Immunoglobulin G isolation by fast protein liquid chromatography (FPLC): Method validation and implementation in patients with amyotrophic lateral sclerosis – a preliminary study [version 1; peer review: awaiting peer review]

Basak Aru1, Turkay Simsek1, Burcu Ormeci2, Gizem Gurel1, Katarina Milićević3, Lidija Radenović3, Pavle Andjus3, Gulderen Yanikkaya Demirel1

1Immunology Department, Yeditepe University, Istanbul, 34755, Turkey
2Vocational School of Health Services, Electroneurophysiology Department, Istanbul Gelisim University, Istanbul, Turkey
3Center for Laser Microscopy, Department for Physiology and Biochemistry 'Ivan Djaja', University of Belgrade, Belgrade, Serbia

Abstract
Amyotrophic lateral sclerosis (ALS) is a fatal, heterogenous neurodegenerative disease which is characterized by weakness and muscle atrophy. While more than 50 genes linked with the familial ALS have been identified, sporadic ALS accounts for the majority of cases and genetic factors contributing to the sporadic form of the disease remain largely elusive. Involvement of the immune system in disease progression, including presence of circulating autoimmune complexes, associations with other autoimmune diseases, as well as immunoglobulin (Igs) levels have been described. However, investigations involving immunoglobulins requires isolation of patient antibodies, while so far, there is no validated fast protein liquid chromatography (FPLC) protocol. For this purpose, we evaluated specificity, accuracy and precision parameters; limit of detection, limit of quantification and correlation coefficient were calculated from the calibration curve. Our results indicated the limit of detection and limit of quantification as 0.045 and 0.13 mg respectively, while r² was calculated as 0.9992. Our preliminary data suggest that this protocol can be implemented for the isolation of IgGs of ALS patients' sera samples for in vitro and in vivo analyses, while more studies involving larger patient groups are required.
Keywords
Fast protein liquid chromatography, Immunoglobulin G, amyotrophic lateral sclerosis

This article is included in the Excellent Science gateway.

Corresponding author: Gulderen Yanikkaya Demirel (gulderen.ydemirel@yeditepe.edu.tr)

Author roles: Aru B: Formal Analysis, Investigation, Resources, Writing – Original Draft Preparation; Simsek T: Investigation, Visualization; Ormeci B: Resources; Gurel G: Visualization; Milićević K: Investigation; Radenović L: Funding Acquisition, Project Administration, Supervision, Writing – Review & Editing; Andjus P: Funding Acquisition, Project Administration, Supervision, Writing – Review & Editing; Yanikkaya Demirel G: Conceptualization, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Supervision, Validation, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 778405.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2022 Aru B et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Aru B, Simsek T, Ormeci B et al. Immunoglobulin G isolation by fast protein liquid chromatography (FPLC): Method validation and implementation in patients with amyotrophic lateral sclerosis – a preliminary study [version 1; peer review: awaiting peer review] Open Research Europe 2022, 2:93 https://doi.org/10.12688/openreseurope.14577.1

First published: 03 Aug 2022, 2:93 https://doi.org/10.12688/openreseurope.14577.1
Plain language summary
Amyotrophic lateral sclerosis (ALS) is a rare neurological disease that affects nerve cells extending from the brain to the spinal cord, and leads to the loss of muscle movement. While some ALS patients live 10 or more years after disease onset, most patients die due to respiratory failure in three to five years. Patients may have a genetic background, but the vast majority of cases are sporadic, meaning that people without familial history or genetic risk factors can also develop ALS. The exact cause of the disease is not known, and currently there is no treatment. Immunoglobulins (Igs), proteins produced by a type of white blood cells, were suggested to be involved in ALS, but their mechanism of action is not entirely understood. Purifying Igs, especially their most abundant subset in serum, IgG, from ALS patients, holds a great importance for studies focusing on their role in ALS. However, currently there is no defined protocol for IgG isolation with protein chromatography. For this purpose, we developed and validated a fast protein liquid chromatography method (FPLC) for IgG purification from human serum and evaluated the protocol performance. Our results revealed that FPLC is a suitable approach for IgG isolation if pure IgGs without other serum proteins are required. However, the success of the implementation of this protocol should be further evaluated with a greater number of healthy people and ALS patients. Moreover, the results should be verified by other laboratories around the world using the same instrument.

Introduction
Amyotrophic lateral sclerosis (ALS), a disease characterized by the degeneration in upper and lower motor neurons, is widely recognized as a heterogenous neurodegenerative disease in terms of its genetic, neuropathologic and clinical aspects. Presenting with excess weakness and muscle atrophy, ALS generally occurs in late middle life; however, the age and site of onset and disease progression rate vary highly among patients. Death occurs within two to four years after disease onset. ALS is the most common motor neuron disease (MND) in adults with an incidence of two per 100,000 and prevalence of 5.4 per 100,000 individuals. Patients who have at least one relative diagnosed with the disease are defined as having familial ALS (fALS), accounting for 10% of total cases. Disease is autosomal dominantly inherited in nearly all cases. Advances in sequencing technologies have revealed that approximately 40 to 55% of fALS patients carry gene variants linked with the disease progression. Currently, more than 50 potential genes linked with ALS have been identified; SOD1 (superoxide dismutase 1), C9ORF72 (chromosome 9 open reading frame 72), FUS (RNA-binding protein FUS/TLS), and TARDBP (TAR DNA binding protein) genes are among the most commonly observed while others are relatively rare. A majority (90–95%) of ALS patients do not have family history, and are thus identified as sporadic ALS (sALS). Genetic factors contributing to sALS remain largely elusive.

Many studies including in vivo and in vitro models, as well as ex vivo studies have confirmed the role of the immune system in ALS: some of the findings implicating autoimmune responses include circulating immune complexes, increased frequency of certain MHC molecules, and association with other autoimmune diseases. Shean et al., (2018) revealed an inverse correlation between the ALS progression rate and the number of T-regulatory cells (T-regs), and reported that an increase in T-regs alleviates the disease symptoms and prolongs survival. Since IgA levels are known to be regulated by T-regs, various studies have aimed to reveal a link between the serum IgA levels and the disease progression; however, no significant correlation was observed. Recently, the contribution of IgGs to ALS onset and progression has been shown by many in vitro and in vivo studies. Accounting for 20% of all plasma proteins, IgG is one of the most common serum proteins, and it is divided into four subclasses; IgG1, IgG2, IgG3, and IgG4. IgG subclasses share more than 90% similarity; however, each has different properties as well as reactions against different types of antigens. Both in vivo and in vitro studies evaluating the effects of ALS IgGs would require isolation of patient antibodies; however, up to now, there has been no validated fast protein liquid chromatography (FPLC) protocols for the isolation of human derived IgGs.

Chromatographic methods have been reported to yield products with high purity in antibody isolation process. Relying on the reversible interaction between a protein and a ligand-immobilized matrix, affinity chromatography is a common method in Ig isolation. Coupled with a matrix, the ligand protein G binds to the Fc region of the IgG, and is routinely used for the isolation of monoclonal IgGs, as well as immune complexes involving IgG. FPLC, a form of high-performance liquid chromatography (HPLC), is a research-scale protein purification system with various advantages over HPLC, such as reduced costs and compatibility with a wide range of aqueous buffers, while covering all modes of HPLC including affinity chromatography.

In this study, we aimed to establish and validate a protocol for IgG isolation from human sera samples, with a focus on ALS patients.

Results
The study was conducted according to the guidelines published by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), which has also been adopted by the European Medicines Agency (EMA) and The U.S. Food and Drug Administration (FDA). Parameters for specificity, accuracy and precision were evaluated, and limit of detection (LOD), limit of quantification (LOQ) and correlation coefficient were calculated from the calibration curves.

Specificity
Due to the high specificity of the ligand (protein G) of this chromatography column to total IgG, a single peak on the chromatogram during elution was expected as shown in Figure 1. Both serum (blue) and spiked (pink) samples overlapped when the chromatograms were overlaid, and a single peak on the elution step was observed, indicating the absence of non-specific elution products. Retention times are shown in Table 2. Increased retention times observed in spiked samples are related to the...
Overlaid chromatograms of healthy individuals’ sera and spiked samples. For spiking, 0.4 mg IgG reconstituted in 150 mM NaCl solution was added to 300 µL serum sample. A single peak was observed on every chromatogram and merging spiked with serum chromatograms indicate the peaks are specific to IgG. Numeric data regarding spiked and serum samples are presented in Table 2.

Accuracy
To determine accuracy, we measured the IgG protein amount in standard samples with spectrophotometry, and then measured the IgG protein amount in standards eluted with our chromatography protocol (BioTek Epoch, USA), and the accuracy and percentage relative standard deviation (%RSD) were calculated. Results are presented in Table 3. The accuracy of the chromatography method for the IgG concentrations of 0.2, 0.5, 0.75 and 1 mg were 115.30%, 115.65%, 97.56% and 100.86%, respectively. On the other hand, %RSD values were 11.26%, 1.06%, 6.20% and 1.21% for 0.2, 0.5, 0.75 and 1 mg IgG, respectively.

Precision
Precision was defined as the agreement between the test results when the same sample was evaluated with the same analytical method multiple times at two levels: repeatability and intermediate precision.

Repeatability. Repeatability was assessed by serially diluting the highest IgG sample (1 mg/mL) with the equilibration buffer at 0.75, 0.5 and 0.20 mg, and running each sample two times. Overlaid chromatograms are presented in Figure 2a. Absorbance values are presented in Table 4. We evaluated linearity according to the repeatability evaluations, which indicates the correlation between the measured value and the actual concentration of the analyte in quantitative analytical methods. Repeatability of the protocol was assessed with regression analysis where the equation was calculated as $y = 189.7^x + 1.305$, and the $r^2$ value was 0.9992 (Figure 2b). Performance data are presented in Table 6.

Intermediate precision. Intermediate precision was evaluated using unpaired two-tailed T test, and chromatograms are presented in Figure 3. No significant difference was observed among mAU values between time points ($p=0.460$). In addition, retention times were similar ($p=0.213$ (Table 5).

Limit of detection (LOD)
LOD indicates the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. LOD is calculated according to the formula $LOD=(3.3\times\sigma)/S$ where $\sigma$ denotes standard deviation of y-intercepts and $S$ denotes the slope of the analyte curve. In our study, LOD was 0.045 mg.

Limit of quantification (LOQ)
LOQ defines the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is especially used for determining degradation products and impurities. LOQ is calculated according to the formula $LOQ=(10\times\sigma)/S$, where $\sigma$ denotes standard deviation of y-intercepts and $S$ denotes the slope of the analyte curve. In our study, LOQ was calculated to be 0.13 mg.

Evaluation of the presence of autoantibodies in patient sera
Samples were evaluated using the “Neurology Mosaic – 1” kit (EuroImmunn, Germany), which is recommended for diagnosing paraneoplastic neurological syndromes (PNS), and diseases of the central and peripheral nervous system that occur in response to tumor development. This kit visualizes autoantibodies (IgG, IgA and IgM) present in the serum against Yo, Hu, Ri, CV2, Ma and amphiphysin proteins on medullated and non-medullated nerves. ALS and MNDs are not entirely
considered as established paraneoplastic syndromes, but paraneoplastic syndrome is one of the possible conditions that may lead to motor neuron degeneration. All patients were positive for anti-neuronal nuclear antibody (Hu) and anti-MAG as determined by the nuclear staining of the nerve tissue and anti-glutamic acid decarboxylase (GAD) according to the staining pattern observed in cerebellum tissues (Figure 4). This was subject to confirmation with other methods such as line blot or immunoassays with transfected cells. No staining of the cerebellum tissue was observed in two ALS patients at 1:100 dilutions, whereas the fluorescent signal was

**Figure 2.** a) Overlaid chromatograms of standards’ each measurement. b) Straight-line regression model graphic. The coefficient of determination ($r^2$) was 0.9992.

**Figure 3.** Overlaid chromatogram of four patients’ each measurement evaluated for intermediate precision.
weaker compared to the 1:10 dilution in the third patient. Similarly, staining of the nerve tissue was quite prominent in the second and third patients at 1:10 dilution, but was lower in the first patient. A comparison with positive controls underlined the absence of anti-Yo antigens in all patients (which was observed as the staining of Purkinje fibers found in cerebellar tissue slices).

**Discussion**

Affecting 5% of the population of Western countries, autoimmune diseases consist of more than 70 different disorders and show a wide variety in terms of their target tissues, age of onset and response to treatments. Both humoral and cellular immunity play a role in the autoimmunity mediated tissue damage, and autoimmune diseases are believed to be a result of the interaction between environmental and genetic factors. Autoantibodies against various cellular compartments including nucleic acids, receptor or other functional cellular components are detected in serum samples of patients, and provide important data regarding the diagnosis and disease classification. In addition, patients may have autoantibodies before the manifestation of disease-related symptoms, and thus autoantibody

---

**Figure 4. IIFT results of three ALS patients’ sera samples.** All samples were analyzed in two dilutions, 1:10 and 1:100 with the EuroImmun Neurology Mosaic I Kit.
screening may have a predictive value prior to diagnosis. High levels of IgM and IgG autoantibodies are known to be associated with many autoimmune diseases; well-known examples of this phenomenon are rheumatoid arthritis, where IgM and IgG have been observed in more than 70% of patients with rheumatoid arthritis, and autoantibodies are known to target chromatin structure in systemic lupus erythematosus. Previously, ALS patient-derived IgGs were shown to induce stress in microglial cells, promote glutamate release at neurons, and alter calcium homeostasis in astrocytes in vitro.

Anti-neuronal antibodies are conventionally identified by indirect immunofluorescence (IIF) screening followed by line blots and cell-based assays, and are classified into two groups: group I defines intracellular targets, whereas group II identifies the proteins present on the cell membrane. Autoantibodies against intracellular proteins such as Yo, Hu, GAD and amphiphysin are regarded as the result of T cell activation against tumors expressing neuronal antigens. However, due to the intracellular localization of their target proteins, they are likely to exert no pathogenic activity in vivo, except anti-Hu that have been shown to activate neurons. Cell surface antigens, which are generally non-paraneoplastic, include aquaporin-4, N-methyl-D-aspartate receptor (NMDAR), leucine-rich, glioma inactivated 1 (LG1), Contactin-associated protein-like 2 (CASPR2), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor 1/2 (AMPAR1/2), and are mainly found after inflammatory brain damage. There have been few cases reporting onconeuronal antibodies including Hu, Ri, Yo or CV2/CRMP5 antibodies in patients with MND; Anti-Hu have been reported in five patients with MND; four cases had typical ALS and one had ‘flail arm syndrome’. All patients were male, and their median age was 60, although one was 30 years old. Presence of anti-Yo was reported in a 67-year-old female ALS patient with ovarian cancer whose neurological symptoms did not alleviate after the tumor resection. Here, we investigated the presence of autoantibodies against anti-neuronal antibodies in sera samples of ALS patients by IIF, and we observed that three ALS patients had autoantibodies directed against neuronal antigens; however, this finding requires further evaluation with line blots and transfected cells as well as a higher number of samples.

In a study on immune system alterations between healthy subjects and sALS patients, Zhang et al., (2005) revealed that disease severity is correlated with lower serum IgG levels, while significantly higher IgM levels were observed in both healthy subjects and individuals with severe disease. When considering involvement of IgG subclasses in ALS, Ostermeyer-Shoaib and Pattern (1993) were the first to evaluate IgG subclasses in 25 ALS patients, and reported IgG1 or IgG3 deficiency in 16 patients, while IgG2 and IgG4 levels were normal in all patients. Similarly, Westarp et al., (1994) evaluated immunoglobulins and IgG subclasses’ levels in ALS patients’ sera, and found that while mean serum concentrations of total IgG along with IgA and IgM remained within reference values, the minimum values for IgG subclasses IgG1, IgG2 and IgG3 were below normal values and maxima only for IgG2, and IgG3 exceeded reference values. Being reduced in 80.9% of the patients, IgG3 was reported to be the most altered isotype, followed by IgG1, which was reduced in 33.3% of the patients. When considered together with studies reporting ALS-derived IgGs induced certain alterations in neuronal and glial cells, these data underline the alterations in IgG levels in ALS progression, which should be evaluated further by both in vitro and in vivo studies. For this purpose, pure IgGs of ALS patients as well as their healthy counterparts, instead of whole sera samples, may be required as other proteins found in the serum may interfere with the results.

Herein, we aimed to develop and validate an IgG isolation protocol with FPLC, a fast and easy method for protein purification. IgG purification can also be performed with laboratory-prepared columns where the product is eluted with gravity, but automated chromatography increases the reproducibility of the experiment. Our results revealed that the detection and quantitation limits were 0.045 mg and 0.13 mg, respectively, suggesting FPLC can be implemented to IgG isolation from sera samples, both from healthy individuals and patients with certain neurodegenerative diseases including ALS. However, it should be noted that due to the preliminary nature of this study, the precision between laboratories (reproducibility) was not evaluated, which should be considered in future studies as other laboratories equipped with the same instruments (AKTA Avant Chromatography System coupled with with HiTrap Protein G HP column, in our case) should validate this protocol. Similarly, this protocol should be validated with a certain number of individual samples as well as their healthy controls, while comparisons between different laboratory methods such as nephelometry, enzyme-linked immunosorbent assays (ELISA) or flow cytometric bead arrays for comparing IgG concentrations can be employed. With sufficient sample sizes, differences between characteristics regarding both groups’ chromatograms can be detected, and FPLC can be utilized not just for IgG purification, but also diagnosis. However, this should be confirmed with further studies. Moreover, ALS is a rare disease and most patients are already receiving treatments, which may alter their IgG levels as well as their subtypes. For accurate results, newly diagnosed patients of both genders should be compared with their age and gender-matching healthy controls. Power analysis may be employed for calculating ideal sample sizes, especially if detecting alterations in the IgG compositions in serum samples via chromatography is the aim. This is a seriously challenging task during the COVID-19 pandemic as patients are generally in their late middle life, and very vulnerable to the SARS-CoV-2 virus.
**Methods**

Sample storage

This study was conducted with healthy individuals (subjects who were not diagnosed with an autoimmune disease and did not have an infection during the time the blood sample was collected) and ALS patients in accordance with the Helsinki Declaration; this study was approved by the local ethical committee of the Yeditepe University Hospitals, Yeditepe University (Approval number K-1616-999). Signed informed consent forms were obtained from all patients by the clinicians, which were also approved by the ethical committee. All samples were either immediately analyzed or stored at -80°C in portions until analysis.

Preparation of standards

Standard IgG samples were prepared by reconstituting total IgG (Sigma Aldrich, Catalogue number: I4506-10MG) with sterile 150 mM NaCl solution to obtain a 1 mg/mL solution and stored as 0.1 mg portions at -80°C. Frozen samples were thawed on ice, then brought to room temperature before analysis. Freeze-thaw cycles were avoided.

Chromatography protocol

This protocol was validated according to the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH). All chromatographic studies were conducted with ÄKTA Avant Chromatography System, equipped with HiTrap Protein G HP (1 mL) (both by GE Healthcare Life Sciences). Flow rate was set to 1 mL/min. The chromatography protocol consisted of equilibration (five column volumes) and elution (fifteen column volumes linear gradient, six-column volumes step gradient) steps. The composition of buffers used in this study are given in Table 1. Elution was performed at 4°C. 50 µL neutralization buffer was added to the wells of the elution plate prior the experiment. Analyses were conducted using the Unicorn software (Version 6.3). GraphPad Prism Version 8 was used for statistical analysis.

| Table 1. Buffer compositions. |
|--------------------------------|
| **Equilibration buffer** | 25 mM phosphate buffer + 150 mM NaCl pH:7.4 |
| **Wash buffer** | 50 mM acetate buffer, pH:6 |
| **Elution buffer** | 75 mM acetate buffer, pH:3 |
| **Protein neutralization buffer** | 1 M tris, pH:9 |

| Table 2 Retention times between patients’ samples and spiked samples. |
|--------------------------------|
| **Age** | **Serum sample** | **Spiked sample** |
| Sample 1 | 32 | 22.27 | 22.46 |
| RT start: 15.3 | RT end: 26.97 | RT start: 18.94 | RT end: 27.63 |
| Sample 2 | 26 | 22.16 | 22.36 |
| RT start: 19.8 | RT end: 26.69 | RT start: 18.39 | RT end: 27.61 |
| Sample 3 | 64 | 22.11 | 22.38 |
| RT start: 13.7 | RT end: 27.05 | RT start: 17.89 | RT end: 27.61 |

| Table 3 Comparisons between the IgG amounts, eluted by chromatography and measured by spectrophotometer; and directly measured by spectrophotometer. |
|--------------------------------|
| **Amount (mg)** | **Spectrophotometer (mg)** | **Elute (mg)** | **Mean** | **RSD (%)** | **Accuracy (%)** |
| 0.2 | 0.24 | 0.25 | 0.26 | 0.305 | 0.2825 | 11.26 | 115.30 |
| 0.5 | 0.61 | 0.54 | 0.66 | 0.67 | 0.665 | 1.06 | 115.65 |
| 0.75 | 0.79 | 0.73 | 0.774 | 0.709 | 0.7415 | 6.19 | 97.56 |
| 1 | 1.15 | 1.17 | 1.16 | 1.18 | 1.17 | 1.20 | 100.86 |
Table 4. Absorbance values for four different concentration of standard IgG samples reconstituted in NaCl solution, and further diluted in equilibration buffer. Each measurement was replicated.

| Concentration (mg) | Height (mAU) | Mean height | Standard deviation |
|-------------------|--------------|-------------|--------------------|
| 0.2               | 42.65        | 37.04       | 39.85              |
| 0.5               | 93.71        | 99.32       | 96.51              |
| 0.75              | 136.35       | 145.48      | 140.92             |
| 1                 | 191.59       | 193.78      | 192.69             |

Table 5. Absorbance values and retention times of four different individuals’ samples analysed on two different time points for intermediate precision.

| Sample | Age | Gender | Height (mAU) | Retention time (CV) |
|--------|-----|--------|-------------|---------------------|
| 1      | 32  | F      | 780 682     | 731 22.63 22.27 22.45 |
| 4      | 29  | F      | 677 691     | 684 22.17 22.31 22.24 |
| 5-ALS  | 59  | M      | 921 778     | 849.5 22.60 22.29 22.44 |
| 6-ALS  | 67  | M      | 856 868     | 862 22.45 22.38 22.41 |

Table 6. Performance data of the protocol.

| Criteria evaluated | Value   |
|--------------------|---------|
| y-intercept        | 1.305   |
| x-intercept        | -0.006878 |
| Standard deviation of the slope | 3.847 |
| R²                 | 0.9992  |
| p-value            | 0.0004  |
| Limit of quantification (LOQ) (mg) | 0.13 |
| Limit of detection (LOD) (mg) | 0.045 |

Criteria evaluated
In our study, specificity (the ability of the protocol to determine the analyte in the presence of components which may be present in samples), accuracy (comparisons between the value obtained from chromatography with data of another analytical method) and precision (the ability of the protocol to measure the same value on different timepoints and by different laboratories and/or different operators) of the protocol were evaluated. For specificity, we analyzed two healthy and one ALS individuals’ serum samples and then spiked the same samples with standard IgG samples. We compared the retention times at the beginning and the end of retention. For accuracy, we measured the IgG protein amount in standard samples with spectrophotometry, and then measured the IgG protein amount in standards eluted with our chromatography protocol.

Precision was considered at two levels in this study: repeatability (precision under the same operating conditions over a short time interval) and intermediate precision (precision under different days). For repeatability, standards at four different concentrations were analyzed on the same day. Each standard was serially diluted from the stock IgG (1 mg/mL) with the equilibration buffer. Limit of detection (LOD) and limit of quantification (LOQ) were calculated according to the calibration
curve drawn by the data obtained from standard IgG samples. Linearity was evaluated up to 1 mg with standard IgG sample measurements and performed two times. Intermediate precision was tested by analyzing four serum samples (two healthy, and two patients diagnosed with ALS) 30 days apart. On the same day, 2 mL whole blood was collected into serum collection tubes with clot activator and centrifuged. Tubes were centrifuged on the same day, and serum samples were separated into 2 mL syringes (0.5 mL per syringe). For each sample, one was immediately used while the other was frozen at -80°C. No preservatives were added to the frozen sample to protect protein stability. Reproducibility (precision between laboratories) was not evaluated within the context of this study.

Evaluating the autoantibodies’ presence in the sera samples
For immunofluorescence tests, serum samples of ALS patients (diluted in PBS-Tween-20 at final concentrations of 1:10 and 1:100, as recommended by the EuroImmun Neurology Mosaic (diluted in PBS-Tween-20 at final concentrations of 1:10 and

30 minutes at room temperature, followed by washing and labeling with FITC conjugated goat anti-human IgAGM. The kit included two positive controls for interpretation, both ready to use. All images were obtained on a JuLi Br&FIL station (NanoEnTek, South Korea) with a green fluorescence filter at 4X zoom.

Data availability
Underlying data
Open Science Framework: Immunoglobulin G isolation by fast protein liquid chromatography, https://doi.org/10.17605/OSF.IO/V3GMA

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

Acknowledgements
We sincerely thank to Pervin Yanikkaya Aydemir for carefully reading and editing the manuscript.

References

1. Mejzini R, Flynn LL, Pitout IL, et al.: ALS Genetics, Mechanisms, and Therapeutics: Where Are We Now? Front Neurol. 2019; 13: 1310. PubMed Abstract | Publisher Full Text | Free Full Text
2. Masrori P, Van Damme P: Amyotrophic lateral sclerosis: a clinical review. Eur J Neurol. 2020; 27(10): 1918-29. PubMed Abstract | Publisher Full Text | Free Full Text
3. Chiò A, Logroscino G, Traynor BJ, et al.: Global epidemiology of amyotrophic lateral sclerosis: a systematic review of the published literature. Neuroepidemiology. 2013; 41(2): 118-30. PubMed Abstract | Publisher Full Text | Free Full Text
4. Alsultan AA, Waller R, Heath PR, et al.: Purification of immunoglobulin G. Methods and Protocols. In: Walls D, Loughran ST, eds. New York, NY: Springer New York, 2017; Chapter 2: Unit 2.7. Publisher Full Text | Free Full Text
5. Zou ZY, Zhou ZR, Che CX, et al.: Genetic epidemiology of amyotrophic lateral sclerosis: a systematic review and meta-analysis. J Neurol Neurosurg Psychiatry. 2017; 88(7): 540-9. PubMed Abstract | Publisher Full Text
6. Boylan K: Familial Amyotrophic Lateral Sclerosis. Neuro Clin. 2015; 33(4): 807-30. PubMed Abstract | Publisher Full Text | Free Full Text
7. Shen S, Sayana F, Zhang X, et al.: Genetics of amyotrophic lateral sclerosis: an update. Mol Neurodegener. 2013; 8: 28. PubMed Abstract | Publisher Full Text | Free Full Text
8. Pagani MR, Gonzalez LE, Uchitel OD: Autoimmunity in amyotrophic lateral sclerosis: past and present. Neurol Res. Int. 2011; 2011: 497080. PubMed Abstract | Publisher Full Text | Free Full Text
9. Sheean RK, McKay FC, Cretney E, et al.: Association of Regulatory T-Cell Expansion With Progression of Amyotrophic Lateral Sclerosis: A Study of Humans and a Transgenic Mouse Model. JAMA Neurol. 2018; 75(5): 681-9. PubMed Abstract | Publisher Full Text | Free Full Text
10. Rentzos M, Evangelopoulos ME, Sereti E, et al.: Humoral immune activation in amyotrophic lateral sclerosis patients. Neuro Int. 2013; 5(1): e3. PubMed Abstract | Publisher Full Text | Free Full Text
11. Crayle J, Elmiah M, Sleasman J, et al.: Total serum immunoglobulin A in ALS. Amyotrophic Lateral Scler Frontotemporal Degener. 2021; 22(1-2): 61-65. PubMed Abstract | Publisher Full Text | Free Full Text
12. Calvo AC, Pradat PF, Mendonça DM, et al.: Decoding amyotrophic lateral sclerosis: discovery of novel disease-related biomarkers and future perspectives in neurodegeneration. Biomed Res Int. 2014; 2014: 629630. PubMed Abstract | Publisher Full Text | Free Full Text
13. Vidarsson G, Dekkers G, Rispen T: IgG subclasses and allotypes: from structure to effector functions. Front Immunol. 2014; 5: 520. PubMed Abstract | Publisher Full Text | Free Full Text
14. Andrew SM, Titus JA: Purification of immunoglobulin G. Curr Protoc Immunol. 2001; Chapter 2: Unit 2.7. PubMed Abstract | Publisher Full Text
15. Madadiou A, O’Sullivan S, Sheehan D: Fast Protein Liquid Chromatography. In: Walls D, Loughran ST, eds. Protein Chromatography: Methods and Protocols. New York, NY: Springer New York, 2017; 1485: 365-73. Publisher Full Text
16. ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology. Q2 (R1), 1995. Reference Source
17. Gillings N, Todde S, Behe M, et al.: EANM guideline on the validation of analytical methods for radiotherapeutics. EJNMMI Radiopharm Chem. 2020; 1(1): 7. PubMed Abstract | Publisher Full Text | Free Full Text
18. Fahnestock SR, Alexander P, Nagle J, et al.: Gene for an immunoglobulin-binding protein from a group G streptococcus. J Bacteriol. 1986; 167(3): 870-80. PubMed Abstract | Publisher Full Text | Free Full Text
19. Le THH, Phung TH, Le DC: Development and Validation of an HPLC Method for Simultaneous Assay of Potassium Guaiacolsulfonate and Sodium Benzoate in Pediatric Oral Powder. J Anal Methods Chem. 2019; 2019: 6143061. PubMed Abstract | Publisher Full Text | Free Full Text
20. Bakhshi F, Molavi O, Rashidi MR, et al.: Developing a high-performance liquid chromatography fast and accurate method for quantification of sibulin. BMC Res Notes. 2019; 12(1): 743. PubMed Abstract | Publisher Full Text | Free Full Text
21. Jhang JS, Chang CC, Fink DJ, et al.: Evaluation of linearity in the clinical laboratory. Arch Pathol Lab Med. 2004; 128(1): 44-8. PubMed Abstract | Publisher Full Text | Free Full Text
