て研究参加の同意を得る。

③ 同意が得られた尿潜血陽性者の血清を採取し（健診での残血清を利用）、血中糖鎖異常IgA等を測定する。血中糖鎖異常 IgA 値と他の臨床情報をスコア化し総合的にIgA腎症の可能性を判定する。

④ 判定結果を受診者にフィードバックし、IgA腎症の可能性が高い例では、腎専門医を受診することを勧め、早期診断につなげる。

(倫理面への配慮)

本研究への参加は自由意志に基づいており、いつでも同意撤回ができる。血中糖鎖異常IgA測定やIgA腎症の可能性の判定は、連結可能匿名化を行っており、受診者の個人情報は保護されている。

C.研究結果

平成25～26年度に、山形県内の健診施設5施設（山形市医師会健診センター、やまがた健康推進機構山形検診センター、日本健康協会山形健康管理センター、高畠町役場げんき館、医療法人社団清永会矢吹病院）から協力同意を得た。

上記5施設での尿潜血が陽性となった健診受診者に本研究の説明を行い、平成25年1月～平成26年12月末までで計185例（山形市医師会健診センター24例、やまがた健康推進機構山形検診センター29例、日本健康協会山形健康管理センター101 例、高畠町役場げんき館17例、医療法人社団清永会矢吹病院14例）から同意を得て、血中糖鎖異常IgA値を測定した。

D．考察

山形県の健診施設5施設の協力が得られ、特に大きな問題もなく、計185例の尿潜血陽性者の検体を収集し、判定結果をフィードバックすることが可能であった。

検査指標のスコア化の結果、185例中21例（11.4%）が、IgA腎症の可能性が高いと判定され、腎専門医への受診が勧められた。

今後、さらには他集団との比較や腎生検結果などの追跡調査による解析が進めば、山形県の健診受診者のIgA腎症の可能性が高い集団の頻度、背景因子が明らかになると思われる。

E．結論

本研究により、山形県の尿潜血陽性健診受診者の血液検体と臨床情報を収集することが可能であった。今後、解析が進めば、IgA 腎症の可能性評価における本バイオマーカーの有用性が明らかになると思われる。

F．健康危険情報

（分担研究報告書には記入せず、総括研究報告書にまとめて記入）なし

G．研究発表

1．論文発表

なし

2．学会発表

なし

H．知的財産権の出願・登録状況(予定を含む)

1．特許取得

なし

2．実用新案登録

なし

3．その他

なし

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総合分担研究報告書

沖縄県健診施設および専門外来における適正な研究登録体制の整備と検体管理体制の確立にむけた研究

— 特定健康診査による個人リスク評価に基づく、保健指導と連結した効果的な慢性腎臓病（CKD）地域医療連携システムの制度設計 —

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研究要旨：
住民健診受診者で血尿陽性者を対象に診断スコア法によるIgA腎症患者の割合を調査する目的で、沖縄県内の４つの住民健診施設（ちばなクリニック、沖縄県健康づくり財団、浦添総合病院健診センター、豊見城中央病院）の協力により、1131名より検体の提出を受けた。A判定645名（57%）、B判定147名（13%）、C判定339名（30%）であった。今後、経過観察および一部に腎生検を施行しIgA腎症か否かを追跡し、診断スコアの有用性を検討する必要がある。本研究にてIgA腎症の可能性が高いと判定されたA判定の受診者について県内の医療機関の協力を得て、再検査の情報収集体制を整えている。

A．研究目的

住民健診受診者で血尿陽性者を対象に診断スコア法によるIgA腎症患者の割合を調査する。

B．研究方法

沖縄県内の４つの住民健診施設（ちばなクリニック、沖縄県健康づくり財団、浦添総合病院健診センター、豊見城中央病院）を受診し試験紙法による検尿において血尿陽性であった受診者を対象とする。研究の目的を説明し、同意を得たうえで検体の一部を順天堂大学へ送付する。検査結果については後日、本人へ通知する。本年度までに二次検査成績の結果を持参して各医療機関の受診者について情報収集体制を整えつつある。

（倫理面への配慮）

患者の個人情報は匿名化し、個人を特定できない様にし、集団として公表する。

C．研究結果

研究対象者数

施　設　名	受診種別	健診受診者総数
浦添総合病院健診センター	健康診断	10751
	人間ドック	9690
敬愛会ちばなクリニック	健康診断	445
	人間ドック	8596
豊見城中央病院附属健康管理センター	健康診断	19162
沖縄県健康づくり財団(名称変更)	人間ドック	14735
沖縄県施設総計		63379

施　設　名	受診種別	血尿総数	リクルート数	検体数(参加同意者)
浦添総合病院健診センター	健康診断	262	262	196
	人間ドック	186	186	157
敬愛会ちばなクリニック	健康診断	55	55	25
	人間ドック	575	575	199
豊見城中央病院附属健康管理センター	健康診断	723	469	316
沖縄県健康づくり財団(名称変更)	人間ドック	826	252	247
沖縄県施設総計		2627	1799	1140

施　設　名	受診種別	測定数	解析結果返信数
浦添総合病院健診センター	健康診断	196	353
	人間ドック	157	
敬愛会ちばなクリニック	健康診断	25	224
	人間ドック	199	
豊見城中央病院附属健康管理センター	健康診断	315	315
沖縄県健康づくり財団(名称変更)	人間ドック	247	247
沖縄県施設総計		1139	1139

施　設　名	受診種別	測定数	対象外合計	研究対象者数
浦添総合病院健診センター	健康診断	196	0	196
	人間ドック	157	0	157
敬愛会ちばなクリニック	健康診断	25	0	25
	人間ドック	199	0	199
豊見城中央病院附属健康管理センター	健康診断	315	8	307
沖縄県健康づくり財団(名称変更)	人間ドック	247	0	247
沖縄県施設総計		1139	8	1131

母集団人数把握

施　設　名	受診種別	検体数(参加同意者)	対象外合計	研究対象者数
浦添総合病院健診センター	健康診断	196	0	196
	人間ドック	157	0	157
敬愛会ちばなクリニック	健康診断	25	0	25
	人間ドック	199	0	199
豊見城中央病院附属健康管理センター	健康診断	316	9	307
沖縄県健康づくり財団(名称変更)	人間ドック	247	0	247
沖縄県施設総計		1140	9	1131

施　設　名	受診種別	研究対象者数	生理数(全検	非生理血尿(全	B判定数 全検体中	B判定数 生理中
浦添総合病院健診センター	健康診断	196	14	182	26	2
	人間ドック	157	7	150	27	1
敬愛会ちばなクリニック	健康診断	25	0	25	2	0
	人間ドック	199	17	182	28	2
豊見城中央病院附属健康管理センター	健康診断	307	0	307	42	0
沖縄県健康づくり財団(名称変更)	人間ドック	247	36	211	22	0
沖縄県施設総計		1131	74	1057	147	5

研究対象者の結果報告判定内容

判定内容	各施設名 浦添総合	ちばな	豊見城	健康づくり
A1	154	125	170	151
A2	7	5	1	2
A3	14	5	6	5
A判定	175	135	177	158
B	53	30	42	22
B判定	53	30	42	22
C1	102	56	82	56
C2	15	2	1	7
C3	8	1	5	4
C判定	125	59	88	67
判定数	353	224	307	247
未判定	0	0	0	0
研究検体数	353	224	307	247
男	107	41	91	59
女	246	183	216	188

生理者	21	17	0	36
すべての登録状況				
対象外				
51歳以上				
血清不足				
欠番			1	
尿潜血対象外			8	
2回目健診				
登録数	353	224	316	247
	健診 196	健診 23		
	ドック 157	ドック 196		

D．考察

今後、経過観察および一部に腎生検を施行しIgA腎症か否かを追跡し、診断スコアの有用性を検討する必要がある。各健診センターの病診連携体制は整っているので、紹介先はほぼ完全に把握が可能である。次年度以降に再検査結果の情報を収集する予定である。

E．結論

沖縄県における4か所の健診施設より協力を得て、1131検体提出を得た。今後、A判定とされた645名（全体の57%）について確認調査が必要である。

F．研究発表

1. 論文発表：なし
2. 学会発表：なし

G. 知的財産権の出願・登録状況

1. 特許取得：なし
2. 実用新案登録：なし
3. その他：なし

研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

【書　籍】

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鈴木祐介	「IgA 腎症」　New エッセンシャル	富野康日己	腎臓内科学	医歯薬出版	in press	2015
鈴木祐介、増田稔	有病者の栄養管理　慢性腎臓病（ＣＫＤ）ネフローゼ症候群	富野康日己	スマート栄養管理術１００－栄養管理が重要であるこれだけの理由	医歯薬出版	東京	2014
鈴木祐介	低尿酸血症は、どうして腎臓に悪いのですか？	富野康日己	腎臓病・高血圧と薬剤　実践 Q&A	中外医学社	東京	2013
鈴木祐介	高尿酸血症は、どうして腎臓に悪いのですか？腎臓が悪いから高尿酸血症になるのですか？	富野康日己	腎臓病・高血圧と薬剤　実践 Q&A	中外医学社	東京	2013
鈴木　仁	「蛋白尿を抑える降圧薬」「CHDF の適応」	富野康日己	CKD の診療連携 evidence&tips	中外医学社	東京	2013
鈴木祐介	脂質異常症（高脂血症）が腎臓病・血圧に悪い理由は何ですか？	富野康日己	腎臓病・高血圧と薬剤　実践 Q&A	中外医学社	東京	2013
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鈴木　仁	「蛋白尿を抑える降圧薬」「CHDF の適応」	富野康日己	CKD の診療連携 evidence&tips.	中外医学社	東京	2013
鈴木　仁、鈴木祐介、富野康日己	IgA 腎症患者扁桃と TLR		Annual Review2013	中外医学社	東京	2013
鈴木祐介	「透析の医療保険はどうなっていますか？」	富野康日己	ナーシングケア Q&A	総合医学社	東京	2012
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鈴木祐介	「IgA 腎症での扁桃摘出術とステロイドパルス併用療法とは何ですか？」	富野康日己	CKD 診療連携―Evidence & Tips―	中外医学社	東京	2012
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研究関連成果の刊行物・別刷

PLOS ONE

A Panel of Serum Biomarkers Differentiates IgA Nephropathy from Other Renal Diseases

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Abstract

Background and Objectives: There is increasing evidence that galactose-deficient IgA1 (Gd-IgA1) and Gd-IgA1-containing immune complexes are important for the pathogenesis of IgA nephropathy (IgAN). In the present study, we assessed a novel noninvasive multi-biomarker approach in the diagnostic test for IgAN.

Materials and Methods: We compared serum levels of IgA, IgG, Gd-IgA1, Gd-IgA1-specific IgG and Gd-IgA1-specific IgA in 135 IgAN patients, 79 patients with non-IgAN chronic kidney disease (CKD) controls and 106 healthy controls. Serum was collected at the time of kidney biopsy from all IgAN and CKD patients.

Results: Each serum marker was significantly elevated in IgAN patients compared to CKD ($P<0.001$) and healthy controls ($P<0.001$). While 41% of IgAN patients had elevated serum Gd-IgA1 levels, 91% of these patients exhibited Gd-IgA1-specific IgG levels above the 90th percentile for healthy controls (sensitivity 89%, specificity 92%). Although up to 25% of CKD controls, particularly those with immune-mediated glomerular diseases including lupus nephritis, also had elevated serum levels of Gd-IgA1-specific IgG, most IgAN patients had elevated levels of Gd-IgA1-specific antibody of both isotypes. Serum levels of Gd-IgA1-specific IgG were associated with renal histological grading. Furthermore, there was a trend toward higher serum levels of Gd-IgA1-specific IgG in IgAN patients with at least moderate proteinuria (≥1.0 g/g), compared to patients with less proteinuria.

Conclusions: Serum levels of Gd-IgA1-specific antibodies are elevated in most IgAN patients, and their assessment, together with serum levels of Gd-IgA1, improves the specificity of the assays. Our observations suggest that a panel of serum biomarkers may be helpful in differentiating IgAN from other glomerular diseases.

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Introduction

IgA nephropathy (IgAN) is the most common type of primary glomerulonephritis worldwide [1,2]. IgAN has a significant morbidity, culminating in end-stage kidney disease in about 40% of patients within 20 years of diagnosis [3]. Renal biopsy is required for the diagnosis of IgAN. Typical histological features include granular mesangial deposits of IgA, usually accompanied by C3, a variable presence of IgG and/or IgM, and diverse degrees of mesangial cellular proliferation and expansion of the extracellular matrix [4].

Several recent studies suggest that aberrant *O*-glycosylation of circulatory IgA1 is vital in the pathogenesis of IgAN. The *O*-linked glycans in the hinge region of IgA1 are generally composed of *N*-acetylgalactosamine (GalNAc) and galactose; sialic acid may be attached to either or both sugars. IgA1-producing cells secrete a mixture of IgA1 *O*-glycofoms. Studies in different populations have shown that IgAN patients have significantly higher levels of circulating IgA1 with galactose-deficient, *O*-linked, hinge-region glycans [5–9]. Depending on the population studied, 50–75% of IgAN patients have levels above the 90th percentile for healthy controls. In addition, IgA1 eluted from renal tissues of IgAN patients also exhibits a galactose deficiency in the *O*-linked glycans in the hinge-region [10,11].

The serum level of IgA1-containing circulating immune complexes is elevated in patients with IgAN [12–14]. These

complexes contain galactose-deficient IgA1 (Gd-IgA1) bound by IgG and/or IgA antibodies [14,15]. Recently, we have shown that the IgG auto-antibodies that recognize glycan-containing epitopes on Gd-IgA1 exhibit unique features in the complementarity-determining region 3 of the variable region of their heavy chains [16]. Furthermore, the serum levels of IgG autoantibodies specific for Gd-IgA1 correlated with disease severity, as assessed by magnitude of proteinuria. However, the serum levels of Gd-IgA1-containing circulating immune complexes may differ widely among IgAN patients [15]. Furthermore, some IgAN patients do not show glomerular deposition of IgG, but rather only IgA. Therefore, it is difficult to explain the pathogenesis of IgAN by an elevated serum level of glycan-specific antibodies of only the IgG isotype. These latter features may be explained by our observation that some patients with IgAN have complexes generated by glycan-specific antibodies of the IgA1 isotype [15]. Whereas the serum levels of IgA, Gd-IgA1 and glycan-specific IgG are higher in patients with IgAN compared to healthy controls, the levels of these parameters have not been systematically studied in patients with other forms renal disease with clinical features similar to those of IgAN. We therefore examined the prevalence of elevated serum levels of IgA, Gd-IgA1 and glycan-specific IgG and IgA in IgAN patients and a large cohort of CKD controls to assess the utility of these biomarkers for the non-invasive diagnosis of IgAN. Our data revealed that this panel of biomarkers is helpful in differentiating patients with IgAN from patients with other glomerular diseases.

Materials and Methods

Ethics Statement

This study was performed according to the Declaration of Helsinki and approved by the Ethics Review Committee of Juntendo University Faculty of Medicine. All study participants provided written informed consent.

Patients and controls

A cross-sectional study was performed using serum samples collected at Juntendo University Hospital in Japan from 2006 to 2010 at the time of renal biopsy from 135 patients with IgAN and 79 patients with other renal diseases as shown in Table 1. We collected serum samples from 106 healthy volunteers who had never exhibited any abnormality by urinalysis in medical examinations from 2009 to 2011. All patients and healthy volunteers were Japanese, and the demographic and clinical features are summarized in Table 2. Histological prognostic stages of IgAN were assessed by the Clinical Guidelines for IgA Nephropathy in Japan (second version) [17], and are as follows: Group I: good prognosis, Group II: relatively good prognosis, Group III: relatively poor prognosis, and Group IV: poor prognosis.

Subgroups of disease controls

As the urinary protein/creatinine ratio in disease controls was higher than that in IgAN patients, we divided CKD controls into two subgroups: one with urinary protein/creatinine ratio $\geq$2.5 g/g (high-proteinuria) and the second with urinary protein/creatinine ratio $<$2.5 g/g (low-proteinuria). Then, we compared the levels of each biomarker between the two subgroups.

Measurement of serum biomarkers

Serum level of Gd-IgA1. The serum level of Gd-IgA1 was measured by lectin ELISA using GalNAc-specific lectin from *Helix aspersa* (HAA; Sigma, St. Louis, MO) as previously reported [9,18,19]. Diluted sera were added 100 ng per well of serum IgA. The captured IgA was treated with 10 mU/ml neuraminidase (Roche Diagnostic Corp. Indianapolis, IN) to remove terminal sialic acid residues [9,19]. The desialylated IgA1 was then reacted with biotin-labeled HAA and subsequently developed; absorbance was measured at 490 nm. The HAA reactivity of IgA1 in each sample was then calculated as OD units/100 ng of serum IgA. Naturally galactose-deficient IgA1 (Ale) myeloma protein [9] treated with neuraminidase and was used as the standard. Serum level of total Gd-IgA1 was expressed in relative Units, calculated by multiplying the normalized HAA reactivity by the amount of IgA in the serum sample (mg/ml).

Serum level of Gd-IgA1-specific IgG. ELISA plates were coated with the Fab fragment of Gd-IgA1 myeloma protein (Ste) generated with an IgA-specific protease from *Haemophilus influenzae* HK50 [15]. The amount of total IgG used for the analyses was normalized in all samples and added to each well. Captured IgG

Table 1. Renal diseases in chronic kidney disease (CKD) controls.

Renal disease	N
Diabetic nephropathy	33
Membranous nephropathy	9
Non-IgA proliferative nephropathy	9
Lupus nephritis	8
Minimal change nephrotic syndrome	6
Nephrosclerosis	4
Interstitial nephritis	2
Focal segmental glomerular sclerosis	2
ANCA-related nephritis	1
Scleroderma renal crisis	1
HCV-related nephritis	1
Fabry's disease	1
Acute post-streptococcal nephritis	1
Minor glomerular abnormality	1

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Table 2. Characteristics of patients with IgAN and CKD controls.

	IgAN patients	CKD controls	Healthy controls
Numbers	135	79	106
Age (years)	34.6±11.9	50.9±19.4	32.6±5.2
Sex (male/female)	56/79	42/37	61/45
Serum creatinine (mg/dl)	0.93±0.53	2.26±2.52	ND
eGFR (ml/min/1.73 m^2)	76.9±25.8	55.7±39.2	ND
Urine P/C (g/g)	0.65±0.84	3.01±3.19	ND
Hematuria	4.08±2.28	1.60±2.23	ND

Values are mean ±SD.
eGFR, estimated glomerular filtration rate; P/C, protein/creatinine ratio; ND, not determined.
Hematuria: Assessed by assigning scores according to number of red blood cells per high-power field (RBC/HPF).
≤5 RBC/HPF =0, 6–10 RBC/HPF =1, 11–15 RBC/HPF =2, 16–20 RBC/HPF =3, 21–25 RBC/HPF =4, 26–30 RBC/HPF =5, >30 RBC/HPF =6.
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was detected with a biotin-labeled $F(ab')_2$ fragment of goat IgG anti-human IgG antibody (BioSource; Invitrogen, San Diego, CA). Avidin–horseradish peroxidase conjugate (ExtrAvidin; Sigma-Aldrich) was then added, and the reaction was developed [16]. Serum levels of Gd-IgA1-specific IgG were expressed in Units (1 unit as OD 1.0 measured at 490 nm).

Serum level of Gd-IgA1-specific IgA. ELISA plates were coated with Fab fragment of Gd-IgA1 (Ste) described above [15]. Captured antibodies were detected by incubation with mouse monoclonal antibody to human IgA (Fc-specific) (Applied Biological Materials Inc., Richmond, BC) and detected by Peroxidase-conjugated AffiniPure Goat Anti-Mouse IgG (H+L) (Jackson Immuno Research, West Grove, PA). Serum levels of Gd-IgA1-specific IgA were expressed in Units (1 unit as OD 1.0 measured at 490 nm).

Statistical analysis

Data are expressed as means ± SD. Comparison of groups was performed using univariate ANOVA, and *post hoc* Bonferroni correction was used for multiple comparisons. Correlation between two groups was performed by regression analysis. $P<0.05$ was considered significant. These statistical analyses were performed using the Prism software (GraphPad Software Inc., La Jolla, CA). The discriminatory ability of the biomarkers and clinical data combination was estimated with the area under the receiver operating characteristic curve (AUC-ROC). We additionally used Akaike's Information Criterion (AIC) to select the best predictive model [20]. The sensitivity, specificity, and positive and negative predictive values were calculated based on the ROC curves with the cut-off selected at the 90th percentile biomarker value for healthy controls. We interpreted the model with the lowest AIC as the most useful in differentiating cases from controls. These analyses were performed with IBM SPSS Statistics release 19.0.

Results

Clinical profiles, serum IgA, IgG and Gd-IgA1

The present study was comprised of 135 IgAN patients, 79 CKD controls and 106 healthy controls (Table 2). Age, serum creatinine concentration, and the urinary protein/creatinine ratio were higher for the CKD controls than for IgAN patients.

The serum IgA concentration was significantly higher for the IgAN patients (3.60±1.47 mg/ml) compared to those of the CKD controls (2.71 ±1.04 mg/ml, P<0.001) and healthy controls (2.17±0.75 mg/ml, P<0.001). Half of the patients with IgAN had a serum IgA concentration higher than the 90th percentile for healthy controls (3.06 mg/ml), consistent with a prior publication [21]. There was no significant difference in serum IgG concentration between the different groups.

Also consistent with prior reports, the serum level of Gd-IgA1 for the IgAN patients was significantly higher compared to that for the CKD controls (P<0.001) and healthy controls (P<0.001) (Figure 1A). Fifty-six of 135 IgAN patients (41%) but only eight nineof 79 (11%) CKD controls had a serum Gd-IgA1 level higher than the 90th percentile for healthy controls ($P=5.5\times10^{-7}$ for differences in distribution in IgAN patients *vs.* CKD controls).

Serum levels of IgG and IgA antibodies against Gd-IgA1

IgAN patients had significantly higher levels of serum Gd-IgA1-specific IgG compared with those of the CKD controls (P<0.001) and healthy controls (P<0.001) (Figure 1B). Most IgAN patients (123/135, 91%) had a serum level of Gd-IgA1-specific IgG higher than the 90th percentile for healthy controls (1.48 Units). The differences in the distribution of Gd-IgA1-specific IgG in IgAN patients *vs.* disease controls were highly significant ($P=3\times10^{-24}$).

Serum levels of Gd-IgA1-specific IgA were elevated in IgAN patients compared with CKD controls (P<0.001) and healthy controls (P<0.001) (0.890±0.840 Units for IgAN patients, 0.482 ±0.483 Units for CKD controls and 0.419±0.289 Units for healthy controls; Figure 1C). The serum level of Gd-IgA1-specific IgA was higher than the 90th percentile for healthy controls (0.611 Units) for 43% of IgAN patients and 14% of CKD controls.

While the serum levels of Gd-IgA1-specific IgG or IgA were significantly higher in IgAN patients, about 25% of CKD controls also had a serum level of Gd-IgA1-specific IgG higher than the 90th percentile for healthy controls. Many of IgAN patients (56/135, 41%) had elevated levels of both Gd-IgA1-specific IgG and IgA. In contrast, elevated levels of both IgG and IgA antibody against Gd-IgA1 were seen in only 5 of 79 CKD controls who had an immune- mediated glomerulonephritis, such as lupus nephritis or membranous nephropathy (Table 3). Twenty-three of 38 CKD controls with an immune-mediated kidney disease had an elevated serum level of Gd-IgA1-specific IgG or IgA. In contrast, only 8 of 41 CKD controls with non-immune-mediated renal disease had an elevated serum level of Gd-IgA1-specific IgG or IgA ($p=6\times10^{-4}$ for differences in distribution between the two groups).

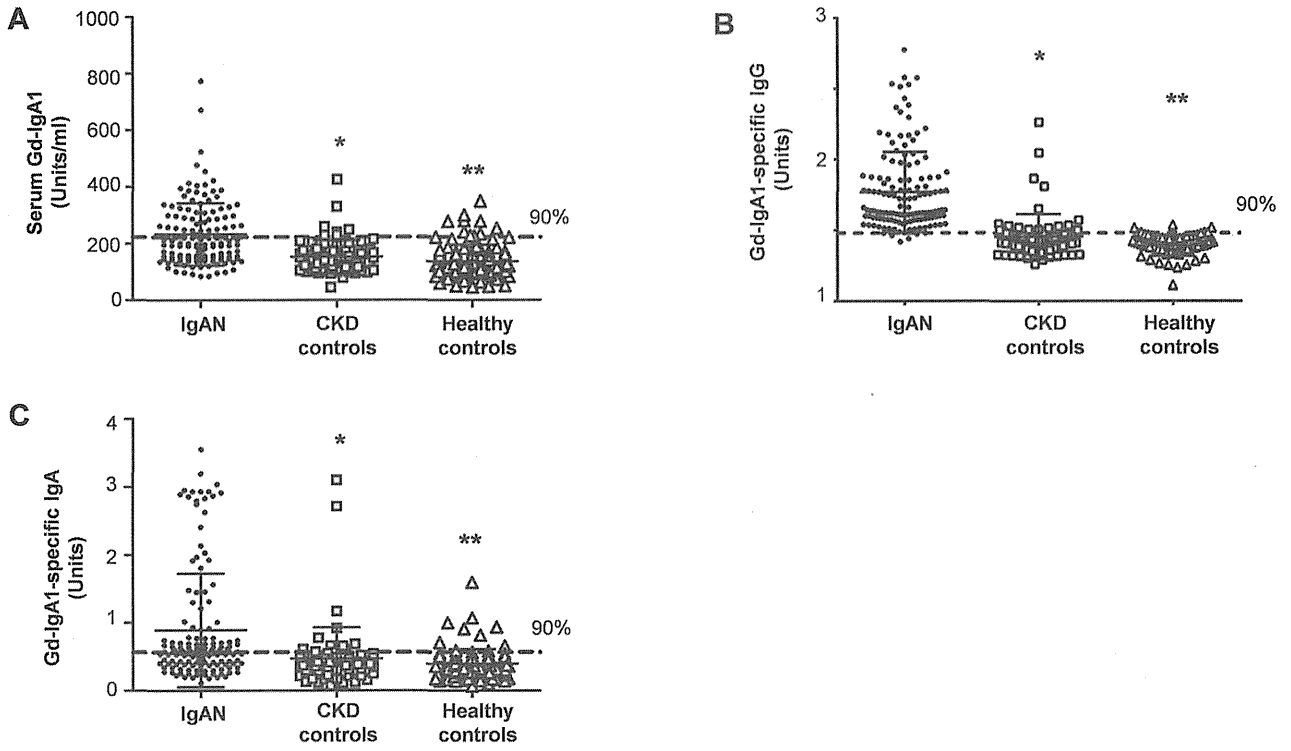

Figure 1. Distribution of serum levels of (A) Gd-IgA1, (B) Gd-IgA1-specific IgG and (C) Gd-IgA1-specific IgA in patients with IgAN (n = 135), CKD controls (n = 79) and healthy controls (n = 106). Each biomarker was measured by capture ELISA. The serum levels of Gd-IgA, Gd-IgA1-specific IgG and Gd-IgA1-specific IgA were higher in IgAN patients compared with those of the CKD controls (*P<0.001) and healthy controls (**P<0.0001).
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Relationship between Gd-IgA1 and Gd-IgA1-specific IgG

The serum levels of Gd-IgA1-specific IgG were significantly higher in IgAN patients with elevated Gd-IgA1 levels (P<0.001). However, about 91% of IgAN patients with a normal serum Gd-IgA1 level also had an elevated level of Gd-IgA1-specific IgG, suggesting that auto-antibody production may occur independently of serum Gd-IgA1 levels (Figure 2).

Comparison of biomarker levels with clinical and pathological findings

We assessed each biomarker for possible correlation with histological findings and the clinical manifestations at the time of renal biopsy. Proteinuria was inversely correlated with serum immunoglobulin levels (IgA, and Gd-IgA1-specific IgG and IgA autoantibodies) in CKD controls (r = −0.17 to −0.28). This

Table 3. Serum levels of Gd-IgA1-specific antibodies in patients with IgAN, CKD controls and healthy controls.

	Gd-IgA1-specific IgG (Units)	Gd-IgA1-specific IgA (Units)
IgAN (N = 135)	1.77±0.28	0.89±0.84
Lupus nephritis WHO class III (N = 2)	1.57±0.09	0.47±0.26
Lupus nephritis WHO class IV (N = 4)	1.62±0.19	0.47±0.20
Lupus nephritis WHO class V (N = 2)	1.91±0.49	0.50±0.04
Membranous nephropathy (N = 9)	1.52±0.19	0.48±0.20
Non-IgA proliferative glomerulonephritis (N = 9)	1.43±0.10	0.36±0.15
Diabetic nephropathy (N = 33)	1.43±0.08	0.35±0.13
Nephrosclerosis (N = 4)	1.40±0.07	0.44±0.33
Minimal-change nephrotic syndrome (N = 6)	1.52±0.27	0.66±0.28
Interstitial nephritis (N = 2)	1.33±0.02	0.58±0.01
Other immune mediated renal diseases (N = 6)	1.47±0.07	0.71±0.75
Other non-immune mediated renal diseases (N = 2)	1.39±0.09	0.59±0.10
Healthy controls (N = 106)	1.39±0.07	0.42±0.29

WHO, World Health Organization.
doi:10.1371/journal.pone.0098081.t003

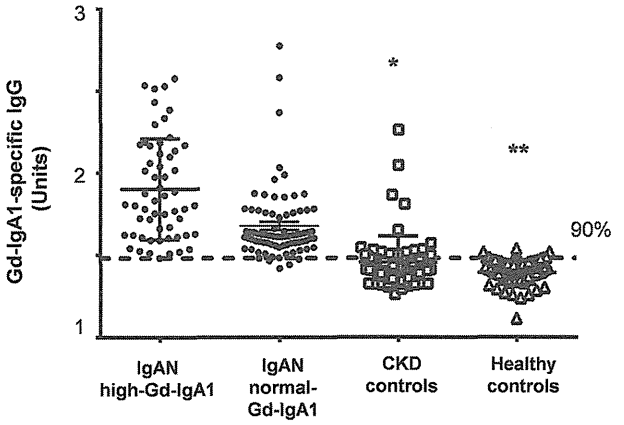

Figure 2. Serum levels of Gd-IgA1-specific IgG in IgAN patients with high-Gd-IgA1 or normal-Gd-IgA1 in comparison to CKD and healthy controls. We divided IgAN patients into two subgroups: patients with serum levels of Gd-IgA1 ≥ the 90th percentile for healthy controls (high-Gd-IgA1 group; n = 56) and patients with levels Gd-IgA1 < the 90th percentile for healthy controls (normal-Gd-IgA1 group; n = 79). Although serum levels of Gd-IgA1-specific IgG were significantly higher in IgAN patients with high Gd-IgA1 levels (*vs.* CKD controls; *P< 0.0001, *vs.* healthy controls; **P<0.0001), IgAN patients with normal Gd-IgA1 levels also had elevated Gd-IgA1-specific IgG (*vs.* CKD controls; *P<0.0001, *vs.* healthy controls; **P<0.0001).
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association was most strongly driven by the CKD controls, who had significantly more proteinuria than did the IgAN patients. To assess whether the serum levels of these immunogloubulins differed based on the magnitude of proteinuria, we divided the CKD disease controls into high-proteinuria and low-proteinuria subgroups, and compared the levels of each biomarker between the two subgroups. There was no significant difference in serum levels of IgA, Gd-IgA1, Gd-IgA1-specific IgG, and Gd-IgA1-specific IgA between the two subgroups (Table S1).

The serum level of Gd-IgA1-specific IgA correlated with degree of mesangial IgA deposits in patients with IgAN ($R^2 = 0.61$) (Figure 3A). In addition, the histological prognostic stage according to the clinical guidelines for IgAN in Japan [17] correlated with proteinuria ($R^2 = 0.71$) (Figure 3B) and the percentage of glomeruli with crescents ($R^2 = 0.69$) (Figure 3C). As we previously reported [16], there was no significant correlation between the serum levels of Gd-IgA1-specific IgG and the degree of mesangial IgG deposition. However, there was a trend for the serum levels of Gd-IgA1-specific IgG to be higher in patients with greater mesangial IgG deposition (2+) (1.937±0.464 Units, n = 9) than in patients with no mesangial IgG deposition (1.747±0.326 Units, n = 13). Furthermore, the mean serum level of Gd-IgA1-specific IgG in the histological prognostic group 4 (n = 35, 1.821±0.318 Units) was high, compared to that in the histological prognostic group 1 (n = 8, 1.663±0.107 Units).

Utility of biomarkers for non-invasive diagnosis of IgAN

We estimated the sensitivity and specificity of each biomarker for prediction of IgAN, using the cut-off value of the 90th percentile for healthy controls. The serum level of Gd-IgA1-specific IgG performed best, providing a sensitivity of 89% and specificity of 92% for diagnosis of IgAN. The ROC curves showed excellent performance in comparison of IgAN vs. healthy controls (Figure 4, with corresponding statistics summarized in Tables 4 and 5). The areas under the ROC curve (C-statistics) for anti-Gd-IgA1 IgG were 0.965 (95%CI: 0.943–0.987) for discriminating IgAN cases *vs.* healthy controls, 0.973 (95%CI: 0.948–0.999) for non-immune-mediated CKD controls, and 0.813 (95%CI: 0.730–0.895) for immune-mediated CKD controls. In addition, the AIC for models testing Gd-IgA1-specific IgG were 57.4 for discriminating IgAN cases *vs.* healthy controls, 75.3 for non-immune-mediated CKD controls, and 145.2 for immune-mediated CKD controls.

These data suggest that although serum levels of Gd-IgA1-specific autoantibodies have excellent predictive properties to discriminate IgAN patients from healthy controls and patients with non-immune-mediated kidney disease, the specificity is lower for discriminating IgAN patients from patients with other types of immune-mediated kidney disease. Altogether, elevated Gd-IgA1-specific IgG provided positive predictive values of 92%, 96%, and 72%, and negative predictive values of 89%, 90%, and 82% for healthy controls and patients with non-immune kidney disease, and immune-mediated kidney disease, respectively.

Discussion

IgAN is the most common type of primary glomerulonephritis worldwide and is often detected by urinary abnormalities, such as microscopic hematuria and low-grade proteinuria. The clinical features of IgAN vary between patients with IgAN. In biopsy specimens, glomerular deposits of IgA1 are sometimes accompanied by IgG and/or IgM. Although microscopic hematuria and low-grade proteinuria are frequent laboratory findings in patients with IgAN, some cases manifest a rapidly progressive clinical course with crescentic glomerulonephritis while others exhibit acute nephritic syndrome or nephrotic syndrome. The long-term prognosis is not benign; about 40% of IgAN patients progress to end-stage kidney disease within 20 years [3]. Thus, early diagnosis and treatment are necessary to prevent or slow disease progression. A noninvasive approach for the diagnosis of IgAN has been sought worldwide, especially in countries where the routine immunohistological processing of renal biopsy tissue is prohibitively expensive.

A pathway of multiple events has been proposed for the pathogenesis of IgAN [16,22]. Immune complexes formed by the binding of Gd-IgA1 by Gd-IgA1-specific IgG or IgA in the circulation deposit in the mesangium and activate resident cells [12,23–26]. Gd-IgA1-containing immune complexes from sera of patients with IgAN induce proliferation of human mesangial cells in culture whereas Gd-IgA1 alone does not [27,28]. Activated mesangial cells produce inflammatory cytokines that cause glomerular injury [29,30]. These reports suggest that Gd-IgA1-containing immune complexes play an essential role in the pathogenesis of IgAN. Therefore, we measured serum levels of the key biomarkers, IgA, Gd-IgA1, Gd-IgA1-specific IgG and Gd-IgA1-specific IgA to assess their utility in distinguishing patients with IgAN from patients with other forms of kidney disease and healthy controls.

The present study showed that serum levels of IgA, Gd-IgA1, Gd-IgA1-specific IgG and Gd-IgA1-specific IgA were elevated in patients with IgAN compared with those of healthy and CKD controls, suggesting that these parameters may be useful for the diagnosis of IgAN. However, several interesting limitations of these tests have emerged.

First, elevated serum levels of Gd-IgA1 have been found in healthy relatives of individuals with IgAN, suggesting that such levels alone are not sufficient to cause the disease [31]. Among patients with an established diagnosis of IgAN, the serum levels of Gd-IgA1 are variable [9]. Notably, longitudinal trajectories of serum Gd-IgA1 levels have not yet been established in prospective