lee KE, Hunter DJ, Sardell RJ, Mitchell P, Merriam JE, Cipriani V, Hoffman JD, Schick T, Lechanteur YT, Guymer RH, Johnson MP, Jiang Y, Stanton CM, Buitendijk GH, Zhan X, Kwong AM, Boleda A, Brooks M, Gieser L, Ratnapriya R, Branham KE, Foerster JR, Heckenlively JR, Othman MI, Vote BJ, Liang HH, Souzeau E, McAllister IL, Isaacs T, Hall J, Lake S, Mackey DA, Constable IJ, Craig JE, Kitchner TE, Yang Z, Su Z, Luo H, Chen D, Ouyang H, Flagg K, Lin D, Mao G, Ferreyra H, Stark K, von Strachwitz CN, Wolf A, Brandl C, Rudolph G, Olden M, Morrison MA, Morgan DJ, Schu M, Ahn J, Silvestri G, Tsironi EE, Park KH, Farrer LA, Orlin A, Brucker A, Li M, Curcio CA, Mohand-Said S, Sahel JA, Audo I, Benchaboune M, Cree AJ, Rennie CA, Goverdhan SV, Grunin M, Hagbi-Levi S, Campochiaro P, Katsanis N, Holz FG, Blond F, Blanché H, Deleuze JF, Igo RP Jr, Truitt B, Peachey NS, Meuer SM, Myers CE, Moore EL, Klein R, Hauser MA, Postel EA, Courtenay MD, Schwartz SG, Kovach JL, Scott WK, Liew G, Tan AG, Gopinath B, Merriam JC, Smith RT, Khan JC, Shahid H, Moore AT, McGrath JA, Laux R, Brantley MA Jr, Agarwal A, Ersoy L, Caramoy A, Langmann T, Saksens NT, de Jong EK, Hoyng CB, Cain MS, Richardson AJ, Martin TM, Blangero J, Weeks DE, Dhillon B, van Duijn CM, Doheny KF, Romm J, Klaver CC, Hayward C, Gorin MB, Klein ML, Baird PN, den Hollander AI, Fauser S, Yates JR, Allikmets R, Wang JJ, Schaumberg DA, Klein BE, Hagstrom SA, Chowers I, Lotery AJ, Léveillard T, Zhang K, Brilliant MH, Hewitt AW, Swaroop A, Chew EY, Pericak-Vance MA, DeAngelis M, Stambolian D, Haines JL, Iyengar SK, Weber BH, Abecasis GR, Heid IM: A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. *Nat Genet* 48: 134–143, 2016
24. Raychaudhuri S, Iartchouk O, Chin K, Tan PL, Tai AK, Ripke S, Gowrisankar S, Vemuri S, Montgomery K, Yu Y, Reynolds R, Zack DJ, Campochiaro B, Campochiaro P, Katsanis N, Daly MJ, Seddon JM: A rare penetrant mutation in CFH confers high risk of age-related macular degeneration. *Nat Genet* 43: 1232–1236, 2011

See related article, "Rare Variants in the Complement Factor H-Related Protein 5 Gene Contribute to Genetic Susceptibility to IgA Nephropathy," on pages 2894–2905.

The Search for a Biomarker of Relapse in ANCA-Associated Vasculitis

Meghan E. Free and Ronald J. Falk

University of North Carolina Kidney Center, University of North Carolina, Chapel Hill, Chapel Hill, North Carolina

J Am Soc Nephrol 27: 2551–2553, 2016.
doi: 10.1681/ASN.2016030347

Biomarkers are invaluable tools in clinical medicine that aid in disease diagnosis, natural history, prognosis, and response to

Published online ahead of print. Publication date available at www.jasn.org.

Correspondence: Dr. Meghan E. Free, University of North Carolina Kidney Center, 5020 Burnett Womack/CB#7155, University of North Carolina, Chapel Hill, NC 27599-7155. Email: meghan_free@med.unc.edu

Copyright © 2016 by the American Society of Nephrology

therapy. Because of their importance in patient care, potential biomarkers must undergo analytic validation (determination of the analytic performance of the assay itself) and clinical validation (determination of the ability of the test to predict the clinical condition that it is intended to detect). After analytic validation is established, clinical validation of biomarkers requires testing in three different populations: (1) the targeted disease population, (2) disease controls who are difficult to distinguish from the targeted disease population by means other than the candidate biomarker, and (3) demographically matched healthy controls.

ANCAs have proven to be useful biomarkers of small vessel vasculitis,¹ in that they have repetitively gone through all of the steps required for validation. These autoantibodies participate in the pathogenesis of the disease² and are a B lymphocyte target of therapy using rituximab.^{3–8} In fact, the disorder is now termed ANCA-associated or ANCA vasculitis.

The field of ANCA-associated vasculitis, like so many autoimmune diseases, is one in which cycles of relapse and remission are common. The ability to detect relapsing disease before overt clinical manifestations and organ damage with a biomarker of relapse or remission has been a focus of much investigation. The study by O'Reilly *et al.*⁹ in this issue of the *Journal of the American Society of Nephrology* examines the biomarker potential of urinary soluble CD163 (sCD163) to predict active renal vasculitis. In very carefully performed studies, elevated urinary sCD163 seems to distinguish active renal vasculitis from patients in remission and those with active vasculitis characterized by nonrenal symptoms. The test was similarly applied to patients with other forms of glomerular disease. Rigorous testing of sCD163 is evident, starting from a proof of principle experiment in a rat model of vasculitis through detection of CD163 expression in kidney biopsy specimens and finally, through urinary detection of sCD163 by ELISA in both an inception cohort and two validation cohorts (one internal and one external). A limitation of this study, however, is evident in the validation cohorts.⁹ There seems to be some patients with SLE and antiglomerular basement membrane disease who also had elevated levels of CD163 on kidney biopsy, which poses the question as to whether sCD163 can distinguish active ANCA-associated vasculitis from disease controls. A disease control that was not examined is the patient with vasculitis and an increasing creatinine in whom the etiology of kidney injury is not overtly apparent. In these patients, in whom a decline in GFR is not attributable to active vasculitis, the gold standard would be a kidney biopsy (for example, AKI caused by a variety of other causes, with the renal biopsy being the only distinguishing definitive diagnosis). A prime example of this would be a patient with ANCA-associated vasculitis and AKI who has an infection. As shown in this study,⁹ elevated sCD163 is noted in some patients with sepsis, therefore limiting the value of sCD163 to discern causes of renal injury. Taken together, although urinary sCD163 is certainly elevated in renal vasculitic flares, there are confounding elevations in other patient groups that remain unresolved.

The detection of sCD163 during inflammation makes biologic sense. CD163 is expressed on macrophages and monocytes, and it is released in its soluble form in the presence of proinflammatory stimuli.¹⁰ Macrophages are frequently found within glomerular crescents, lending credibility to the hypothesis that sCD163 emanates from glomerular macrophages that could be induced during localized inflammatory events. O'Reilly *et al.*⁹ clearly show that CD163 is expressed in glomerular crescents in a rat model of vasculitis and that CD163 seems to localize with markers indicative of M2 macrophages.

M2 macrophages are known for their anti-inflammatory properties. The colocalization of CD163 with M2 macrophages in the inflammatory milieu of the vasculitic glomerulus is not by chance. These cells may serve to modulate the inflammatory response. What is known is that a variety of inflammatory mediators induces the release of CD163 and that sCD163 dampens T cell proliferation and cytokine production.¹¹ Therefore, although it seems that urinary sCD163 is a potential biomarker of renal flare, in a true biologic sense, it could be a marker of disease resolution.

Would the existing data pertaining to a urinary sCD163 biomarker elicit a change in clinical care? O'Reilly *et al.*⁹ note that detection of sCD163 will never be a surrogate of diagnosis or eradicate the necessity of kidney biopsy in vasculitis. However, it may well provide a new tool as a possible early predictor of renal flare. This study makes a strong case for the positive prediction of this marker for active renal vasculitis but not extrarenal vasculitic activity. In the rat model studies, O'Reilly *et al.*⁹ noted that sCD163 was detectable in the urine by day 28, whereas pathologic kidney disease was most severe at day 56. If a rise in urinary sCD163 could be detected in patients before renal flare, clinicians could intercede and preemptively begin treatment, which may have a large effect on limiting renal damage and preserving function. To fully use sCD163 as a biomarker, a prospective longitudinal study is required to examine the predictive value of urinary sCD163 detection for renal flare.

There are a number of questions that a prospective validation study would help to address. What is the consequence of therapy on the detection of urinary sCD163? O'Reilly *et al.*⁹ note that, in some patients followed longitudinally, sCD163 levels diminished quite quickly after initiation of therapy. Furthermore, what happens in those patients who flare while concomitantly on therapy? Would urinary sCD163 still be useful as a biomarker of renal flare in these patients?

Any new biomarker must be better and perhaps, less expensive than current techniques to detect relapse. It comes as no surprise that urinary protein excretion did not prove to be a good predictor of renal flare, because proteinuria may be a consequence of active disease, glomerular scarring, or tubular interstitial damage. The real question is how well this biomarker performed against the finding of hematuria or other formed elements in the urine as assessed by an experienced clinician or laboratory technician examining freshly voided

and spun urine samples under a microscope. Urine is commonly evaluated microscopically in kidney center clinics in which there is an available microscope with experienced clinicians to perform the evaluations. The importance of hematuria has been debated in the literature, but the degree of hematuria has been shown to reflect not only disease activity but also, the severity of glomerular damage.¹² More recent reports have questioned the specificity of hematuria as it relates to disease activity.¹³ Of concern is that hematuria is being assessed solely by the use of a dipstick, a measure of hematuria fraught with error. Any biomarker of relapsing kidney disease, including sCD163, should be evaluated in a prospective study that compares the potential biomarker with the very inexpensive test of microscopic examination of freshly voided urine.

Despite its limitations in the clinic, dipstick urine tests for detecting blood in the urine are likely useful for home monitoring. In individuals who are in remission and no longer have hematuria, the presence of dipstick positivity for blood prompts patients to provide a fresh urine sample to their provider to determine whether there are signs of active glomerular disease under the microscope. In our practice, this has proven to be the most inexpensive and useful approach to the early identification of a flare of renal vasculitis. Urinary sCD163 must then also be compared with this practical approach.

Biomarkers of relapse of ANCA vasculitis also need to pass the hurdle of whether a clinical biomarker of relapse is better than the patient's perception of whether they have a relapse. Tomasson *et al.*¹⁴ investigated the predictive value of the patients' perception of their disease and found that patients could predict disease flare 3 months before the physician or any existing clinical test. Although renal vasculitis is notorious for silent flares from the patient's perspective, it is important to prove that any biomarker is better than the patient's self-report of changes in symptoms and their own keen awareness of the potential resurgence of disease activity.

Despite these concerns, the examination of sCD163 in the urine as a predictor of renal vasculitis in the setting of known ANCA vasculitis is biologically plausible and on first pass, has potential clinical usefulness as a marker of active vasculitis in the kidney. O'Reilly *et al.*⁹ should be commended for their detailed and careful analysis. A prospective study is now needed that validates this test with all of the appropriate disease controls and other common and inexpensive markers of a renal vasculitic flare.

DISCLOSURES

None.

REFERENCES

1. Jennette JC, Wilkman AS, Falk RJ: Anti-neutrophil cytoplasmic autoantibody-associated glomerulonephritis and vasculitis. *Am J Pathol* 135: 921–930, 1989

2. Xiao H, Heeringa P, Hu P, Liu Z, Zhao M, Aratani Y, Maeda N, Falk RJ, Jennette JC: Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. *J Clin Invest* 110: 955–963, 2002
3. Stone JH, Merkel PA, Spiera R, Seo P, Langford CA, Hoffman GS, Kallenberg CG, St Clair EW, Turkiewicz A, Tchao NK, Webber L, Ding L, Sejsmundo LP, Mieras K, Weitzkamp D, Ikle D, Seyfert-Margolis V, Mueller M, Brunetta P, Allen NB, Fervenza FC, Geetha D, Keogh KA, Kissin EY, Monach PA, Peikert T, Stegeman C, Ytterberg SR, Specks U; RAVE-ITN Research Group: Rituximab versus cyclophosphamide for ANCA-associated vasculitis. *N Engl J Med* 363: 221–232, 2010
4. Rhee EP, Laliberte KA, Niles JL: Rituximab as maintenance therapy for anti-neutrophil cytoplasmic antibody-associated vasculitis. *Clin J Am Soc Nephrol* 5: 1394–1400, 2010
5. Keogh KA, Wylam ME, Stone JH, Specks U: Induction of remission by B lymphocyte depletion in eleven patients with refractory antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum* 52: 262–268, 2005
6. Jayne D: Treatment of ANCA-associated systemic small-vessel vasculitis. *APMIS Suppl* 127: 3–9, 2009
7. Jones RB, Ferraro AJ, Chaudhry AN, Brogan P, Salama AD, Smith KG, Savage CO, Jayne DR: A multicenter survey of rituximab therapy for refractory antineutrophil cytoplasmic antibody-associated vasculitis. *Arthritis Rheum* 60: 2156–2168, 2009
8. Jones RB, Tervaert JW, Hauser T, Luqmani R, Morgan MD, Peh CA, Savage CO, Segelmark M, Tesar V, van Paassen P, Walsh D, Walsh M, Westman K, Jayne DR; European Vasculitis Study Group: Rituximab versus cyclophosphamide in ANCA-associated renal vasculitis. *N Engl J Med* 363: 211–220, 2010
9. O'Reilly VP, Wong L, Kennedy C, Elliot LA, O'Meachair S, Coughlan AM, O'Brien EC, Ryan MM, Sandoval D, Connolly E, et al.: Urinary soluble CD163 in active renal vasculitis. *J Am Soc Nephrol* 27: 2906–2916, 2016
10. Møller HJ, Nielsen MJ, Maniecki MB, Madsen M, Moestrup SK: Soluble macrophage-derived CD163: A homogenous ectodomain protein with a dissociable haptoglobin-hemoglobin binding. *Immunobiology* 215: 406–412, 2010
11. Högger P, Sorg C: Soluble CD163 inhibits phorbol ester-induced lymphocyte proliferation. *Biochem Biophys Res Commun* 288: 841–843, 2001
12. Fujita T, Ohi H, Endo M, Ohsawa I, Kanmatsuse K: Level of red blood cells in the urinary sediment reflects the degree of renal activity in Wegener's granulomatosis. *Clin Nephrol* 50: 284–288, 1998
13. Magrey MN, Villa-Forte A, Koenig CL, Myles JL, Hoffman GS: Persistent hematuria after induction of remission in Wegener granulomatosis: A therapeutic dilemma. *Medicine (Baltimore)* 88: 315–321, 2009
14. Tomasson G, Davis JC, Hoffman GS, McCune WJ, Specks U, Spiera R, St Clair EW, Stone JH, Merkel PA: Brief report: The value of a patient global assessment of disease activity in granulomatosis with polyangiitis (Wegener's). *Arthritis Rheumatol* 66: 428–432, 2014

See related article, "Urinary Soluble CD163 in Active Renal Vasculitis," on pages 2906–2916.