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ORIGINAL ARTICLE

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Abnormal glycosylation of serum IgG in patients with IgA nephropathy

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Abstract
Background. We postulated that IgA nephropathy (IgAN) involved alterations of serum IgG. The present study was undertaken to elucidate changes in serum IgG oligosaccharide structure analysis and to assess the diagnostic usefulness of this analysis in IgAN.
Methods. The subjects were 28 children who were definitively diagnosed as having IgAN on the basis of renal biopsy and who had not received treatment for this disease; 27 healthy children; 15 untreated adults definitely diagnosed as having IgAN; 5 patients with other nephropathies; and 61 healthy adults. Oligosaccharide analyses of IgG were performed by reverse-phase high-performance liquid chromatography (HPLC) developed by Takahashi and colleagues.
Results. In both the children and the adults, the peak area ratio of isomers with two different galactosyl-N-acetylglucosamine (Gal-GlcNAc) binding sites was significantly lower in the presence of IgAN than in the healthy subjects ($P < 0.05$ in children and $P < 0.001$ in adults). The ratio of Gal-free oligosaccharides to Gal-positive oligosaccharides did not differ according to the presence or absence of IgAN in children or in adults.
Conclusions. The analysis of the oligosaccharide structure of serum IgG seems to be useful in diagnosing IgAN.

Key words IgA nephropathy · Serum IgG · Oligosaccharide structure · High-performance liquid chromatography

Introduction

IgA nephropathy (IgAN) is the most common primary glomerulonephritis in the world. It is a major cause of endstage renal disease, with approximately 50% of all IgAN patients developing endstage renal failure within 10 to 15 years of diagnosis.[1] IgAN is characterized by a predominance of mesangial IgA deposition, often in conjunction with deposits of C3 and IgG and/or IgM.[2] It has been reported that, in patients with persistent microscopic hematuria or proteinuria, or a serum IgA greater than 315 mg/dl in adults, the probability of a diagnosis of IgAN exceeds 80%.[3] However, no means other than renal biopsy is available for making a definitive diagnosis of IgAN.

The mechanism of the formation of macromolecular IgA is not yet well understood. A significant increase in serum IgA possessing an affinity for human IgA has been reported in patients with IgAN.[4] In recent years, attention has been paid to the finding that the molecular structure, especially the oligosaccharide structure of IgA1 (a subclass of serum IgA), shows variations in patients with IgAN.[5–7]

It has been reported that serum IgG charges in patients with IgAN differ from those in normal individuals,[8] and that serum IgA1-IgG complex levels are increased in patients with IgAN.[9] Based on these findings, we hypothesized that IgAN involves alterations of serum IgG.

Human IgG contains one asparagine-linked oligosaccharide in each C-terminal half of the heavy chain dimers of the (Fc) region. Takahashi et al.[10] reported detailed glycoform profiles of IgG, with the use of reverse-phase high-performance liquid chromatography (HPLC), and elucidated the structures of 16 neutral oligosaccharides.

The present study was undertaken to assess the usefulness of serum IgG oligosaccharide structure analysis in patients with IgAN, and to compare the findings in children with IgAN and adults with IgAN.

Subjects and methods

Subjects

The subjects were 28 children who were definitively diagnosed as having IgAN on the basis of renal biopsy and who

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had not received any treatment for this disease (16 boys and 12 girls, between 5 and 21 years of age, with a mean age of 13.8 years); 27 healthy children, belonging to the families of staff working at the authors' hospital (10 boys and 17 girls, between 4 and 22 years of age, with a mean age of 16.1 years); 15 untreated adults definitely diagnosed as having IgAN (7 men and 8 women between 17 and 57 years of age, with a mean age of 33.0 years); 2 patients with membranoproliferative glomerulonephritis (MPGN); 1 patient with membranous nephropathy (MN); 2 patients with focal segmental glomerulosclerosis (FGS); and 61 healthy adults (36 men and 25 women between 20 and 83 years of age, with a mean age of 41.6 years). We obtained informed consent from all of the subjects or the families of the subjects.

Oligosaccharide analyses of IgGs

IgG was extracted from serum, using HiTrap TM Protein G affinity column chromatography (Pharmacia Biotech, Uppsala, Sweden). IgG was heated for 1 h to remove sialic acid. The oligosaccharide moieties were released by enzymatic cleavage with glycoamidase A (EC 3.5.1.52; Seikagaku Kogyo, Tokyo, Japan). The supernatant was subjected to Bio Gel P-4 (Bio-Rad, Hercules, CA, USA) gel filtration. The reducing ends of the oligosaccharides were reductively aminated with 2-aminopyridine (Wako Pure Chemicals, Osaka, Japan) by use of sodium cyanoborohydride (Aldrich Chemicals, Milwaukee, WI, USA). The pyridylamino (PA) oligosaccharides were purified by gel filtration on a Sephadex G-15 column (Pharmacia, Piscataway, NJ, USA). PA-oligosaccharide was isolated by HPLC on a Shim-pack CLC-octadecylsilyl (ODS)-silica column (6 mm × 150 mm; Shimadzu, Kyoto, Japan) with fluorescence detection.

Data analysis

The peak area ratio was calculated for each oligosaccharide chart obtained by HPLC on the ODS-silica column. This ratio was compared between children with IgAN and healthy children, and between adults with IgAN and

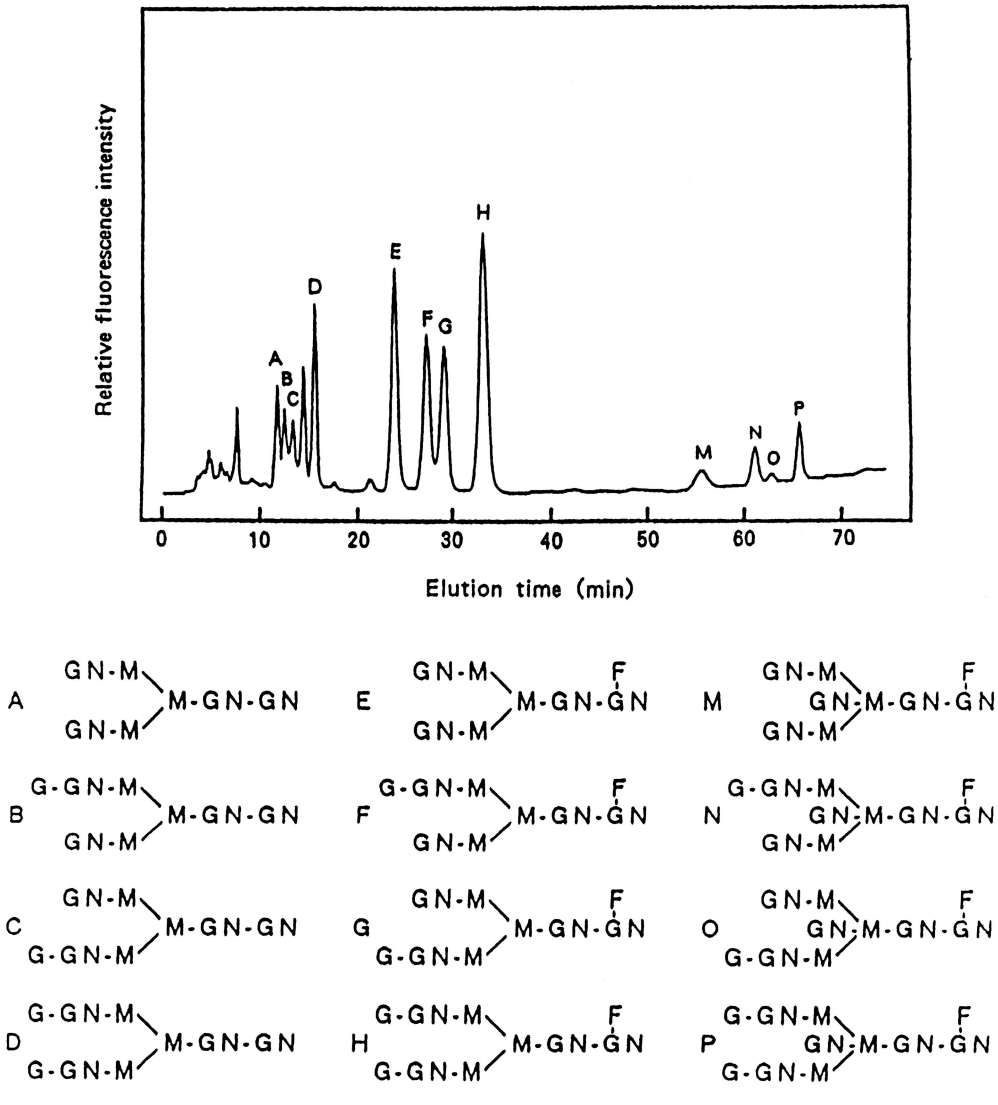

Fig. 1. Typical HPLC profile of the pyridylamino (PA) derivatives of IgG oligosaccharides purified from healthy adults. Oligosaccharides were separated into 12 fractions and classfied into three groups. group I, biantennary without fucose (*peaks A*, *B*, *C*, *and D*); group II, biantennary with fucose (*peaks E*, *F*, *G*, *and H*); and group III, biantennary with fucose and bisecting GlcNAc (*peaks M*, *N*, *O*, *and P*). *G*, galactose; *M*, mannose; *F*, fucose; *GN*, N-acetylglucosamine

healthy adults. The Mann-Whitney *U*-test was used to test the significance of differences. We used Pearson's correlation coefficient to analyze the correlation between the peak area ratio and the clinical data. $P < 0.05$ was regarded as significant.

Results

Figure 1 shows a typical HPLC profile of PA derivatives of IgG oligosaccharides purified from healthy adults. Oligosaccharides were separated into 12 fractions and classfied into three groups as shown in Fig. 1: group I (peaks A, B, C, and D), group II (peaks E, F, G, and H), and group III (peaks M, N, O, and P), which corresponded to the following oligosaccharides: biantennary without fucose (F) (group I), biantennary with F (group II), and biantennary with F and bisecting GlcNAc (GN) (group III). The volume in groups I and III was less than that in group II. Peaks I, J, K, and L (without F and with bisecting GN) were scarcely observed in human IgG.

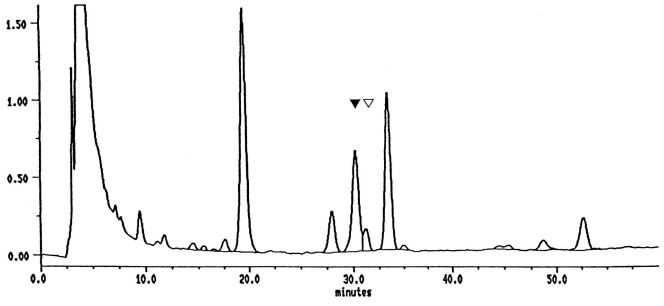

Fig. 2. HPLC profile of the PA derivatives of IgG oligosaccharides purified from patient with IgA nephropathy (IgAN). The ratio of the peak G(*open triangle*)/F(*closed triangle*) was significantly lower in the presence of IgAN than in healthy subjects

Peaks F and G are important for distinguishing IgA patients from healthy subjects. In healthy subjects, the ratio of the peak G/F (the peak area ratio of isomers with different Gal (G)-GlcNAc (GN) binding sites) was a small quantity, less than equal. In both children and adults, the ratio of the peak G/F was significantly lower in the presence of IgAN (0.425 ± 0.149 in children, 0.433 ± 0.089 in adults) than in healthy subjects (0.556 ± 0.196 in children, 0.672 ± 0.236 in adults; Fig. 2; $P < 0.05$ in children (Fig. 3) and $P < 0.001$ in adults (Fig. 4). The ratios of the peak G/F were 0.536 and 0.619 in patients with MPGN, 0.559 in the patient with MN, and 0.632, and 0.665 in the patients with FGS. That is, in these other primary renal diseases the ratio of the peak G/F tended to be closer that in healthy subjects than IgAN.

The ratio of Gal(G)-free oligosaccharide (peak E) to Gal(G)-positive oligosaccharides (peaks F, G, and H) [Gal(–)/Gal(+) ratio] did not differ between patients with IgAN and healthy subjects, either in children or in adults (Figs. 3 and 4).

The other ratio, of bisecting GlcNAc(GN)-positive oligosaccharide (group III; peaks M, N, 0, and P) to bisecting GlcNAc(GN)-free oligosaccharides (peaks E, F, G, and H; group II) did not differ between patients with IgAN and healthy subjects (data not shown).

Tables 1 and 2 show the relationship between the clinical data (serum IgG and IgA, urinary protein, serum creatinine, blood urea nitrogen, and creatinine clearance) in IgAN patients and the ratio of the peak G/F. Neither children nor adults with IgAN showed any particular relationship between the ratio of the peak G/F and the clinical data (in childen, $r = -0.406$; $P = 0.095$ for serum IgG; $r = -0.192$; $P = 0.451$ for serum IgA; $r = 0.283$; $P = 0.260$ for urinary protein; $r = 0.109$; $P = 0.672$ for serum creatinine; $r = -0.020$; $P = 0.940$ for blood urea nitrogen; and $r = 0.203$; $P = 0.425$ for creatinine clearance; in adults, $r = 0.456$; $P = 0.088$ for serum IgG; $r = -0.439$; $P = 0.103$ for serum IgA; $r = 0.257$; $P = 0.431$ for urinary protein; $r = 0.450$; $P = 0.092$ for serum

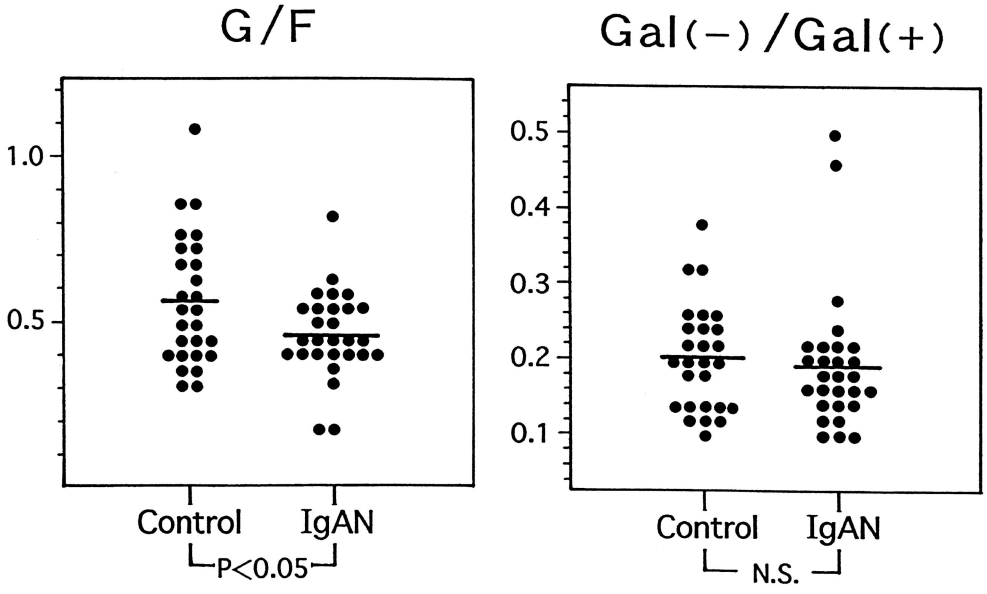

Fig. 3. In children, the ratio of the peak G/F was significantly lower in the presence of IgAN than in healthy subjects ($P < 0.05$), while the ratio of Gal(G)-free oligosaccharides (peak E) to Gal(G)-positive oligosaccharides (peaks F, G, and H) [*Gal*(–)/*Gal*(+) *ratio*] did not differ between patients with IgAN and healthy subjects. *N.S.*, not significant

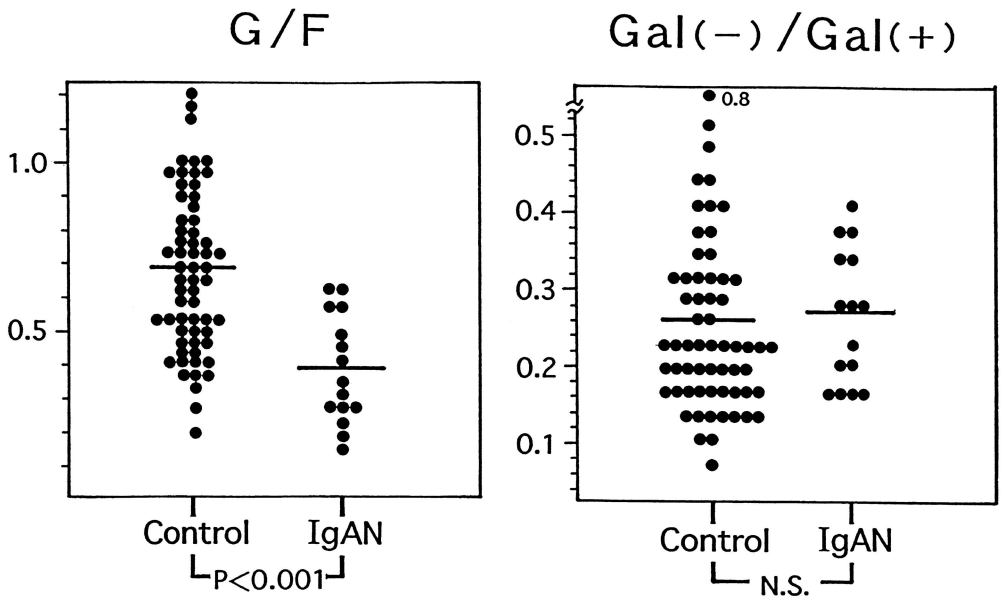

Fig. 4. In adults, the ratio of the peak G/F was significantly lower in the presence of IgAN than in healthy subjects ($P < 0.001$), while the ratio of Gal(G)-free oligosaccharide (peak E) to Gal(G)-positive oligosaccharides (peaks F, G, and H) [*Gal*(−)/*Gal*(+) *ratio*] did not differ between patients with IgAN and normal subjects

Table 1. Relationship between the clinical data of children with IgAN and the peak area ratios of G/F and G(−)/G(+)

Patient no	S-IgG (mg/dl)	S-IgA (mg/dl)	U-protein (g/day)	Cre (mg/dl)	BUN (mg/dl)	Ccr (ml/min)	G/F	G(−)/G(+)
1	948	229	1.05	0.3	15	74.0	0.791	0.462
2	1490	328	0.21	0.5	6	79.7	0.408	0.111
3	757	454	1.04	0.6	12	68.3	0.561	0.096
4	913	239	0.13	3.9	16	75.3	0.567	0.206
5	1110	254	0	0.8	9	59.1	0.149	0.104
6	1134	269	0.54	0.6	8	71.0	0.379	0.219
7	1225	225	0.9	0.5	9	89.8	0.375	0.211
8	1598	672	0.43	0.8	21	58.6	0.351	0.141
9	1298	200	0.79	0.7	11	94.4	0.387	0.215
10	1090	197	0.22	0.7	19	67.2	0.295	0.161
11	855	245	1.43	1.0	18	77.0	0.413	0.173
12	1140	226	0.12	0.1	8	91.8	0.554	0.266
13	1440	252	0.16	0.5	12	95.3	0.403	0.229
14	1120	224	0.16	0.5	14	96.4	0.398	0.189
15	1380	523	0.34	0.5	15	72.8	0.176	0.088
16	1020	334	0	0.6	10	84.9	0.550	0.181
17	1490	332	0.13	0.4	13	109.3	0.419	0.504
18	1470	379	0.13	0.3	13	82.4	0.476	0.143

S-IgG, serum-IgG; S-IgA, serum-IgA; U-protein, urinary protein; Cre, creatinine; BUN, blood urea nitrogen; Ccr, creatinine clearance

Table 2. Relationship between the clinical data of adults with IgAN and the peak area ratios of G/F and G(−)/G(+)

Patient no	S-IgG (mg/dl)	S-IgA (mg/dl)	U-protein (g/day)	Cre (mg/dl)	BUN (mg/dl)	Ccr (ml/min)	G/F	G(−)/G(+)
1	1801	251	1.69	0.8	11	140.2	0.532	0.397
2	835	354		0.7	15		0.442	0.204
3	1501	214	0.35	0.7	10		0.376	0.181
4	767	326	2.45	1.8	19	51.0	0.499	0.158
5	1225	344	2.08	1.1	21	44.2	0.398	0.171
6	1552	295	1.07	2.1	26	32.0	0.533	0.170
7	1293	520	0.41	0.8	10	119.0	0.365	0.371
8	813	382	0.85	0.8	7	91.0	0.391	0.208
9	1540	180	0.77	1.3	11	58.0	0.477	0.350
10	1268	423	0.36	1.3	20	55.9	0.319	0.292
11	1776	249	1.26	2.2	42	20.5	0.567	0.276
12	1097	453	0.27	0.6	7	74.1	0.316	0.268
13	622	196	3.00	1.0	17	80.0	0.340	0.222
14	1189	294		1.1	18	139.3	0.379	0.337
15	1198	295		0.8	13		0.563	0.330

Blank spaces indicate no data

creatinine; $r = 0.437$; $P = 0.104$ for blood urea nitrogen; and $r = -0.295$; $P = 0.362$ for creatinine clearance).

Discussion

Recently it has been worthy of notice that the oligosaccharide structure of serum IgAl in patients with IgAN differs from that in healthy subjects. Mestecky et al.[5] have reported that a decrease in the amount of Gal contained in the oligosaccharides bound to the hinge portion of IgAl plays an important role in the metabolism of IgAl molecules and their glomerular deposition. Hiki et al.[6] found an increase of asialo type oligosaccharides in IgAl isolated from the sera of patients with IgAN. Also, Hashim et al.[11] have reported that the O-linked oligosaccharide moieties of serum IgA1 in patients with IgAN are generally lacking in galactose and sialic acid residues. It is considered that galactose and its associated sialic acid are reduced because of a defect in β-1,3-galactosyltransferase activity in IgAN.[12]

Patients with IgAN sometimes have high-electron-density deposits, not only in the mesangium but also in the subepithelial and subendothelial tissue. In patients showing subendothelial deposits, the IgG deposited on the wall of Henle's loop is reported to increase the activity level of IgAN. This suggests that IgG is closely related to IgAN. However, few studies have examined serum IgG in patients with IgAN. None of the studies published to date referred to the oligosaccharide structure of IgG found in these patients. Based on the view that serum IgG has been altered in patients with IgAN, we paid attention to the oligosaccharide structure of serum IgG and analyzed it, using HPLC developed by Takahashi et al.[10]

A number of diseases which involve altered oligosaccharide structures of serum IgG have been discovered. Chronic inflammatory diseases such as rheumatoid arthritis (RA) have been found to show a decrease in galactose. This is because an increase in serum IgG causes a decrease of the percentage of Gal-bound IgG.[13] The galactose deficiency of serum IgG in patients with RA is considered to result from the altered β-1,4-galactosyltransferase activity in IgG-producing B cells.[14] The extent of the changes correlates with the severity of the disease, and the level of an autoantibody to galactose-deficient IgG is a good biochemical marker, clinically, for RA.[15] Apart from that in RA, increased expression of agalactosyl IgG has also been reported in patients with tuberculosis,[16] Crohn's disease,[17] juvenile RA,[18] Sjögren's syndrome,[18] and myotonic dystrophy.[19]

In the present study, the G/F ratio in the serum was lower in patients with IgAN than in healthy subjects; that is, the percentage of isomers with altered Gal-GlcNAc binding sites differed between these two groups. This finding was noted in both children and adults, and no other diseases showing a reduced G/F ratio have been reported. Therefore, it appears to be specific to this disease.

The mechanism by which this ratio decreases seems to involve IgG subclasses. The G/F ratio for IgG1 and IgG4 is lower, and the G/F for IgG2 and IgG3 is higher than that of polyclonal IgG.[20] Therefore, the percentage of IgG1 and IgG4 is higher or that of IgG2 and IgG3 is lower in the presence of IgAN. In adult IgAN, IgG1 and IgG2 were significantly lower than in controls.[21] Human serum IgG3 binds preferentially to IgA1 in IgAN,[22] and this binding is possibly related to the change in the G/F ratio. Other possible mechanisms underlying the change in the G/F ratio include changes in galactosyltransferase activity similar to IgA1 glycosylation in IgAN or IgG glycosylation in RA, and changes in the metabolism of IgG due to altered Gal-GlcNAc binding sites, although none of these mechanisms have been established.

The relationship between the G/F ratio and renal histology, and the prognosis of IgAN, requires further investigation.

In conclusion, analysis of the oligosaccharide structure of serum IgG seems to be useful in assisting with the diagnosis of IgAN.

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