



**СРПСКИ АРХИВ**  
ЗА ЦЕЛОКУПНО ЛЕКАРСТВО  
**SERBIAN ARCHIVES**  
OF MEDICINE

Address: 1 Kraljice Natalije Street, Belgrade 11000, Serbia

+381 11 4092 776, Fax: +381 11 3348 653

E-mail: [office@srpskiarhiv.rs](mailto:office@srpskiarhiv.rs), Web address: [www.srpskiarhiv.rs](http://www.srpskiarhiv.rs)

**Paper Accepted\***

**ISSN Online 2406-0895**

**Original Article / Оригинални рад**

Dejana Kosanović<sup>1</sup>, Marija Vukadinović<sup>1</sup>, Biljana Ristić<sup>1</sup>, Marijana Kovačić<sup>1</sup>,  
Milan Prodanović<sup>2</sup>, Olivera Savić<sup>3</sup>, Rajna Minić<sup>2</sup>, Irina Maslovarić<sup>1</sup>, Dragana Marković<sup>1</sup>,  
Ivana Drvenica<sup>1</sup>, Vesna Ilić<sup>1,\*</sup>

**Expression of terminal galactose and sialic acid on serum IgA in  
IgA multiple myeloma**

Експресија терминалне галактозе и сијалинске киселине на серумским IgA у  
мултиплом мијелому IgA типа

<sup>1</sup>University of Belgrade, National Institute of Republic of Serbia, Institute for Medical Research, Belgrade, Serbia;

<sup>2</sup>Torlak Institute of Virology, Vaccines and Sera, Belgrade, Serbia;

<sup>3</sup>Institute for Blood Transfusion of Serbia, Belgrade, Serbia

**Received: April 8, 2025**

**Revised: July 20, 2025**

**Accepted: July 21, 2025**

**Online First: July 22, 2025**

**DOI: <https://doi.org/10.2298/SARH250408057K>**

\* **Accepted papers** are articles in press that have gone through due peer review process and have been accepted for publication by the Editorial Board of the *Serbian Archives of Medicine*. They have not yet been copy-edited and/or formatted in the publication house style, and the text may be changed before the final publication.

Although accepted papers do not yet have all the accompanying bibliographic details available, they can already be cited using the year of online publication and the DOI, as follows: the author's last name and initial of the first name, article title, journal title, online first publication month and year, and the DOI; e.g.: Petrović P, Jovanović J. The title of the article. *Srp Arh Celok Lek*. Online First, February 2017.

When the final article is assigned to volumes/issues of the journal, the Article in Press version will be removed and the final version will appear in the associated published volumes/issues of the journal. The date the article was made available online first will be carried over.

**\*Correspondence to:**

Vesna ILIĆ

University of Belgrade, Institute for Medical Research, Dr Subotića 4, 11129 Belgrade, Serbia

[vesnai@imi.bg.ac.rs](mailto:vesnai@imi.bg.ac.rs)

## Expression of terminal galactose and sialic acid on serum IgA in IgA multiple myeloma

Експресија терминалне галактозе и сијалинске киселине на серумским IgA у мултиплом мијелому IgA типа

### SUMMARY

**Introduction/Objective** IgA multiple myeloma has a poor prognosis, and altered glycosylation of myeloma IgA may be one of contributing factors. This study examined the expression of terminal galactose and sialic acid (SA) on serum IgA oligosaccharides in patients with IgA myeloma, compared to healthy control sera.

**Methods** Serum samples from 15 IgA myeloma patients and pooled serum from 100 healthy donors were analyzed. IgA was purified using peptide M affinity chromatography. Terminal galactose and SA expression on isolated IgA was analyzed by RCA I and SNA lectin blotting.

**Results** IgA heavy chains from both healthy individuals and all myeloma patients expressed galactose. SA was present in healthy control and in 14 out of 15 myeloma patients. Compared to controls, myeloma IgA showed 12–63% a reduction in galactose and a 67–97% reduction in SA expression on heavy chains. Notable galactosylation of IgA light chains was observed in only 3, while weak SA expression was seen in 14 myeloma cases. Healthy IgA was predominantly monomeric and expressed both galactose and SA. Myeloma IgA existed in both and polymeric forms of myeloma expressing detectable galactose level, though with different expression levels among individuals. At the same time SA was undetectable.

**Conclusion** The results of this study showed altered glycosylation of myeloma IgA. Compared to healthy control, myeloma IgA heavy chains expressed reduced terminal galactose and sialic acid. Notable galactosylation of light chains was observed in three cases. Unlike sialic acid, galactose was detectable on intact monomeric and polymeric multiple myeloma IgA.

**Keywords:** multiple myeloma; IgA; glycosylation; sialic acid; galactose

### САЖЕТАК

**Увод/Циљ** Сматра се да мање повољна прогноза IgA мултиплог мијелом у односу на друге форме мијелом може бити последица и промењене гликозилације IgA у овој болести. Анализирана је експресија терминалне галактозе и сијалинске киселине на гликанима IgA изолованим из серума болесника са мијеломом и упоредили експресију ова два моносахарида на мијеломским IgA са њиховом експресијом на IgA серума здравих особа.

**Метод** IgA су изоловани из серума 15 особа са IgA мијеломом и збирног узорка серума 100 здравих особа афинитетном хроматографијом на пептиду М. Експресија терминалане галактозе и сијалинске киселине је анализирана лектинским блотом, на основу везивања RCA I и SNA лектина.

**Резултати** Тешки ланци серумских IgA здравих особа и свих 15 особа са мијеломом су експримирали галактозу, док је сијалинска киселина детектована код здравих особа и 14 особа са мијеломом. Експресија галактозе и сијалинске киселине на тешким ланцима мијеломских IgA је била за 12–63% и 67–97% нижа у односу на њихову експресију на IgA здравих особа. Снажна галактозилација лакких ланаца детектована је само код три мијеломска IgA, док је слаба експресија сијалинске киселине на лаким ланцима детектована код 14 од 15 мијеломских IgA. IgA молекули здравих особа су били преобладајуће у форми мономера и експримирали су и галактозу и сијалинску киселину. Мијеломски IgA су били присутни у форми и мономера и полимера. Обе форме су експримирале детектабилни ниво галактозе (иако је ниво експресије варирао међу узорцима) али не и сијалинске киселине.

**Закључци** Гликозилација IgA измењена. Тешки ланци IgA у мултиплом мијелому имају нижу експресију и галактозе и сијалинске киселине, док су лаки ланци појединих мијеломских IgA снажно галактозиловани. Галактоза, али не и сијалинска киселина, су присутне у детектабилном нивоу на интактним мономерним и полимерним мијеломским IgA.

**Кључне речи:** мултипли мијелом; IgA; гликозилација; сијалинска киселина; галактоза

## INTRODUCTION

Multiple myeloma is an immunoproliferative disease characterized by the accumulation of malignant plasma cells in the bone marrow. The presence and the level of monoclonal immunoglobulins - known as M-proteins or paraproteins - in serum and urine are related to the outcome of patients with myeloma. IgA paraprotein occurs in up to 20% of myeloma cases [1]. Although IgA myeloma is rarer than IgG, the clinical course is more aggressive with a prognostically less favorable outcome compared to IgG isotype [1].

Immunoglobulins are glycoproteins, and the presence of oligosaccharides of Fc region is essential in regulating the immune response [2]. IgA is one of the most heavily glycosylated antibodies. Unlike IgG, which possesses a single conserved N-glycosylation site, IgA is characterized by multiple N-glycosylation sites. Additionally, IgA1 possesses nine potential O-glycosylation sites located in its hinge region. To date, 16 N-linked glycan structures on IgA, exhibiting significant heterogeneity due to variations in terminal galactose and sialic acid content, have been identified [3, 4].

The IgA glycans have been shown to have a significant effect on its immune function [5]. Paraproteins may have an altered carbohydrate profile compared to normal serum immunoglobulins of the same isotype. Renfrow et al. [5, 6] showed that the decrease in galactose content of IgA1 subclass myeloma is the result of aberrant galactosylation of O-linked glycans in the hinge region. Additionally, Bosseboeuf et al. [7] showed that sialic acid expression in myeloma IgA was reduced compared to healthy individuals. However, the location of sialylated oligosaccharides on IgA molecules was not analyzed in this study. Some of the complications in IgA myeloma may be linked to the structural characteristics of monoclonal IgA, including the expression of glycans on their  $\alpha$  heavy chains of both monomeric and polymeric forms of IgA. Given the importance of pathogenic potential of

immunoglobulin glycans [2], the primary aim of the study was to assess the expression of terminal galactose and sialic acid on heavy and light chains of IgA molecules isolated from sera of multiple myeloma patients and to compare it with the expression of these monosaccharides on IgA from healthy individuals.

## METHODS

### Serum samples

Pooled serum samples of 100 healthy blood donors from the Institute of Transfusion and Haemobiology, Military Medical Academy were used in this study. Ethical approval (No. 17/2020) was granted by the Military Medical Academy Ethics Committee. Serum from 15 individuals with multiple myeloma (*C90.0 - myeloma multiplex*) was used with ethical approval (No. 213/23) from the Institute for Blood Transfusion of Serbia Ethics Committee.

### Paraprotein detection and the isotype analysis

Serum samples from multiple myeloma patients were analyzed during diagnostics at the Institute for Blood Transfusion of Serbia. The presence of paraproteins was confirmed by serum protein agarose gel electrophoresis [8] and immunochemically identified by immunoelectrophoresis [9]. The concentrations of IgG, IgA, and IgM in myeloma sera were determined by immunonephelometry on the Siemens BN ProSpec System analyzer. The subclass of IgA paraproteins was determined by dot blot at the Institute for Medical Research [10].

## Isolation of IgA

Myeloma patients and healthy donors' serum IgA was isolated by affinity chromatography on peptide M agarose (InvivoGen, San Diego, CA) [11]. Chromatography was performed according to the manufacturer's instruction. Protein concentration was determined with the NanoPhotometer P-330 (Implen GmbH, Germany).

## Western and lectin blot

Vertical SDS-PAGE, under both non-reducing and reducing conditions of 0.5 µg isolated IgA was performed on a SE 260 Mighty Small II Vertical Unit, as described in [12]. Proteins on gels were visualized by Coomassie brilliant blue (CBB) staining or transferred to nitrocellulose membrane using a semi-wet transfer method (Hoefer TE70X; Hoefer Inc. Holliston, MA). The transfer efficacy was verified by Ponceau S staining.

Polyclonal goat anti-human IgA antibodies conjugated with peroxidase (Boster Biological Technology Co. Ltd, Pleasanton, CA) were used to detect affinity-isolated IgA. Galactose and sialic acid expression on IgA molecules were examined by lectin blotting with *Ricinus communis* agglutinin I (RCA I) and *Sambucus nigra* agglutinin (SNA) both purchased from Vector Laboratories, Newark, CA.

Nitrocellulose membranes were incubated in 3% BSA in TBS-Tween to block nonspecific binding. For Western blotting the membranes were incubated with 10,000x diluted anti-human IgA antibody. After the washing with TBS-Tween, the bands were visualized by chemiluminescence and imaged with ChemiDoc 2.0 (Bio-Rad, Hercules, CA). For lectin blotting, membranes were incubated with RCA I or SNA lectins (1 µg/ml and 2.5 µg/ml, respectively), followed by incubation with 1,000-time diluted avidin-peroxidase (Vector

Laboratories, Newark, CA). The chemiluminescence intensity from both western and lectin blotting was quantified with Image Master Total Lab software (GE HealthCare).

Sugar specificity of RCA I or SNA lectins was confirmed by inhibitory dot blot in the presence of 200 mM galactose and 400 mM N-acetylneuraminic acid (sialic acid). The concentration of inhibitory sugars was recommended by the manufacturer (Vector Laboratories, Newark, CA).

**Ethics:** The investigations were approved by the the Military Medical Academy Ethics Committee (No. 17/2020) and the Institute for Blood Transfusion of Serbia Ethics Committee (No. 213/23)

## RESULTS

### **Expression of galactose and sialic acid on total IgA isolated from the serum of healthy individuals.**

In this study we first analyzed glycosylation of IgA isolated from a pooled serum sample of 100 healthy individuals, using affinity chromatography on peptide M agarose. Given the significant heterogeneity of immunoglobulin molecules and substantial individual variations in glycan expression on them [2,3], this analysis of pooled serum samples provides more reliable information about the structure of immunoglobulin molecules in a particular population and minimizes physiological variations that may arise in a small subset of healthy individuals.

Following SDS-PAGE under non-reducing conditions we observed a 160 kDa anti-human IgA antibody reactive protein, corresponding to IgA monomer (Figure 1.A1-2). After SDS-PAGE under reducing conditions, proteins of 60 kDa and 25 kDa, corresponding to IgA  $\alpha$  heavy and light chains, were detected (Figure 1.B1). The presence of IgA  $\alpha$  heavy chain was confirmed

by Western blot with anti-human IgA antibodies (Figure 1.B2).

The lectin blotting showed that isolated IgA monomer expressed both galactose and sialic acid (Figure 1.A3-4), with expression primarily localized on the  $\alpha$  heavy chains (Figure 1.B3-4). The expression of these two sugars on proteins corresponding to IgA light chains was detectable, but weak. Besides IgA, “additional” RCA I or SNA reactive glycoproteins were detected in healthy human serum IgA isolate (Figures 1.A3; 1.B2-B4). These proteins, which might represent serum proteins in complexes with IgA or nonspecifically bound proteins, were not further analyzed.

#### **Expression of galactose and sialic acid on total IgA isolated from the serum of individuals with IgA multiple myeloma**

We analyzed galactose and sialic acid expression on IgA molecules isolated from 15 myeloma serum samples with confirmed IgA paraproteins. The patients' age, serum concentrations of  $\beta$ 2-microglobulin, total serum IgA, IgM, and IgG, and the ratio of  $\kappa$  and  $\lambda$  light chains are given in Table 1. The concentration of IgA represents the sum of the concentrations of IgA paraproteins (i.e., monoclonal IgA) and residual polyclonal IgA. All 15 IgA paraproteins belonged to the IgA1 subclass.

We analyzed total, polyclonal and monoclonal, IgA molecules isolated by affinity chromatography from the serum of individuals with IgA multiple myeloma. Additional purification steps to separate monoclonal from most of residual polyclonal IgA molecules were not performed. SDS-PAGE under reducing conditions confirmed the presence of two protein bands of 60 kDa and 25 kDa, corresponded to IgA heavy and light chains (Figure 2A). Western blot confirmed the fraction of 60 kDa as IgA heavy chain (Figure 2B), and RCA I lectin blotting

showed strong reaction with these heavy chains (Figure 2C). Conversely, SNA reaction, i.e. sialic acid expression, was weak and detected in 14 out of 15 isolates (Figure 2D). Light chains reacted strongly with RCA I in only three out of 15 isolated IgA, indicating that the expression of terminal galactose on 12 remaining IgA light chains was below the detection limit of the method applied in our study. The results of the lectin blotting performed with SNA showed low, but detectable level of sialic acid on heavy chains in 14 and on light chains in 14 out of 15 IgA isolates. The specificity of RCA I and SNA was tested with an inhibitory dot-blot (Figure 3), confirming that the observed RCA I and SNA reactivity originate from binding to terminal galactose and sialic acid on IgA oligosaccharides.

Densitometric analysis showed significant individual variations in galactose and sialic acid expression. The sialic acid expression levels were 67–97% lower in myeloma IgA compared to healthy individuals. Similarly, galactose expression was reduced by 12–63% in 13 out of 15 analyzed myeloma IgA heavy chains relative to healthy controls (Figure 4). Compared to IgA from healthy individuals, the sialic acid to galactose expression ratio was lower on all analyzed myeloma IgA heavy chains (Figure 4).

In contrast to IgA from pooled serum of healthy individuals (Figure 1A1), sera from individuals with multiple myeloma were separated into two or more fractions after SDS-PAGE under non-reducing conditions, one with a molecular weight of 160 kDa, and other(s) with higher molecular weights. (Figure 5). RCA I lectin blotting demonstrated that both monomeric and polymeric forms of myeloma IgA expressed galactose. The intensity of expression varies depending on the sample (Figure 4C,4D). Sialic acid expression on either monomeric or polymeric forms of myeloma IgA was below the detection limit of SNA lectin blot performed after SDS-PAGE under non-reducing conditions.



## DISCUSSION

We analyzed the expression of galactose and sialic acid on IgA molecules from 15 serum samples of myeloma patients with IgA paraproteins, all of which were of IgA1 subclass. The frequency of IgA2 paraproteins is lower than expected based on their concentration in healthy sera [13], so the absence of IgA2 paraproteins in our study was not unexpected. In 13 out of the 15 analyzed sera the concentration of total IgA was higher than for individuals of similar age [14]. Immunoparesis, a hallmark of multiple myeloma characterized by the suppression of immunoglobulin isotypes different from the paraprotein isotype [15], was also observed.

For the purpose of analyzing the expression of galactose and sialic acid on IgA molecules, IgA was isolated from the pooled healthy human serum and sera of IgA myeloma patients using affinity chromatography on peptide M agarose. Peptide M binds with high affinity monomeric and dimeric human IgA serum, secretory IgA, both IgA1 and IgA2, and IgA in complexes with antigens [11] allowing isolation of all molecular forms of IgA molecules. With Peptide M affinity chromatography, we isolated both monoclonal and residual polyclonal IgA from myeloma serum samples. Additional purification steps for separation of monoclonal from most residual polyclonal IgA molecules, as described in [7], were not performed in this study although we are aware that, for IgG myeloma was shown that the level of sialylation on residual polyclonal serum IgG might be higher than on IgG paraprotein [16].

The results of our study showed intense reaction between the heavy chains of healthy IgA molecules and RCA I and SNA indicating a notable expression of galactose and sialic acid. All 15 analyzed myeloma IgA heavy chains reacted with RCA I and 14 of them reacted with SNA but densitometric analysis showed lower expression of both galactose and sialic acid on heavy chains of myeloma IgA than on healthy IgA. Bosseboeuf et al. [7] also showed a reduced level of sialic acid in IgA of myeloma patients. Ding et al. [3] suggested that hyposialylation of IgA

in multiple myeloma might affect the binding efficiency of IgA to FcαRI, consequently affecting IgA effector functions. Moreover, enzymatic desialylation of IgA1 of healthy people leads to proinflammatory profile of IgA1 [17]. It has long been known that reduced expression of galactose on O-linked glycans in the hinge region of heavy chain of IgA1 molecules is associated with IgA nephropathy. Renfrow et al. [5, 6] showed that decreased content of galactose in myeloma IgA1 is the result of aberrant galactosylation of O-linked glycans in the hinge region, with (similar to our results) reduced galactose content varying among the analyzed monoclonal IgA1 molecules. It remains unclear whether the immune complexes in IgA myeloma, like in IgA nephropathy [18], are formed between galactose-deficient O-glycans in IgA1 and anti-glycan IgG autoantibodies, leading to their deposition in the glomeruli and subsequent initiation of renal injury.

The expression of galactose and sialic acid on IgA light chains was weak, and most likely reflected the rare carbohydrates immunoglobulin light chains occurring as the consequence of a variable region somatic mutation creating an Asn-[X≠Pro]-Ser/Thr [19] sequence to which carbohydrate group is attached to the asparagine (Asn) residue of this triplet sequence [20]. Whether strong RCA I reactivity observed only on three IgA light chains compared to weak reactivity on other myeloma IgA light chains reflects somatic mutation of glycan binding site within IgA variable (V) regions, or germline-encoded V region sequences containing glycan binding motive is not clear. Independently of their origin, glycosylation of monoclonal immunoglobulin light chains has been indicated as a potential risk for progression of monoclonal gammopathy of undetermined significance (MGUS) to myeloma and immunoglobulin light chain amyloidosis [21].

In this study, we confirmed that serum IgA of healthy individuals predominantly exists in monomeric form [22], with detectable expression of both galactose and sialic acid. In contrast,

myeloma IgA existed in both monomeric and polymeric forms. Although polymeric IgA could represent complexes of IgA with some serum proteins, we consider it unlikely: non-covalent interactions between IgA and these proteins break down in the elution buffer used in affinity chromatography and non-reducing SDS-PAGE, but dimeric and polymeric forms of IgA are linked via J chains by covalent bonds that cannot be broken in the buffers. However,  $\alpha$ 1-antitrypsin covalently bound to IgA molecules forming IgA polymers was described in IgA myeloma sera [23], so the presence of such complexes cannot be entirely excluded. Polymeric forms of IgA are believed to be responsible for hyperviscosity syndrome [24] and represent predominant components of immune complexes detected in circulation and glomerular deposits in IgA nephropathy [25]. A similar role of polymeric IgA in multiple myeloma cannot be excluded. In IgA nephropathy, it has been shown that polymeric IgA significantly reduces the expression of Fc $\alpha$ R on macrophages compared to monomeric IgA. This consequently decreases the binding of IgA1 and IgA1 immune complexes to Fc $\alpha$ R and their degradation in macrophages, resulting in increased circulating levels of these complexes as well as proliferation of mesangial cells and glomerular sclerosis [26]. By RCA I lectin blotting, we demonstrated that both monomeric and polymeric myeloma IgA forms expressed galactose with sample-dependent intensity. Inversely, a very weak sialic acid expression was detected on heavy and light chains, and none on intact monomers or polymers of IgA. This “loss” of sialic acid expression might reflect methodological limitations: non-reducing SDS-PAGE cannot reach the “concentration” effect on proteins that SDS-PAGE under reducing conditions can. Therefore, the extremely small amount of sialic acid detected on separated heavy and light chains, which is now “divided” between the fractions of monomeric and polymeric IgA, may be less accessible to the SNA lectin due to the preserved structure of the complete molecules, and could not be detected by lectin blotting applied.

The pathogenic potential of differently galactosylated monomeric and polymeric myeloma

IgA1 forms remains unclear. In IgA nephropathy, heavy chains of polymeric and monomeric IgA are differently N-glycosylated. Oligomannose-type glycan, an important signaling molecule for the activation of the complement system via the lectin pathway and the induction of inflammation in renal glomeruli, is exclusively present on polymeric IgA [27]. Pathogenic significance of complement activation via lectin pathway in IgA multiple myeloma remains obscure.

This study contributes to our understanding of glycosylation patterns of IgA in multiple myeloma, an area that remains less extensively characterized than IgG glycosylation in both physiological and pathological conditions. Despite the limited number of IgA myeloma samples and lack of clinical stratification by disease stage or treatment, due to the nature of obtaining blood samples (Methods; Serum samples), we demonstrate that galactose and sialic acid expression is markedly reduced on IgA heavy chains from myeloma patients compared to healthy individuals. This finding is aligned with and extending previous reports [5, 6, 7], suggesting that altered glycosylation of myeloma IgA may carry functional relevance. Additionally, we believe that introducing clinical stratification by disease stage or treatment in a small cohort would risk misinterpretation. However, one should be also cautious when interpreting these results since both IgA subclasses are present in the peripheral blood of healthy individuals (90% IgA1 and 10% IgA2) [22] whereas analyzed paraproteins were classified in IgA1 subclass, and the level of residual IgA2 was not determined.

The use of lectin blotting in our analysis enabled us to examine the intact IgA glycoprotein, non-enzymatically digested, providing insights into the steric accessibility and composition of glycans on glycoproteins [28]. While this method does not distinguish between N- and O-linked monosaccharides or localize them to specific regions (e.g., CH2, hinge, or variable regions of IgA heavy chains), it offers a valuable insight into the functional availability of these

sugars for interactions with immunoglobulin receptors or complement proteins, and their effector functions.

Immense heterogeneity of IgA glycoforms [3-4] limits the ability to draw even generalized conclusions from our study or other studies [5-7] performed on relatively small samples number. To provide a more comprehensive understanding of IgA glycosylation in multiple myeloma and pathogenic significance of IgA oligosaccharides, the type of glycans (N- or O linked) and precise binding site of IgA glycans (hinge region, Fc or Fab region), the monosaccharides composition analysis of myeloma IgA oligosaccharides on a notably larger number of IgA isolated from peripheral blood of myeloma patients an advanced glycoproteomics with classical bio- and physicochemical approaches and with incorporated detailed clinical metadata is needed.

## CONCLUSION

Myeloma IgA has altered glycosilation. Lectin blot analysis revealed that, compared to healthy human IgA, the expression of both galactose and sialic acid was reduced on heavy chains myeloma-derived IgA. The light chains of myeloma IgA were predominantly weakly sialylated; however, notable galactosylation of the light chains was observed in approximately one-fifth of the cases. In contrast to the predominantly monomeric IgA found in healthy individuals, myeloma sera contained both monomeric and polymeric forms. Lectin blotting detected galactose on both monomeric and polymeric myeloma IgA, although expression levels varied among patients. The sialic acid, however, was not detectable on either the monomeric or polymeric forms of myeloma IgA.

## ACKNOWLEDGMENT

**Funding:** This work was supported by the Ministry of Science, Technological Development, and Innovations of The Republic of Serbia, (Contract No 451-03-66/2024-03/200015).

**Conflict of interests:** None declared.

Paper accepted

## REFERENCES

1. Visram A, Vaxman I, Saleh ASA, Parmar H, Dispenzieri A, Kapoor P, et al. Disease monitoring with quantitative serum IgA levels provides a more reliable response assessment in multiple myeloma patients. *Leukemia*. 2021;35(5):1428–37. [DOI:10.1038/s41375-021-01180-x] [PMID:33623138]
2. Abduh MS. An overview of multiple myeloma: A monoclonal plasma cell malignancy's diagnosis, management, and treatment modalities. *Saudi J Biol Sci*. 2024;31(2):103920. [DOI:10.1016/j.sjbs.2023.103920] [PMID:38283805].
3. Ding L, Chen X, Cheng H, Zhang T, Li Z. Advances in IgA glycosylation and its correlation with diseases. *Front Chem*. 2022;10:974854. [DOI:10.3389/fchem.2022.974854] [PMID:36238099]
4. Hansen AL, Reily C, Novak J, Renfrow MB. Immunoglobulin A Glycosylation and Its Role in Disease. *Exp Suppl*. 2021;112:433–77. [DOI:10.1007/978-3-030-76912-3\_14] [PMID:34687019]
5. Renfrow MB, Cooper HJ, Tomana M, Kulhavy R, Hiki Y, Toma K, et al. Determination of aberrant O-glycosylation in the IgA1 hinge region by electron capture dissociation fourier transform-ion cyclotron resonance mass spectrometry. *J Biol Chem*. 2005;280(19):19136–45. [DOI:10.1074/jbc.M411368200] [PMID:15728186]
6. Renfrow MB, Mackay CL, Chalmers MJ, Julian BA, Mestecky J, Kilian M, et al. Analysis of O-glycan heterogeneity in IgA1 myeloma proteins by Fourier transform ion cyclotron resonance mass spectrometry: implications for IgA nephropathy. *Anal Bioanal Chem*. 2007;389(5):1397–407. [DOI:10.1007/s00216-007-1500-z] [PMID:17712550]
7. Bosseboeuf A, Seillier C, Mennesson N, Allain-Maillet S, Fourny M, Tallet A, et al. Analysis of the targets and glycosylation of monoclonal IgAs From MGUS and myeloma patients. *Front Immunol*. 2020;11:854. [DOI:10.3389/fimmu.2020.00854] [PMID:32536913]
8. Johansson BG. Agarose gel electrophoresis. *Scand J Clin Lab Invest Suppl*. 1972;124:7–19. [DOI:10.3109/00365517209102747] [PMID:4114361]
9. Sam-Yellowe TY. Exercise 10: Immunodiffusion and Immunoelectrophoresis. In: *Immunology: Overview and Laboratory Manual*. Springer, Cham. 2021;311–8. [DOI:10.1007/978-3-030-64686-8\_34]
10. Djukić T, Drvenica I, Kovačić M, Milanović S, Majerić D, Šefik-Bukilica M, et al. Exploring the Link between Hydrodynamic Size and Immunoglobulins of Circulating Immune Complexes in Rheumatoid Arthritis. *Int. J. Mol. Sci*. 2024;25(6):3138. [DOI:10.3390/ijms25063138] [PMID:38542112]
11. Sandin C, Linse S, Areschoug T, Woof JM, Reinholdt J, Lindahl G. Isolation and detection of human IgA using a streptococcal IgA-binding peptide. *J Immunol*. 2002;169(3):1357–64. [DOI:10.4049/jimmunol.169.3.1357] [PMID:12133959]
12. Djukić T, Drvenica I, Kovačić M, Minić R, Vučetić D, Majerić D, et al. Dynamic light scattering analysis of immune complexes in sera of rheumatoid arthritis patients. *Anal Bioch*. 2023;674:115194. [DOI:10.1016/j.ab.2023.115194] [PMID:37279816]
13. Lee AYS, Lin MW. Polymeric IgA paraprotein on agarose gel electrophoresis immunofixation identifies a unique subset of IgA myeloma patients. *Clin Chim Acta*. 2021;512:112–6. [DOI:10.1016/j.cca.2020.10.031] [PMID:33127346]
14. Khan SR, Chaker L, Ikram MA, Peeters RP, van Hagen PM, Dalm VASH. Determinants and Reference Ranges of Serum Immunoglobulins in Middle-Aged and Elderly Individuals: a Population-Based Study. *J Clin Immunol*. 2021;41(8):1902–14. [DOI:10.1007/s10875-021-01120-5] [PMID:34505230]
15. Chahin M, Branham Z, Fox A, Leurinda C, Keruakous AR. Clinical Considerations for Immunoparesis in Multiple Myeloma. *Cancers (Basel)*. 2022;14(9):2278. [DOI:10.3390/cancers14092278] [PMID: 35565407]
16. Mittermayr S, Bones J, Le GN, O'Gorman P. Characterization of myeloma paraprotein glycosylation reveals functional insights and increased sialylation of residual polyclonal antibodies. *Blood*. 2015;126:1775. [DOI:10.1182/blood.V126.23.1775.1775]
17. Steffen U, Koeleman CA, Sokolova MV, Bang H, Kleyer A, Rech J, et al. IgA subclasses have different effector functions associated with distinct glycosylation profiles. *Nat Commun*. 2020;11(1):120. [DOI:10.1038/s41467-019-13992-8] [PMID:31913287]
18. Suzuki H, Yasutake J, Makita Y, Tanbo Y, Yamasaki K, Sofue T, et al. IgA nephropathy and IgA vasculitis with nephritis have a shared feature involving galactose-deficient IgA1-oriented pathogenesis. *Kidney Int*. 2018;93(3):700–5. [DOI:10.1016/j.kint.2017.10.019] [PMID:29329643]
19. Grigaite R, Iles JK, Harding S, Patel R, Wallis G, Iles RK. Degree of immunoglobulin kappa light chain glycosylation of anti-spike SARS CoV-2 antibodies correlates with COVID-19 severity. [Preprint]. medRxiv. 2023 [DOI: 10.1101/2023.01.06.23284259]
20. Singh G, Xu H, Bollag RJ. Monoclonal Light Chains in Multiple Myeloma: The Sinister Immunoglobulin. *Int J Pathol Clin Res* 2022;8(2):134. [DOI:10.23937/2469-5807/1510134] [PMID:4193390]
21. Dispenzieri A, Larson DR, Rajkumar SV, Kyle RA, Kumar SK, Kourelis T, et al. N-glycosylation of monoclonal light chains on routine MASS-FIX testing is a risk factor for MGUS progression. *Leukemia*. 2020;34(10):2749–53. [DOI:10.1038/s41375-020-0940-8] [PMID:32594098]

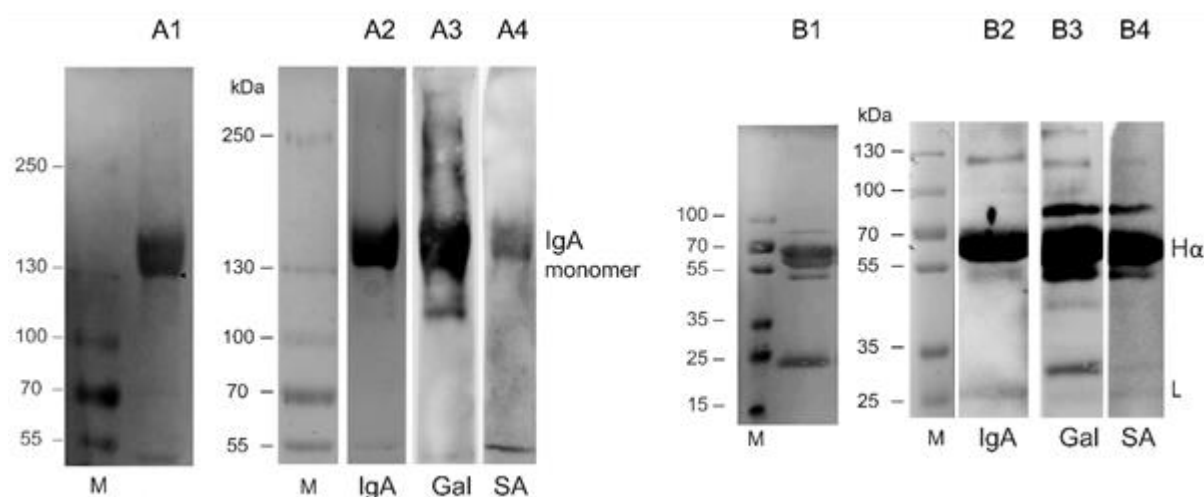
22. Wang Y, Xiao J. Recent advances in the molecular understanding of immunoglobulin A FEBS J. 2024;291(16):3597–603. [DOI:10.1111/febs.17089] [PMID:38329005]
23. Laurell CB, Thulin E. Complexes in Human Plasma between  $\alpha$ 1-Antitrypsin and IgA, and  $\alpha$ 1-Antitrypsin and Fibrinogen. Scand J Immunol. 1975;4:7–12. [DOI:10.1111/j.1365-3083.1975.tb03802.x]
24. Perez Rogers A, Estes M. Hyperviscosity Syndrome. 2023. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025. [PMID: 30085505]
25. Hernando P, Egido J, de Nicolas R, Sancho J. Clinical significance of polymeric and monomeric IgA complexes in patients with IgA nephropathy. Am J Kidney Dis. 1986;8(6):410–6. [DOI:10.1016/s0272-6386(86)80167-6] [PMID:2949605]
26. Liu Y, Gong Y, Xu G. The role of mononuclear phagocyte system in IgA nephropathy: pathogenesis and prognosis. Front. Immunol. 2023;14:1192941. [DOI:10.3389/fimmu.2023.1192941] [PMID:37529043]
27. Oortwijn BD, Roos A, Royle L, van Gijlswijk-Janssen DJ, Faber-Krol MC, Eijgenraam JW, et al. Differential glycosylation of polymeric and monomeric IgA: a possible role in glomerular inflammation in IgA nephropathy. J Am Soc Nephrol. 2006;17(12):3529–39. [DOI:10.1681/ASN.2006040388] [PMID:17050773]
28. Geyer H, Geyer R. Strategies for analysis of glycoprotein glycosylation. Biochim Biophys Acta. 2006;1764(12):1853–69. [DOI:10.1016/j.bbapap.2006.10.007] [PMID:17134948]



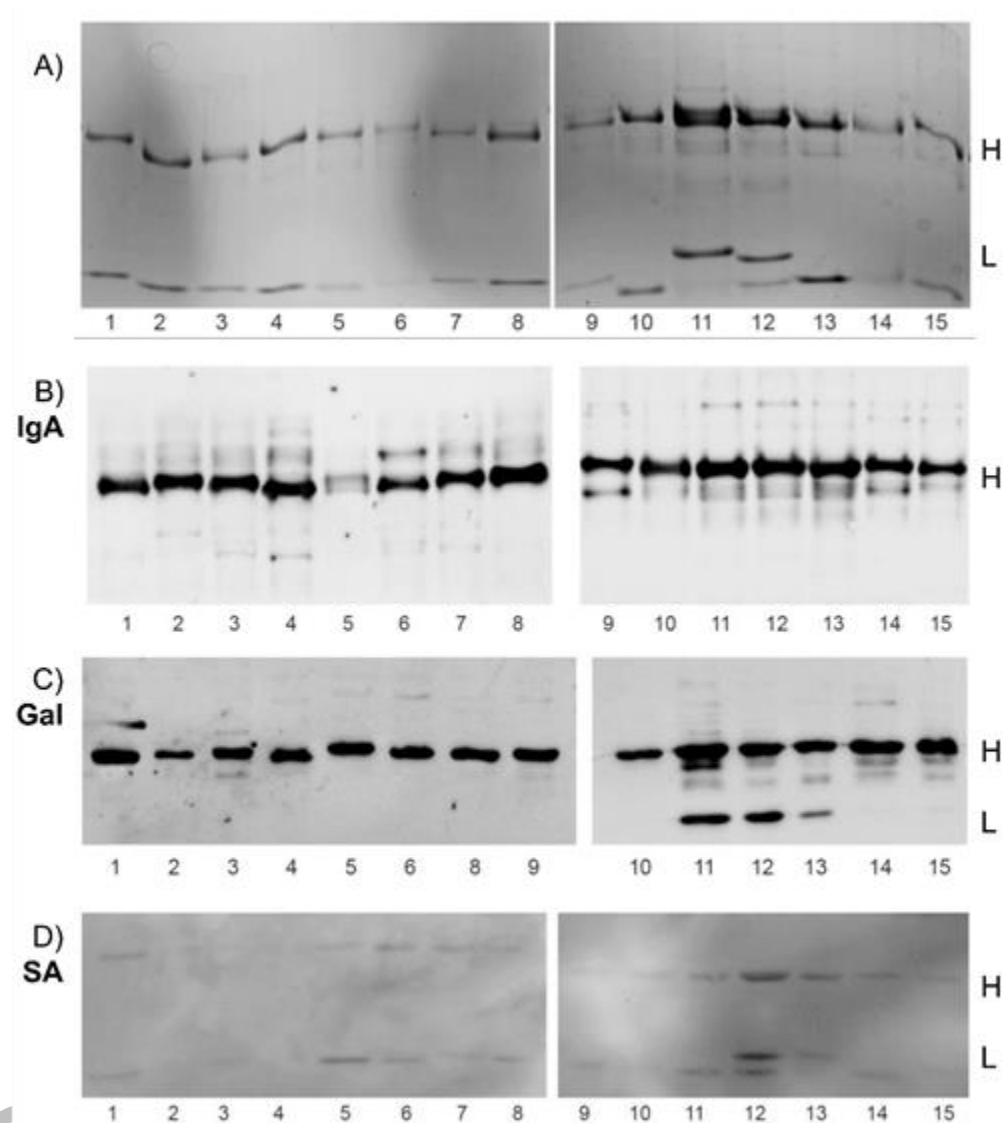
**Table 1.** Paraprotein isotype, and demographic and clinical data of individuals with IgA multiple myeloma

Sample	Paraprotein	Age	Gender	$\beta$ 2micro (mg/L)	IgA (g/L)	IgM (g/L)	IgG (g/L)	$\kappa/\lambda$
1	mIgA1 $\kappa$	70	M	2.44	44.40	0.38	2.72	40.80
2	mIgA1 $\kappa$	50	M	5.92	63.10	< 0.18	1.61	0.02
3	mIgA1 $\kappa$	76	M	3.20	8.82	< 0.18	4.72	8.33
4	mIgA1 $\kappa$ + $\kappa$ LC	67	F	1.93	18.90	< 0.18	4.84	11.60
5	mIgA1 $\kappa$	81	F	12.8	1.99	0.29	12.80	2.10
6	mIgA1 $\lambda$	88	M	2.65	3.96	2.17	7.20	0.84
7	mIgA1 $\kappa$	88	M	7.02	4.79	< 0.18	2.64	6.21
8	mIgA1 $\kappa$	81	F	3.53	20.70	0.27	5.96	11.00
9	mIgA1 $\lambda$	73	M	1.66	10.20	0.38	6.02	0.36
10	mIgA1 $\kappa$ + $\kappa$ LC	69	F	3.64	34.40	< 0.18	7.76	6.60
11	mIgA1 $\kappa$	80	M	3.48	12.90	0.44	6.19	9.52
12	mIgA1 $\kappa$ + $\kappa$ LC	81	F	7.49	25.20	< 0.18	5.25	12.00
13	mIgA1 $\kappa$	86	M	7.70	31.70	0.44	23.00	5.60
14	mIgA1 $\lambda$	52	F	8.85	7.20	1.31	10.20	1.32
15	mIgA1 $\lambda$	85	F	2.49	5.04	1.79	6.69	0.74
(Q1 – 25%)		69		2.49	5.04	0.17	4.72	0.84
<b>Median</b>		<b>80</b>		<b>3.53</b>	<b>12.90</b>	<b>0.29</b>	<b>6.02</b>	<b>6.21</b>
(Q3 – 75%)		85		7.49	31.70	0.44	7.76	11.00
Reference	Min			0.70	0.86	0.29	6.22	0.26
Values [17]	Max			1.80	4.69	2.60	15.10	1.65

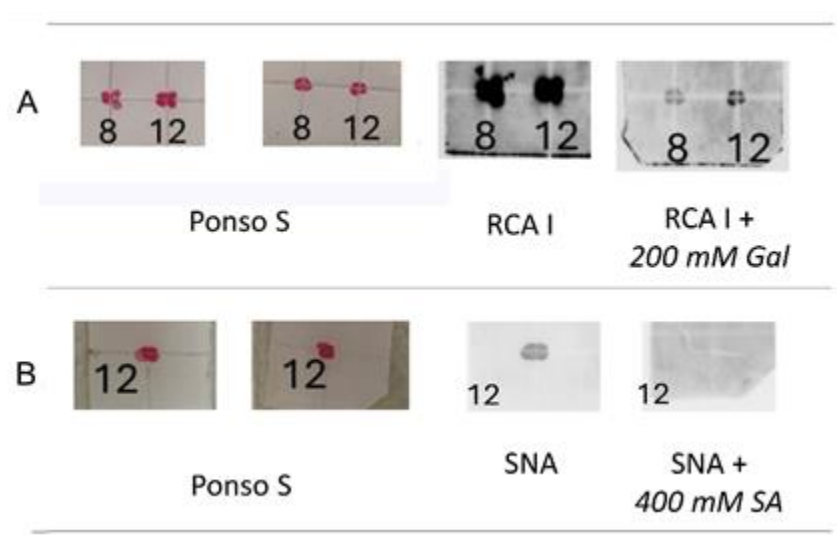
M – male; F – female; LC – free light chains; Q1 – the first quartile, indicating the lower range (25%) of the distribution for the analyzed parameters; Q3 – the third quartile, 75% of the values observed are below this value;  $\beta$ 2micro –  $\beta$ 2-microglobulin;  $\kappa/\lambda$  – kappa/lambda light chain ratio



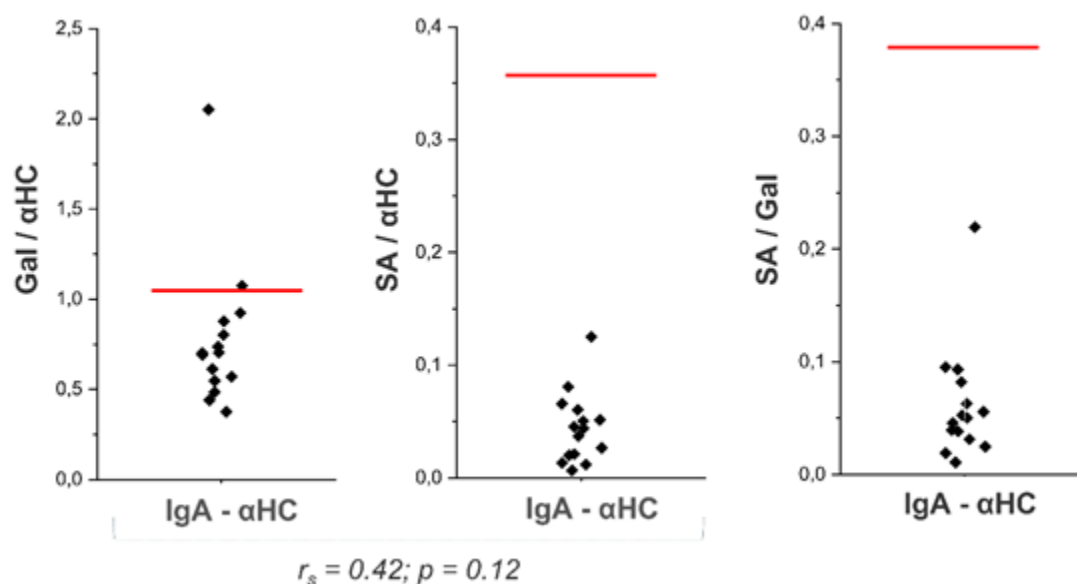
**Figure 1.** Expression of galactose and sialic acid on IgA from pooled healthy human serum; Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) under non-reducing (A1) or reducing conditions (B1); Western blot with anti-human IgA antibody (A2 and B2); Lectin blot with galactose (Gal) specific *Ricinus communis* agglutinin I (RCA I) (A3 and B3); Lectin blot with sialic acid (SA) specific *Sambucus nigra* agglutinin (SNA) (A4 and B4); M – molecular weight protein markers. H $\alpha$  – IgA heavy chains, L – IgA light chains



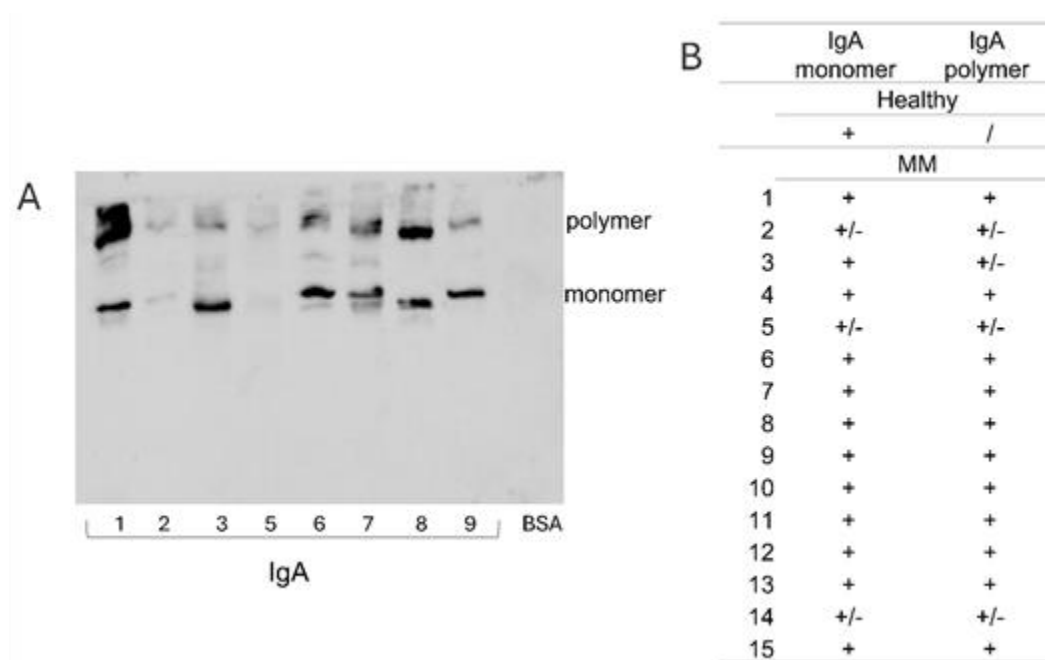
**Figure 2.** Expression of galactose (Gal) and sialic acid (SA) on light and heavy chains of total IgA isolated from the serum of individuals with IgA multiple myeloma; A – non-reducing Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE); B – Western blot performed with anti-human IgA antibody; C – Lectin blots performed with galactose (gal) specific *Ricinus communis* agglutinin I (RCA I); D – sialic acid (SA) specific – *Sambucus nigra* agglutinin (SNA); H – heavy chains; L – light chains of IgA



**Figure 3.** Confirming *Ricinus communis* agglutinin I (RCA I) and *Sambucus nigra* agglutinin (SNA) specificity with inhibitory dot blot; (A) – inhibition of RCA I binding to IgA isolates #8 and #12 with 200 mM galactose (Gal); (B) – inhibition of SNA binding to IgA isolate #12 (showing the highest SNA signal) with 400 mM sialic acid; Ponceau S – indicates the amount of dotted IgA



**Figure 4.** Densitometric quantification of the expression of galactose (Gal) and sialic acid (SA) on heavy chains (HC) of total serum IgA of individuals with IgA multiple myeloma and healthy individuals; the expression of monosaccharides presented as a ratio of chemiluminescence intensity (*i.e.*, pixel count) in the lectin blot with *Ricinus communis* agglutinin I (RCA I) or *Sambucus nigra* agglutinin (SNA) to the chemiluminescence intensity in the Western blot with anti-human IgA antibody



**Figure 5.** Expression of galactose (Gal) on monomeric and polymeric IgA isolated from the serum of individuals with IgA multiple myeloma (MM); A – *Ricinus communis* agglutinin (RCA I) lectin blot; B – expression of galactose on monomeric and polymeric forms of IgA; (+) – strong expression; (+/-) – weak expression; BSA – bovine serum albumin – the assay negative control