Urine Biomarkers to Predict Response to Lupus Nephritis Therapy in Children and Young Adults

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ABSTRACT. ***Objective.*** To delineate urine biomarkers that forecast response to therapy of lupus nephritis (LN).

Methods. Starting from the time of kidney biopsy, patients with childhood-onset systemic lupus erythematosus who were diagnosed with LN were studied serially. Levels of 15 biomarkers were measured in random spot urine samples, including adiponectin, α-1-acid glycoprotein (AGP), ceruloplasmin, hemopexin, hepcidin, kidney injury molecule 1, monocyte chemotactic protein-1, lipocalin-like prostaglandin D synthase (LPGDS), transforming growth factor-β (TGF-β), transferrin, and vitamin D binding protein (VDBP).

Results. Among 87 patients (mean age 15.6 yrs) with LN, there were 37 treatment responders and 50 nonresponders based on the American College of Rheumatology criteria. At the time of kidney biopsy, levels of TGF-β ($p < 0.0001$) and ceruloplasmin ($p = 0.006$) were significantly lower among responders than nonresponders; less pronounced differences were present for AGP, hepcidin, LPGDS, transferrin, and VDBP (all $p < 0.05$). By Month 3, responders experienced marked decreases of adiponectin, AGP, transferrin, and VDBP (all $p < 0.01$) and mean levels of these biomarkers were all outstanding (area under the receiver-operating characteristic curve ≥ 0.9) for discriminating responders from nonresponders. Patient demographics and extrarenal disease did not influence differences in biomarker levels between response groups.

Conclusion. Low urine levels of TGF-β and ceruloplasmin at baseline and marked reduction of AGP, LPGDS, transferrin, or VDBP and combinations of other select biomarkers by Month 3 are outstanding predictors for achieving remission of LN. If confirmed, these results can be used to help personalize LN therapy. (First Release June 15 2017; J Rheumatol 2017;44:1239–48; doi:10.3899/jrheum.161128)

Key Indexing Terms:
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Systemic lupus erythematosus (SLE) is a multisystem inflammatory autoimmune disease, and lupus nephritis (LN) is one of the main determinants of poor prognosis[1,2,3,4]. Although data from large-scale epidemiological studies are lacking, an estimated 10% of the children and adolescents will develop endstage renal disease (ESRD) within 10 years

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of LN diagnosis[5], and 22% of children in ESRD from LN will die within 5 years of requiring renal replacement therapy[6]. A major factor leading to such unsatisfactory LN outcomes is a lack of noninvasive clinical and laboratory measures to accurately gauge LN status in terms of activity and response to therapy.

Several urine biomarkers have been described that hold much promise to improve the surveillance of LN compared to the current clinical and laboratory measures[7,8]. We have shown that the urine concentrations of adiponectin, ceruloplasmin, hemopexin, kidney injury molecule 1 (KIM-1), monocyte chemotactic protein 1 (MCP-1), and neutrophil gelatinase–associated lipocalin (NGAL) can be used to calculate the Renal Activity Index in Lupus (RAIL); this index has excellent accuracy in estimating histological activity of LN in both children and adults[9,10]. However, biomarkers that reflect LN activity cross-sectionally are not necessarily the best measures of response to therapy. Indeed, other promising biomarkers of LN response include α-1-acid glycoprotein (AGP), cystatin-C, hepcidin, lipocalin-like prostaglandin D synthase (LPGDS), liver-type fatty acid-binding protein 1 (LFABP), osteopontin, transforming growth factor-β (TGF-β), transferrin, and vitamin D binding protein (VDBP)[10,11,12,13,14,15,16,17].

Although research suggests that the above-mentioned biomarkers may be suited to reflect or even anticipate patient response to LN therapy, this has not been studied well in children. Further, the importance of patient demographics, LN histology, and medications when using these urine candidate biomarkers to determine clinically relevant improvement of LN has not been studied in depth.

The objectives of our study were (1) to delineate urine biomarkers that can forecast the response to therapy of LN; and (2) to determine whether histological severity of LN, patient demographics, or extrarenal disease activity influence the ability of these urine biomarkers to anticipate LN response. We hypothesized that some of the candidate urine biomarkers, individually or combined, can be used to predict the course of pediatric LN, with focus on patient response at 6 months after kidney biopsy.

MATERIALS AND METHODS

Patients. Patients diagnosed with childhood-onset SLE (cSLE)[18] requiring a kidney biopsy as part of standard of care participated in this longitudinal study. Random spot urine samples were collected in regular intervals, starting from the time of kidney biopsy. Prospectively, relevant clinical information and traditional measures of LN were recorded, including the glomerular filtration rate (GFR)[19,20] and proteinuria. All patients received therapy for cSLE at the time of the urine collection and biopsy.

The renal domain scores of the Systemic Lupus Disease Activity Index (SLEDAI; range 0–16; 0 = inactive LN)[21] and the British Isles Lupus Activity Group index[22] were completed to serve as measures of LN clinical activity. We also measured extrarenal disease activity using the SLEDAI as previously described[23]. The study was approved by the institutional review boards of all of the participating institutions. Cincinnati Children's Hospital served as the institutional review board of reference (CHMC 2010-1919; CHMC 2008-0635); patients and/or caregivers provided informed assent and consent prior to commencement of any study-related activities.

Kidney histology and response to therapy. The histological characteristics of available kidney biopsies were interpreted in a blinded fashion by an expert nephropathologist (DW) as per the International Society for Nephrology/Renal Pathology Society (ISN/RPS) Classification[24,25]. In line with what is proposed by the American College of Rheumatology (ACR), complete response to LN therapy was defined as the presence of an inactive urine sediment plus decrease of proteinuria (estimated by protein-to- creatinine ratio) to ≤ 0.2 g/day plus normal or stable GFR based on the modified Schwartz formula[26,27].

Urinary biomarker assays. The following 15 biomarkers were assayed: adiponectin, AGP, ceruloplasmin, cystatin-C, hemopexin, hepcidin, KIM-1, LPGDS, LFABP, MCP-1, NGAL, osteopontin, TGF-β, transferrin, and VDBP. Laboratory personnel assaying the biomarkers were blinded to clinical and histological information. Spun urine samples were stored at 0°C within 1 h of collection and frozen at –80°C within 24 h prior to batch processing.

Unless stated otherwise, biomarkers were quantified using commercial ELISA kits as per the manufacturers' instructions, and a 4-measure logistic curve fit was used to fit the standard curve. The intraassay and interassay variability is expressed in percent of the coefficient of variation (CV intra/inter).

Adiponectin (CV intra/inter: 4.0%/9.9%) was measured using the Quantikine ELISA Human HMW Adiponectin/Acrp30 (R&D Systems), AGP (CV intra/inter: 5.0%/8.5%) by ELISA (R&D Systems), ceruloplasmin (CV intra/inter: 4.1%/7.1%) by ELISA (Assaypro), hemopexin (CV intra/inter: 4.8%/7.3%) with the AssayMax Human Hemopexin ELISA Kit (Assaypro), and hepcidin-25 (CV intra/inter: 3.5%/3.4%) by ELISA (R&D Systems). The KIM-1 assay was constructed using commercially available reagents (Duoset DY1750) as described previously[28]. We quantified LFABP (CV intra/inter: 6.1%/10.9%) by ELISA (CMIC Co.), MCP-1 (CV intra/inter: 5.0%/5.9%) by ELISA (R&D Systems), NGAL (CV intra/inter: 1.0%/9.1%) by ELISA (Human NGAL ELISA; Bioporto), osteopontin (CV intra/inter: 7.8%/9.0%) with the DuoSet Human EPCR kit (R&D Systems), and VDBP (CV intra/inter: 5.1%/6.2%) by ELISA (R&D Systems).

TGF-β (CV intra/inter: 2.6%/8.3%) was measured by ELISA (R&D Systems) after acid activation. Briefly, 20 µl of 1N HCl was added to 100 µl of urine sample, mixed by inversion and incubated at room temperature for 10 min. Next, the acidified sample was neutralized by adding 20 µl of 1.2 N NaOH/0.5 M HEPES buffer. Then the assay was immediately run per manufacturer's instructions (CV intra/inter: 2.0%/7.8%). Using immunonephelometry (Siemens, BNII), we measured cystatin-C (CV intra/inter: 2.3%/2.5%), transferrin (CV intra/inter: 2.5%/3.4%), and LPGDS (CV intra/inter: 2.3%/6.5%). We also determined levels of urine creatinine using an enzymatic creatinine assay (CV intra/inter: 0.65%/4.48%) on a Dimension RXL Clinical Analyzer (Siemens).

Raw concentrations of the urine biomarkers (in ng/ml for NGAL, AGP, ceruloplasmin, LFABP, VDBP, osteopontin, hemopexin, and hepcidin; in pg/ml for adiponectin, KIM-1, MCP-1, and TGF-β; in ng/dl for transferrin and LPDGS; in ng/l for cystatin-C) are presented as well as biomarker concentrations standardized for urine creatinine levels (in mg/ml).

Statistical analysis. All biomarker levels and microalbumin were found right skewed in their distributions, but their nature log transformed variables were symmetrically distributed and fit the conditions for parametric statistical models. Hence, all the analyses were performed using (nature) log transformed biomarker levels.

The primary statistical model was a mixed-effect model. In particular, each dependent variable, i.e., the log transformed biomarker, was assessed for its associations to the major fixed effects of interest, the response effect (yes vs no), the time effect (a categorical variable of months 0, 3, 6, 9, and 12), and its interaction in the mixed-effect model. A random effect was used to account for within-person correlation caused by the repeated measurements over the visits. Posthoc means of the dependent variable were based

on the effect-model framework. Mixed-effect models were repeated after adjusting biomarker levels by urine creatinine concentrations. Because the findings from the analyses considering urine creatinine-adjusted biomarkers agreed with those based on unadjusted urine biomarkers, only results from unadjusted biomarkers are presented herein.

The study also included subset analyses using the primary mixed-effect models in subgroups stratified by LN class as well as the treatment with either cyclophosphamide (CYC) or mycophenolate mofetil (MMF). To test whether the association between the dependent variable and the response effect were importantly influenced by possible moderators (age, sex, race, angiotensinogen-blocking medications), we modified the mixed-effect models by letting the moderator interact with the response factor while nesting them under the time effect.

Receiver-operating characteristic (ROC) curves were used to investigate the performance of individual biomarkers in discriminating responders from nonresponders. The overall accuracy of each biomarker was evaluated using the areas under the ROC curve (AUC). Biomarker accuracy was considered outstanding, excellent, good, and fair if the AUC was in the range of 0.9–1.0, 0.81–0.90, 0.71–0.80, and 0.61–0.70, respectively.

For the RAIL biomarkers, we developed a multiple logistical regression model to predict LN response using levels of NGAL, MCP-1, KIM-1, ceruloplasmin, adiponectin, and hemopexin as predictors; the respective ROC curve and AUC using the logit score (or RAIL score) were also calculated from the multiple logistical regression model, as previously described[9,29]. Chi-square tests were done to compare rates between groups. Categorical and numerical variables at baseline were summarized using frequency in percent and mean (SD or SE). All statistical analyses were computed using an SAS 9.4 package. P values < 0.05 were considered statistically significant.

RESULTS

Patient characteristics and features of kidney biopsy. Details about the study cohort are provided in Table 1. Eighty-seven patients with LN were included; all required a kidney biopsy as part of clinical care for cSLE[18]. The patients' mean (SD) age at the time of kidney biopsy was 15.6 years (2.9), and the average extrarenal disease activity as measured by the SLEDAI was 6.7 (6.8). None had ISN/RPS Class 1 or Class 6 LN. MMF and CYC at recommended doses[23] were generally used for LN therapy, and the majority of the patients were prescribed an angiotensin system–blocking drug soon after their kidney biopsy.

Responders versus nonresponders to LN therapy. Of the 87 patients enrolled, 37 were responders and 50 failed to respond to treatment. At baseline, response groups (nonresponders, responders) were similar in histological activity (p = 0.917) and histological chronicity (p = 0.952) as measured by the US National Institutes of Health Activity and Chronicity Indices[30], respectively. There were no important baseline differences between groups for renal function (GFR; p = 0.459) or the degree of proteinuria (albumin-to-creatinine ratio; p = 0.122). Nonresponders had markedly higher extrarenal disease activity than the responder group at baseline (Table 1).

Table 1. Demographics and clinical information of the patients at the time of urine collection and the time of kidney biopsy. Values are arithmetic means (SD), unless stated otherwise.

Variable	Category	Total, n = 87	Responders, n = 37	Nonresponders, n = 50	p##
Sex, n (%)	Female	68 (78.2)	27 (73.0)	41 (82.0)	0.314
Race/ethnicity, n (%)					
	White	29 (33.3%)	19 (51.4)	10 (20.0)	0.015
	Black	33 (38.0)	12 (32.4)	21 (42.0)	
	Hispanic	10 (11.5)	2 (5.4)	8 (16.0)	
	Mixed racial and others	15 (17.2)	4 (10.8)	11 (22.0)	
Medications started for LN therapy around kidney biopsy, n (%)	Mycophenolate mofetil	47 (54.0)	17 (46.0)	30 (60.0)	0.194
	Azathioprine	7 (8.1)	5 (13.5)	2 (4.0)	
	Cyclophosphamide	33 (37.9)	15 (40.5)	18 (36.0)	
Angiotensin system–blocking drug, n (%)	Yes	51/87 (58.6)	20 (54)	31 (62)	0.202
GFR, ml/min/m^2		135.6 (57.4)	141.0 (66.4)	131.6 (50.1)	0.459
Renal SLEDAI		8.0 (5.2)	5.4 (4.7)	9.8 (4.7)	< 0.0001
Renal BILAG		9.9 (4.0)	8.2 (5.1)	11.2 (2.1)	0.0003
Microalbumin/ creatinine ratio**		1.16 (2.04)	0.80 (2.23)	1.54 (1.83)	0.122
ISN/RPS, n (%) #	Class 2	13 (14.9)	5 (13.5)	8 (16.0)	0.634
	Class 3 or 4	47 (54.0)	22 (59.5)	25 (50.0)	
	Class 5	27 (31.0)	10 (27.0)	17 (34.0)	
NIH-AI ‡		7.6 (6.5)	7.7 (6.0)	7.5 (6.9)	0.917
NIH-CI Δ		1.6 (1.9)	1.6 (1.4)	1.6 (2.1)	0.952
Extrarenal SLEDAI*		6.7 (6.8)	3.8 (3.3)	8.8 (8.0)	0.004

P values are from t tests to compare means or chi square tests to compare rates (in %). # International Society for Nephrology/Renal Pathology Society Class; there were no biopsies consistent with Class 1 or 6. ‡ US National Institutes of Health (NIH) Activity Index; range 0–24; 0 = inactive LN; available in only 76 patients. Δ NIH Chronicity Index; range 0–12; 0 = LN without chronic changes; available in only 62 patients. * Measured by the SLEDAI summary score minus the SLEDAI renal domain score. ** Natural log transformed. GFR: glomerular filtration rate; LN: lupus nephritis; SLEDAI: Systemic Lupus Disease Activity Index; BILAG: British Isles Lupus Activity Group.

As early as Month 3 of LN therapy, there were significant differences between responders and nonresponders in the change of the albumin-to-creatinine ratio from baseline ($p = 0.013$; Supplementary Table 1, available with the online version of this article), and differences in proteinuria persisted over time.

Select urine biomarkers differed between responders and nonresponders at the time of kidney biopsy. At the time of kidney biopsy, mean concentrations of 7 of the included biomarkers significantly differed between response groups (Table 2). The most pronounced differences were observed for TGF-β and ceruloplasmin (both $p \leq 0.006$), followed by transferrin, AGP, VDBP, hepcidin, and LPGDS. MCP-1 and NGAL showed only trends toward higher levels among nonresponders at baseline. Notably, with the exception of hepcidin, all biomarkers levels were higher among nonresponders than responders.

Mean urine biomarker concentrations differed over time by responder status. Figure 1 depicts mean concentrations of the 6 biomarkers included in the RAIL (panels A–F) and of 3 other biomarkers (transferrin, AGP, VDBP in panels G–I) that markedly differed from responder status (details in Supplementary Table 1, available with the online version of this article). All RAIL biomarkers, except for NGAL, differed significantly by responder status at Month 3. NGAL showed only significant differences at Month 6.

Figure 2 summarizes differences in biomarker mean levels between response groups. Although levels of TGF-β, and to a lesser degree, hepcidin and LFABP, all significantly differed between response groups at baseline, only TGF-β and LFABP continued to show significant differences at months 3 and 6. Osteopontin did not significantly differ between response groups at any timepoint (details in Supplementary Table 1, available with the online version of this article).

Differences in urinary biomarker levels under consideration of LN severity. Biomarker pattern levels showed dependency on LN severity as defined by ISN/RPS class. Given the limited numbers of patients with Class 2 LN, Figure 2 shows only the results of these analyses for proliferative LN (Class 3 or 4) and pure membranous LN (Class 5).

By Month 3, the RAIL biomarker levels differed with responder status, particularly with proliferative LN. There were also significant differences in the urine levels ($p < 0.05$) of AGP and transferrin for more than 1 timepoint between responders and nonresponders with proliferative LN.

For pure Class 5 LN, LPGDS levels differed most markedly with response status (all $p < 0.0001$), but VDBP and adiponectin levels also significantly differed for more than 1 timepoint between responders and nonresponders. Additional details are provided in Supplementary Table 2 (available with the online version of this article).

Absolute changes in biomarker levels between Month 0 and Month 3 by responder status. While Figure 2 describes differences in biomarker mean levels between responders and nonresponders, Figure 3A depicts changes of biomarker concentrations from baseline to Month 3.

All biomarkers, except for osteopontin, decreased over time irrespective of responder status, but reductions were more pronounced among responders: they experienced declines of adiponectin, AGP, transferrin, and VDBP levels by > 2 logs by Month 3. Hepcidin showed the most profound drop in urine levels but also the most variability (large SE), and TGF-β decreases did not significantly differ with responder status.

Accuracy of the biomarkers to discriminate responders from nonresponders. At the time of biopsy, none of the biomarkers or the RAIL biomarkers in combination achieved outstanding accuracy (AUC ≥ 0.9) for anticipating the responder status (also see Supplementary Table 3, available with the online version of this article).

As shown in Figure 3B, adiponectin, AGP, LPGDS, transferrin, and VDBP individually had outstanding ability (AUC > 0.9) to anticipate treatment response as early as Month 3. The RAIL biomarkers also showed outstanding overall accuracy at Month 3 (AUC = 0.92) and also at Month 6 (AUC = 0.91) when adjustments for urine creatinine were performed.

Dependence of urine biomarker levels on patient age, race, sex, and extrarenal disease activity. We found that mean levels of the biomarkers were not importantly or systematically influenced by patient age, race, sex, and extrarenal

Table 2. Biomarker levels differences at the time of biopsy among 37 responders and 50 nonresponders. Values are geometric means ± SE.

Biomarker	Biomarker Levels at the Time of Biopsy		
	Nonresponders	Responders	p*
TGF-β	3.74 ± 0.21	2.61 ± 0.22	< 0.0001
Ceruloplasmin	9.76 ± 0.29	8.58 ± 0.30	0.006
Transferrin	2.12 ± 0.32	0.93 ± 0.34	0.012
AGP	11.44 ± 0.36	10.24 ± 0.38	0.023
VDBP	6.65 ± 0.31	5.65 ± 0.32	0.027
Hepcidin	6.88 ± 0.60	8.70 ± 0.62	0.037
LPGDS	6.19 ± 0.31	5.30 ± 0.28	0.044
MCP-1	6.87 ± 0.21	6.28 ± 0.22	0.057
NGAL	3.77 ± 0.22	3.20 ± 0.23	0.079
KIM-1	7.37 ± 0.22	7.10 ± 0.23	0.402
Osteopontin	4.66 ± 0.32	4.30 ± 0.34	0.432
Hemopexin	8.10 ± 0.25	7.82 ± 0.26	0.443
Cystatin-C	4.35 ± 0.20	4.10 ± 0.26	0.451
Adiponectin	10.65 ± 0.36	10.27 ± 0.37	0.463
LFABP	3.10 ± 0.26	3.09 ± 0.31	0.975

* P values are computed using mixed effect models. NGAL: neutrophil gelatinase associated lipocalin; KIM-1: kidney injury molecule 1; MCP-1: monocyte chemotactic protein 1; AGP: α-1-acid glycoprotein; TGF-β: transforming growth factor-β; LFABP: liver-type fatty acid-binding protein 1; VDBP: vitamin D binding protein; LPGDS: lipocalin-like prostaglandin D synthase.

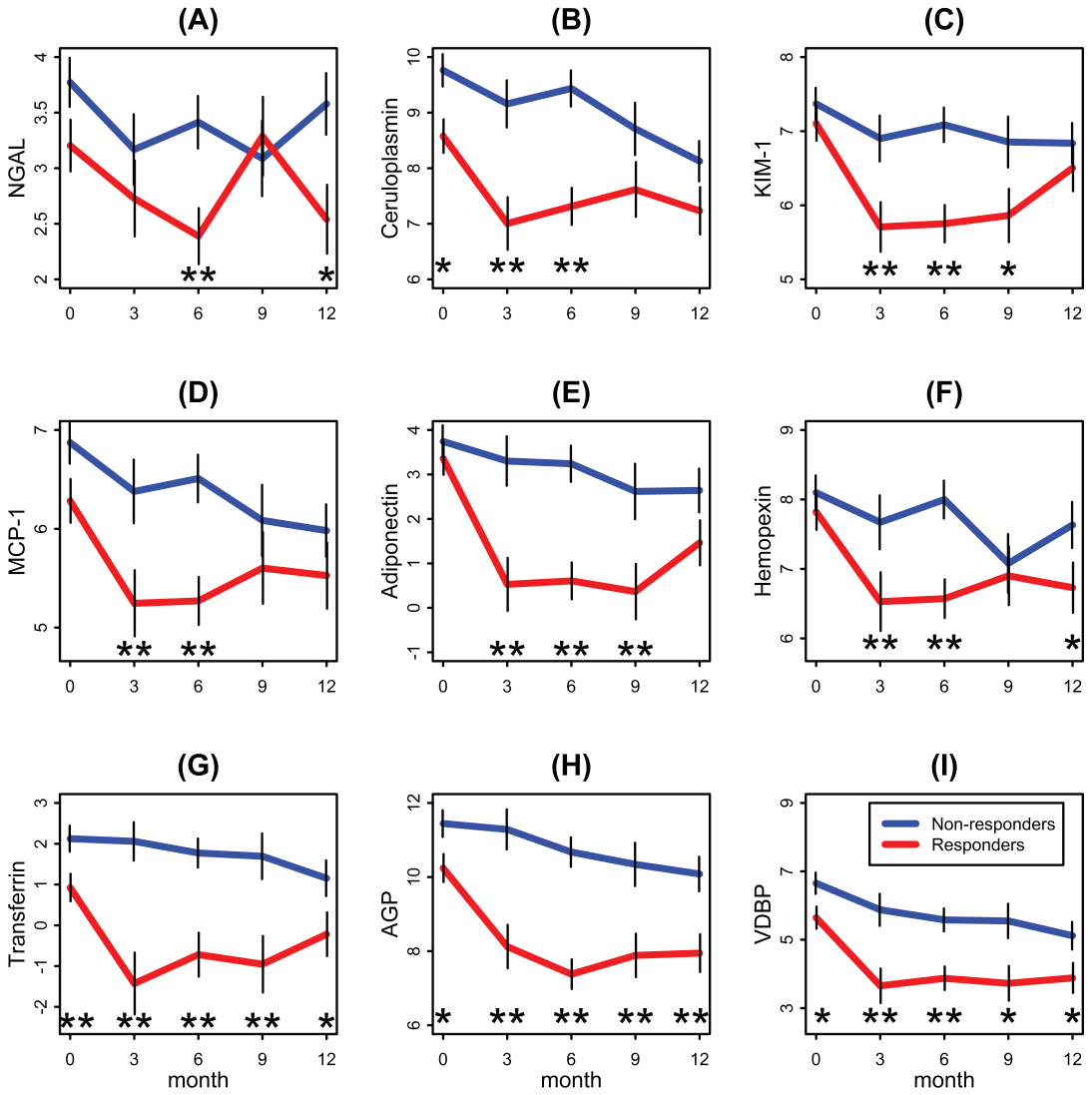

Figure 1. Blue solid lines represent means of biomarkers (natural log transformed) in the group of nonresponders (n = 50). Red solid lines represent means (natural log transformed) among responders to therapy. A. Neutrophil gelatinase (NGAL)– associated lipocalin. B. Ceruloplasmin. C. Kidney injury molecule 1 (KIM-1). D. Monocyte chemotactic protein 1 (MCP-1). E. Adiponectin. F. Hemopexin. G. Transferrin. H. α-1-acid glycoprotein (AGP). I. Vitamin D binding protein (VDBP). Asterisks indicate that the difference of means between responders and nonresponders is statistically significant, with its p value < 0.05 and 0.01, respectively.

disease activity (Supplementary Tables 4 and 5, available with the online version of this article).

Relevance of medication use on urine biomarkers. The use of angiotensinogen system–blocking medications did not importantly influence biomarker levels (Supplementary Table 6, available with the online version of this article). Among 47 patients treated with MMF after kidney biopsy, 17 (46%) were classified as responders. Of the 33 patients initially treated with intravenous CYC, 15 (40.5%) responded to therapy. Early decline of the RAIL biomarker levels occurred more rapidly with CYC therapy than MMF treatment (Figure 4). The same held true for most of other biomarkers we considered (Supplementary Table 7, available with the online version of this article).

DISCUSSION

Currently, accurate assessment of LN activity requires a kidney biopsy, and response to LN therapy in children is generally assessed without confirmation by repeat kidney biopsy. When considering a pool of highly promising biomarkers, we confirmed that select biomarkers that reflect LN histological activity, i.e., those included in the RAIL, are also suited to predict response to LN therapy. We found transferrin, AGP, and TGF-β levels to be early indicators of LN

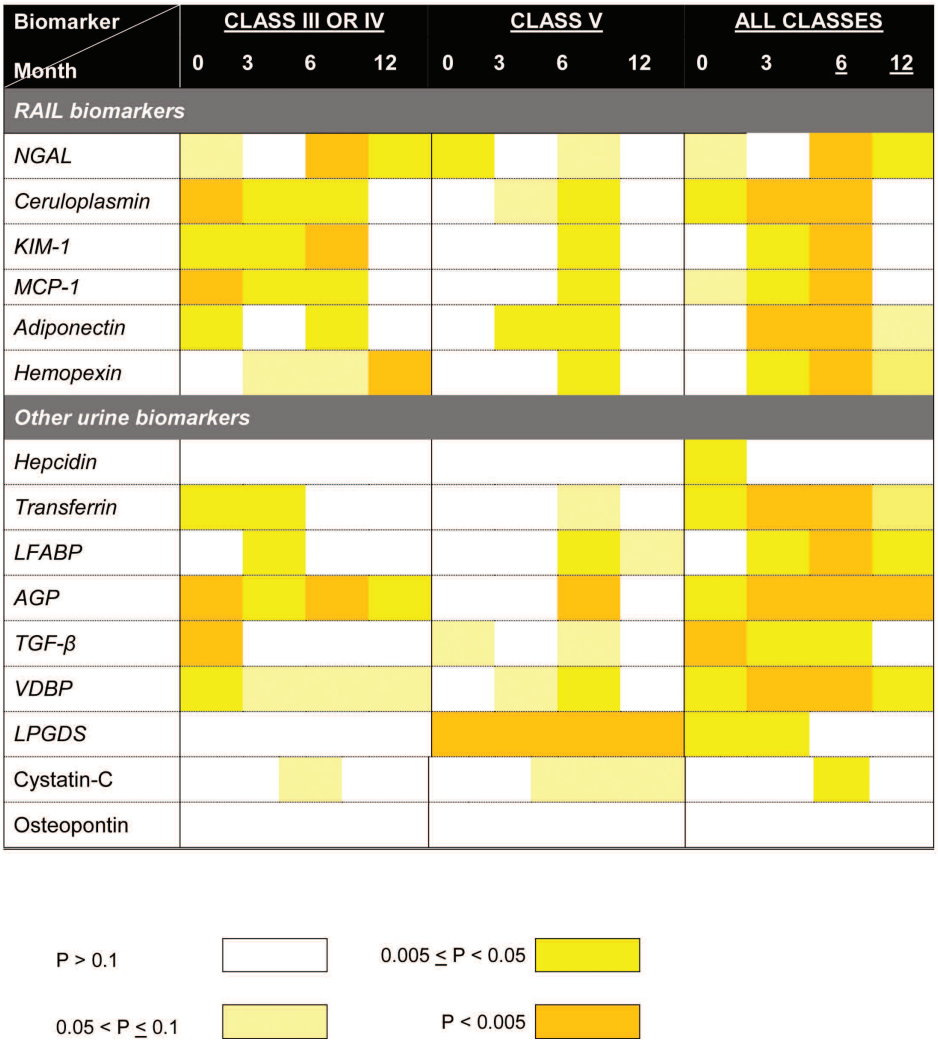

Figure 2. Patterns of differences in the urine biomarker levels over time between responders and non-responders to lupus nephritis therapy. P values from mixed model analysis are compared between groups. RAIL: Renal Activity Index in Lupus; NGAL: neutrophil gelatinase–associated lipocalin; KIM-1: kidney injury molecule 1; MCP-1: monocyte chemotactic protein 1; AGP: α-1-acid glycoprotein; TGF-β: transforming growth factor-β; LFABP: liver-type fatty acid-binding protein 1; VDBP: vitamin D binding protein; LPGDS: lipocalin-like prostaglandin D synthase.

response, while LPGDS seemed especially useful to identify improvement of pure membranous LN.

Achieving complete or even partial response to LN therapy often requires more than 6 months. However, both the ACR and the European League Against Rheumatism recommend adjusting a chosen LN therapy for questionable LN improvement at 3 months[31,32]. We confirmed that several urine biomarkers considered in our study can serve as "early biomarkers" to help identify patients who are at high risk of experiencing a poor response to LN therapy[10,11,12,13,14,15,16].

TGF-β, transferrin, ceruloplasmin, and AGP levels at the time of kidney biopsy differed markedly between responders and nonresponders. However, in contrast with the other biomarkers, decline of TGF-β levels over time was similar among responders and nonresponders. Although TGF-β has been associated with LN activity in the past[17,33], our results are more consistent with the notion that TGF-β is a risk factor of LN damage. Indeed, it has been recognized that LN chronicity progresses even in patients who respond to LN therapy[34] and that TGF-β promotes scarring through accelerated matrix deposition[35].

We previously reported that high levels of LFABP, MCP-1, and transferrin at the time of kidney biopsy are risk factors of future kidney damage[10]. The findings of our current study are in line with that earlier report: continuously high levels of MCP-1 and transferrin are risk factors of nonresponse to LN therapy, hence increased risk of LN damage.

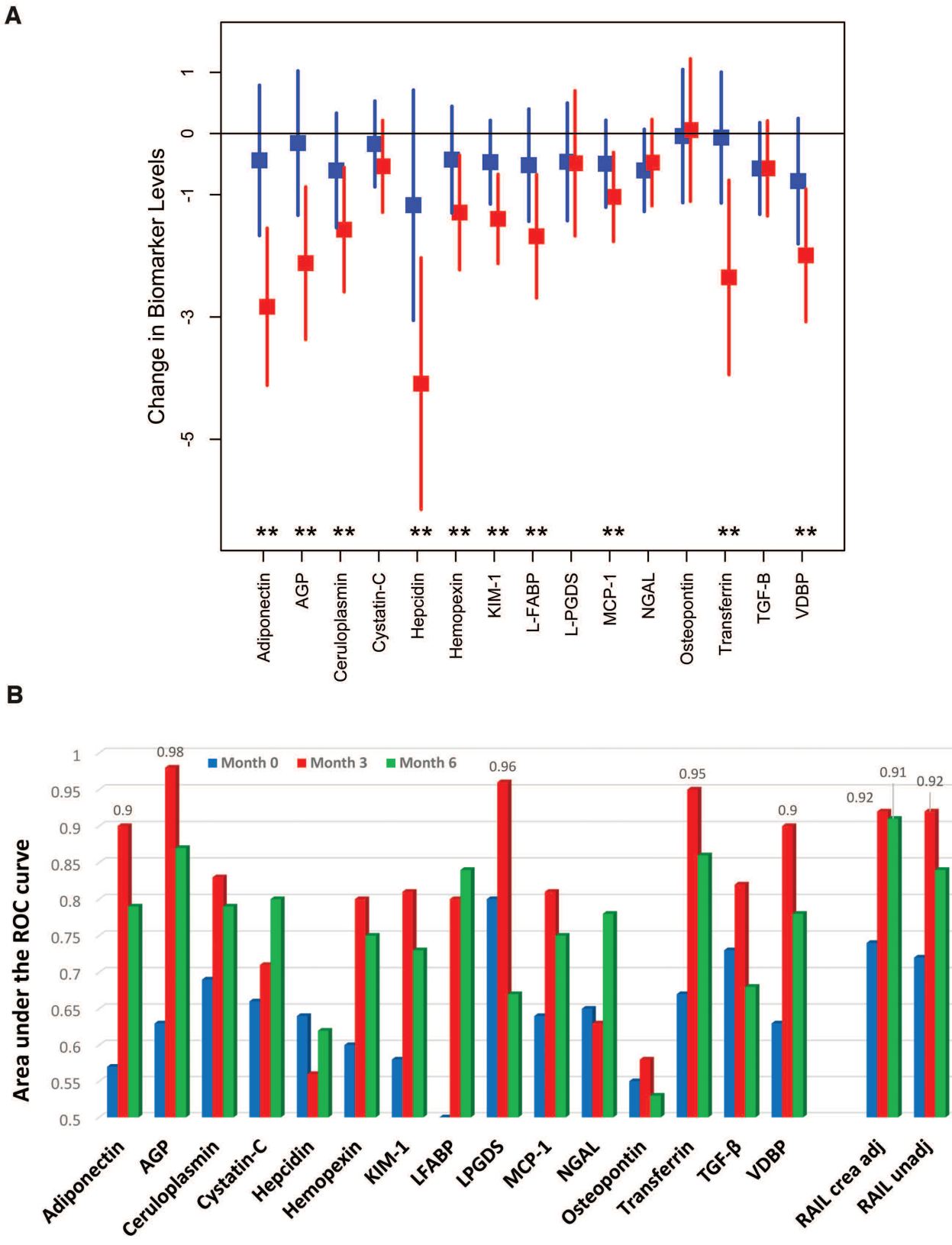

Figure 3A. Blue squares and lines represent changes of urine biomarkers (natural log transformed) means from baseline to Month 3 in patients with response to LN therapy. Red squares and lines represent changes of urine biomarkers (natural log transformed) means from baseline to Month 3 in patients with non-response. Asterisks indicate that the difference of means between respondents and nonrespondents is statistically significant, with its p value < 0.01. LN: lupus nephritis; AGP: α-1-acid glycoprotein; KIM-1: kidney injury molecule 1; LFABP: liver-type fatty acid-binding protein 1; LPGDS: lipocalin-like prostaglandin D synthase; VDBP: vitamin D binding protein; MCP-1: monocyte chemotactic protein 1; NGAL: neutrophil gelatinase–associated lipocalin; TGF-β: transforming growth factor-β; VDBP: vitamin D binding protein.

Figure 3B. Area under the receiver-operating characteristic (ROC) curve (AUC) between responders and nonresponders over time. The RAIL biomarkers (NGAL, MCP-1, adiponectin, KIM-1, ceruloplasmin, and hempexin) combined were excellent to discriminate responders from nonresponders when considering all timepoints. However, adiponectin, AGP, and transferrin combined, and adiponectin and VDBP individually, had excellent ability to anticipate treatment response as early as Month 3. Values are shown only for biomarkers or combination of biomarkers with outstanding accuracy (AUC > 0.9). RAIL: Renal Activity Index in Lupus; NGAL: neutrophil gelatinase–associated lipocalin; MCP-1: monocyte chemotactic protein 1; KIM-1: kidney injury molecule 1; AGP: α-1-acid glycoprotein; TGF-β: transforming growth factor-β; LFABP: liver-type fatty acid-binding protein 1; LPGDS: lipocalin-like prostaglandin D synthase; VDBP: vitamin D binding protein.

Earlier studies reported that LFABP is a sensitive indicator of acute and chronic tubulointerstitial injury and GFR loss[36]. We were unable to confirm these findings and further studies are needed to evaluate the role of LFABP as a marker of LN damage and interstitial injury.

To our knowledge, no association with membranous LN course has been reported in the past. Because our earlier research suggested that LN biomarkers reflect the diverse histopathological changes observed with LN[10,16], it is not surprising that changes in LN biomarkers with therapy would

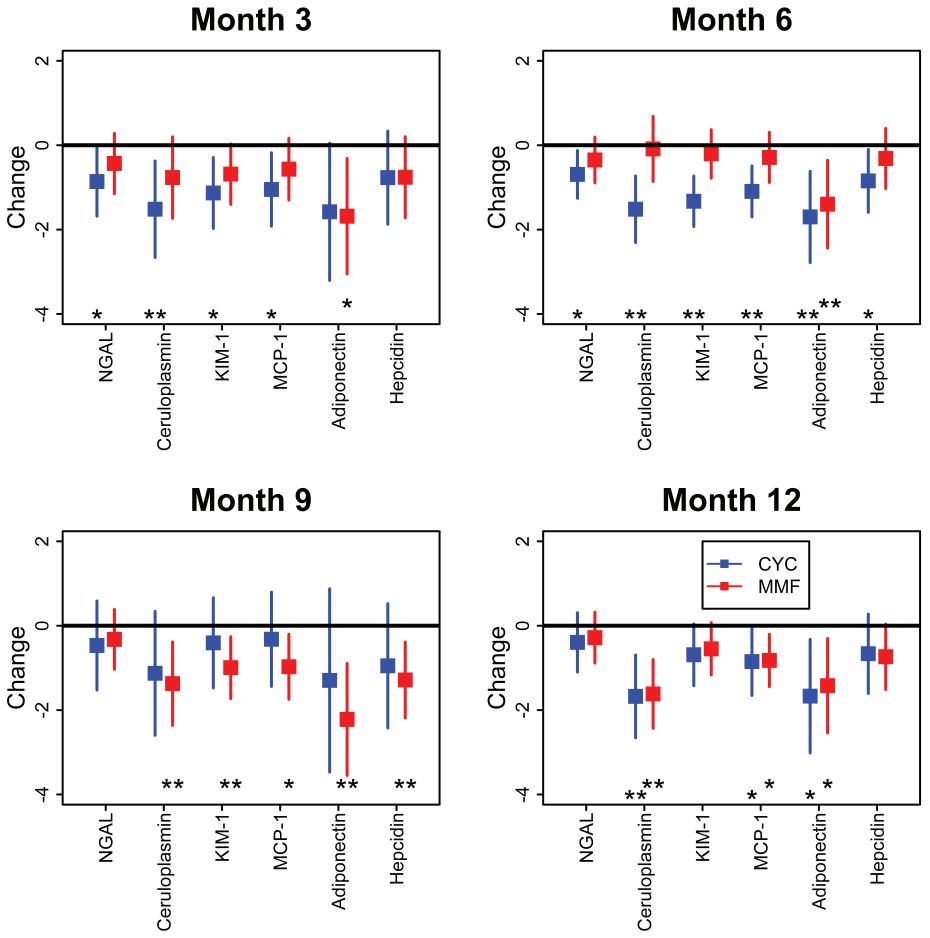

Figure 4. Blue squares and lines represent means and standard errors of the changes of the biomarkers (natural log transformed) from baseline to a followup month with CYC treatment. Red squares and lines represent means and standard errors of the changes of the biomarkers (natural log transformed) from baseline to a followup month with MMF treatment. Asterisks indicate that the difference of means between responders and non-responders is statistically significant, with its p value < 0.05 and 0.01, respectively. CYC: cyclophosphamide; MMF: mycophenolate mofetil; NGAL: neutrophil gelatinase–associated lipocalin; KIM-1: kidney injury molecule 1; MCP-1: monocyte chemotactic protein 1.

be influenced by LN severity. Previous research suggested that LPGDS is strongly associated with GFR decline and ESRD[37,38]. LPGDS is likely locally produced in the proximal tubules, loop of Henle, and glomerulus[37].

Identifying a limited number of urine biomarkers that reflect LN histology and can predict LN flares has been the underlying principle for the selection of the RAIL biomarkers (adiponectin, ceruloplasmin, hemopexin, KIM-1, MCP-1, NGAL)[10,11,16]. Based on the findings of our current study, consideration of AGP, LPGDS, and transferrin also seems sensible for comprehensively determining LN response over time.

Standardizing urine biomarker concentrations by urine creatinine or total protein has been suggested previously. We have not found the latter useful[9], and we confirm our previous reports that creatinine adjustment of the included urine biomarkers is not necessary to accurately measure LN activity over time[9,29].

MMF and CTX are commonly considered equally effective for treating LN in adults[39]. Several urine biomarkers decreased slower with MMF than CTX in our study. This might suggest that MMF was dosed inappropriately in some patients, given the complex pharmacology of MMF and known shortcomings of body-surface–based MMF dosing[40].

Angiotensinogen system blockers are recommended for marked proteinuria with LN[23,31,32]. Although such medications will alter the degree of proteinuria, currently the leading laboratory measure to gauge LN course, this is not the case for the biomarkers included in our study. Indeed, we have shown previously[9] that changes in the urine biomarkers tested in this study cannot be explained by the change of overall proteinuria with LN. Likewise, we did not find any important racial differences in the biomarker levels or consistent association with extrarenal SLE activity. Together, these findings confirm the robustness of the proposed LN biomarkers to reflect LN activity over time[10,11,16].

A special strength of our study is that we included children and young adults who lacked common age-related kidney pathology, which has the potential to influence biomarker identification and verification. Nonetheless, the usefulness of any biomarker found in a pediatric cohort needs to be robust enough to still be useful in adult patient populations. The latter has been shown for the RAIL biomarkers in the past, albeit in smaller studies as well as for transferrin, ceruloplasmin, and LPGDS[9,10,38,41]. We consider another strength of our study the prospective collection of the study cohort with strictly controlled procedures to gather and store urine samples. An additional strength is that the samples were tested at a laboratory certified by the Clinical Laboratory Improvement Amendments, a US program that ensures quality laboratory testing.

Although our cohort constitutes one of the largest to prospectively undergo biomarker evaluation, limitations of our studies include a relatively small sample size. Thus, we were unable to report on potential differences in the biomarkers with proliferative LN with versus without membranous overlap features or report on longer-term LN outcomes. Small sample sizes are difficult to avoid in pediatric studies in general and pediatric orphan diseases, such as LN in children, in particular. Further, we were unable to confirm response to therapy by means of a followup kidney biopsy, a more accurate approach than current laboratory measures to confirm LN response to therapy[42].

We identified a limited number of urine biomarkers that are suited to anticipate response of LN to therapy. If confirmed in large independent cohorts, these "early" biomarkers may prove invaluable for the identification of patients at risk of poor LN outcomes owing to their relative resistance to standard therapies, and may assist in personalizing and optimizing LN care.

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ONLINE SUPPLEMENT

Supplementary material accompanies the online version of this article.

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