A new monoclonal antibody for detecting degalactosylated IgA1 as serum biomarker of IgA nephropathy

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The hallmark of IgA nephropathy (IgAN) is the prevalence of IgA over the other immunoglobulins in glomerular deposits [1]. In IgAN the deposited IgA is mostly of IgA1 subclass and presents with altered gycosylation [2–4].

In addition to the N-linked oligosaccharides typically present at the carboxyl terminus of all classes of immunoglobulins heavy chain, IgA1 contains short O-linked oligosaccharide chains. These glycans are coupled to serine and/or threonine residues present in an insertion of 18 aminoacids in the hinge region connecting the immunoglobulin CH1 and CH2 domains, which is selectively present in IgA1 subclass [5]. The O-glycans consist of a core N-acetyl galactosamine (GalNAc) which occurs alone or extended with β1,3 linked galactose (Gal) or further covered with sialic acid (Neu5Ac) in α2, 3 and/or α2, 6 linkage. In healthy subjects, serum IgA1 consists of a mixture of molecules with different O-glycoforms, whereas in patients with IgAN there is high frequency of galactose–deficient O-glycans (Gd-IgA1) consisting of terminal GalNAc or sialylated GalNAc [6–8].

Gd-IgA1 can react with various antigens and form immune complexes which are deposited in glomeruli of patients with IgAN and activate resident mesangial cells stimulating proliferation and extracellular matrix production, finally leading to glomerular damage [9]. Our group previously reported that IgA glycoforms isolated from patients with IgAN or in vitro prepared Gd-IgA1 IgA can fix complement in fluid phase and act on mesangial cells regulating integrin expression [10] with enhancement of inducible nitric oxide synthase activity, decrease endothelial growth factor synthesis, meanwhile depressing proliferation and increasing apoptosis [11, 12]. Oxidative stress is activated in patients with IgAN in correlation with high levels of Gd-IgA1 [13]. Moreover, we recently found that the supernatants of mesangial cells cultured with IgA1 glycoforms isolated from patients with IgAN are able to activate—via tumour necrosis factor (TNF) production—cultured human podocytes to release mediators potentially favouring proteinuria, including platelet aggregating factor (PAF) [14].

Gd-IgA1 has been reported to be a biomarker of IgAN with a sensitivity of 75% and specificity of 90% [15]. However, high levels of Gd-IgA1 were found also in healthy relatives of familiar and sporadic cases of IgAN [16], suggesting the role of additional co-factors. IgAN is presently considered a multifactorial or ‘complex’ disease in which one or more genes [17, 18], probably in combination with environmental factors, may be responsible for the onset of the disease and in which there is a role for autoimmunity [19]. The formation of Gd-IgA1 is thought to be the first hit. Gd-IgA1 can circulate in monomeric form or participate in macromolecular self-aggregates, or react with antigens and form IgA containing circulating immune complexes. Moreover, Gd-IgA1 elicits an IgG autoimmune response. IgG antibodies were found to recognize GalNAc-containing epitopes on the galactose-deficient hinge region of IgA1, forming mixed IgG/IgA1 immune complexes [20]. Macromolecular Gd-IgA1 or IgG/Gd-IgA1 escape the clearance by hepatic receptors and have a preferential renal deposition due to reactivity with mesangial matrix components [21]. Serum levels of IgG/IgA1 were found to correlate with disease activity and progression in adults and in children with IgAN [22]. It is of interest that these IgG may cross react with bacterial or viral cell-surface GalNAc-containing glycoproteins present on pathogens and cross-react with the GalNAc residues exposed on Gd-IgA1Gal deficient O-glycans detectable in IgAN [23].

The chain of events summarized above, including the production of IgG autoantibody and the effects on mesangial cell proliferation and apoptosis leading to glomerular damage has in any case one clear starting point, i.e. production and circulation of high levels of Gd-IgA1, which is considered the frontline biomarker of IgAN [24].

The most used test to detect Gd-IgA1 is based on lectin binding activity to specific lectins—such as helix aspersa (HAA), helix pomatia (HPA) or vicia villosa (VV)—naturally occurring molecules with high carbohydrate binding capacity, with specificity for GalNAc residues [25]. We isolated different glycoforms of IgA in sera from patients and controls by affinity
chromatography using lectins immobilized on Sepharose. The eluted peaks were fractionated in HPLC to isolate 250–400 kDa glycoproteins [10]. IgAN patients showed increased levels of IgA glycoforms with higher affinity for glycine maximus (SBA), indicating increased exposure of GalNAc and increased binding to Limulus polyphemus (LPA), suggesting increased exposure of sialic acid bound to GalNAc (Neu5Ac-α2,6GalNAc).

The most used lectin to detect increased GalNAc exposed residues on the IgA1 surface is HHA, isolated from snail agglutinin and purified by affinity chromatography. HAA has been used in several researches [15, 26] providing interesting results, however its bioactivity and stability is variable depending on the batch used. This limitation reduces the possibility of comparing results from different laboratories.

In this issue of NDT, Yasutake et al. report the production of a new Gd-IgA1-specific monoclonal antibody (KM55), which was obtained after immunizing rats with human galactose-modified IgA1 hinge region peptide. Using this monoclonal antibody the authors established a new ELISA method for Gd-IgA1 measurement. The test provided significantly higher values of Gd-IgA1 in patients with IgAN in respect to other renal and non-renal diseases. Moreover, KM55 antibody detected IgA glomerular deposits in patients with IgAN. The results provided by this paper support the use of this new monoclonal antibody for clinical use to measure serum levels of Gd-IgA1 by the described ELISA.

However, even though the technique appears to have solid technical ground, the major limitation for clinical application is that Gd-IgA1 can be detected by this ELISA also in healthy subjects as well as in other renal and non-renal disease. Trying to establish the optimal cut-off for upper normal levels, the authors defined as abnormal values those above the maximum value found in the cohort with other renal diseases. In 27.6% only of patients with IgAN this ‘upper normal limit’ of Gd-IgA1 was exceeded. Hence, the value of this test to screen patients with IgAN in order not to proceed to renal biopsy for diagnostic purposes, which was one of the aims of the study, is rather limited and false negative results are expected. However, this work has to be considered as a preliminary report, being performed in a limited number of patients (152 samples) in a cross-sectional analysis, hence it needs validation in large cohorts of patients before any treatment, and during the subsequent follow-up, in different phases of clinical activity.

Apart from these limitations, the paper from Yasutake et al. provides a technically relevant advancement in detecting Gd-IgA1, offering a test which will allows a valuable comparison of data among different series of patients on different continents, which was limited in the past due to the variability of the natural reagents (lectins from naturally variable sources). We expect interesting data from the application of this new ELISA in worldwide cohorts of patients with IgAN.

CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES


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