Screening for Antibody Deficiencies in Adults by Serum Electrophoresis

Cristina Frias Sartorelli de Toledo Piza (conscient.med@gmail.com)
UNIFESP: Universidade Federal de Sao Paulo https://orcid.org/0009-0006-5831-2260
Carolina Sanchez Aranda
Dirceu Solé
Stephen Jolles
Antonio Condino-Neto
https://orcid.org/0000-0002-1069-3117

Research Article

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Abstract

Purpose

This study aimed to investigate the correlation between calculated globulin (CG, total protein level minus albumin level) and the gamma globulin fraction (Gamma), obtained from serum protein electrophoresis with serum IgG levels in adults (≥ 18 years).

Methods

Using linear regression models, analyses of CG and Gamma levels correlation with IgG levels in adults were performed. Receiver-operator curves were created to determine cutoff values and the respective sensitivity and specificity measures.

Results

A total of 886 samples were analyzed. CG and Gamma were positively and statistically correlated with IgG levels (r^2 = 0.4628 for CG, and = 0.7941 for Gamma, $p < 0.0001$ for both analyses). For the detection of hypogammaglobulinemia, i.e., IgG level below the reference value (6 g/L), a CG cutoff value of 24 g/L showed a sensitivity of 86.2% (95% CI 69.4–94.5) and a specificity of 92% (90.0-93.6). A Gamma cutoff value of 7.15 g/L yielded a sensitivity of 100% (88.3–100) and a specificity of 96.8 (95.3-97-8).

Conclusion

Both CG and Gamma levels determined by protein electrophoresis analysis may be used to screen for antibody deficiencies in adults, enabling earlier diagnosis of antibody deficiencies in a routine clinical setting.

Introduction

Antibody deficiencies are a subset of immunodeficiencies and may be either primary or secondary in etiology [1, 2]. Primary antibody deficiencies (PADs) are a heterogeneous group of genetic disorders in which the fundamental defect is an intrinsic impairment in antibody production or function 1. They are the most common primary immunodeficiencies (PIDs), with a prevalence estimated to be approximately 1 in 1.2000 individuals of any age and up to 75% of all patients diagnosed with PIDs [3, 4].

In adults, the most common severe PADs are common variable immunodeficiency disorders (CVIDs), which account for the majority (57%) of all primary immunodeficiency cases, with an overall prevalence estimated of one in 25.000–50.000 [5–9]. Although the disease's clinical presentation varies depending on the clinical phenotype [7], frequent and severe respiratory tract infections are the most common

finding, and the gut and other sites may also be involved [10–12]. In addition, patients with CVIDs also commonly present with noninfectious complications, including allergic disease [5], autoimmune and inflammatory disorders [13], lymphoma, and other malignancies [14].

Despite their prevalence, PADs are important diagnoses because of the need for early therapeutic intervention mainly to prevent infections and their complications, which positively impacts the overall survival of 58% 45 years after diagnosis [15, 16].

With an estimated prevalence 30 times greater than that of PADs [2, 17], secondary antibody deficiencies (SADs) may be caused by underlying conditions and/or immunosuppressive treatments, frequently targeting B cells [2, 17–20]. The most common type of SAD arises from hematologic malignancies such as chronic lymphocytic leukemia, multiple myeloma, and lymphoma, where hypogammaglobulinemia can be present in up to 85% of patients [21,22]. Medication-associated SAD is a growing phenomenon related to immunosuppressive medications, mainly rituximab, veltuzumab, ibrutinib, and imatinib, to manage autoimmune and oncologic/hematologic diseases [17–22]. Other conditions responsible for antibody deficiency are protein-losing disorders associated with renal, gastrointestinal, or cutaneous diseases [2, 17, 18]. The clinical presentation of SAD ranges from a restricted increased susceptibility to infections to severe conditions mainly characterized by airway recurrent chronic, complicated, or systemic infections [2,17–22].

Antibody deficiency diagnosis can be simply established by serum immunoglobulin quantitation [2, 23, 24]. However, on average, most patients experience long delays of 6 to 7 years before an antibody deficiency is suspected, diagnosed, and treated [25, 29–33]. The years-long delay for diagnosis can lead to end-organ damage [26] and higher mortality [27], while timely correct treatment may decrease morbidity and improve survival [28]. In addition, early diagnosis of antibody deficiencies reduces health-care expenses and leads to better health improvement for patients [21, 29]. The factors impacting the delay in antibody deficiency diagnosis are not entirely understood, but the misconception that primary immunodeficiencies are always childhood diseases, combined with the heterogeneity of disease presentation and the number of specialists caring for these patients, negatively impact the establishment of the diagnosis [5, 6, 10, 34]. Secondary antibody deficiencies are often missed despite the occurrence of recurrent and even severe infections. In addition, a paucity of guidance related to its diagnosis screening and management and the diverse etiologies of SAD may contribute to the diagnostic delay in these patients [17–21, 35].

Several strategies have been taken to promote earlier diagnosis of antibody deficiencies. These include education to raise the awareness of physicians and patients [36, 37], systematic population screening [38], and opportunistic screening [39]. The European Society for Immunodeficiency (ESID) has developed diagnostic guidelines for nonimmunologists [36], and the "10 warning signs" of PID of the Jeffrey Model Foundation (JMF) (http://www.info4pi.org) [35, 40] and AAAAI Work Group Report a “Practical guidance for the diagnosis and management of secondary hypogammaglobulinemia” are widely available [17].

However, these documents may be overlooked if physicians do not consider immunodeficiency in the differential diagnosis.

Low immunoglobulin G levels affect a variety of laboratory tests, but a lower limit to the reference range could not be validated for most except for calculated globulin (CG) [41]. CG is derived from the difference between total protein and albumin levels, forms part of the liver function test (LFT) profile [42] and is low in patients with antibody deficiencies [41]. In addition, recent studies in different populations have demonstrated that CG can be used as a low-cost method for screening antibody deficiencies in adults [41–45].

In clinical practice, serum electrophoresis is also commonly used as a screening test in the initial evaluation of patients in internal medicine as part of the investigation of hepatic, renal, or hematological conditions [46, 47]. The gamma globulin fraction (Gamma) is predominantly composed of immunoglobulin G and reflects antibody levels [47].

In this study, we evaluated CG and Gamma values obtained from serum protein electrophoresis, allowing us to correlate those with serum IgG levels in the same patients.

Methods

Participant inclusion procedures

The University of São Paulo and Federal University of São Paulo Ethics Committees approved the protocol (approval numbers 3.340.392 and 3.499.511, respectively) according to the Brazilian Ministry of Health and the Declaration of Helsinki rules and regulations. Individuals were recruited at three different Allergy/Immunology centers in São Paulo state, Brazil. Written informed consent was obtained before the inclusion of participants and blood collection.

A 5 mL blood sample was collected from each individual and used for laboratory analyses. The patients had a free choice of laboratories. All laboratories were contacted to ascertain measurement methods. They were accredited according to the Associação Brasileira de Normas Técnicas (ABNT NBR ISO 15189) [48] and the Brazilian Society of Clinical Pathology (PALC) [49].

Laboratory Measurements

Immunoglobulin G values were determined by immunoturbidimetry (Roche COBAS 6000, Roche Diagnostics International Ltd, CH-6343, Rotkreuz, Switzerland). Protein electrophoresis was performed according to standard methods. CG values were obtained by subtracting the albumin levels from total protein values. The gamma globulin fraction was directly determined by protein electrophoresis. The laboratories used HYDRASYS (Sebia, Paris, France) instruments and HYDRAGEL PROTEIN gels (Sebia, Paris, France). The visualization of the gel provided qualitative analysis, while the reading of the agarose gels on a Sebia reader provided protein profiles for relative quantitative analysis by a Hydrasys 2 Scan (Sebia, Paris, France) scanning system.

Statistical Analysis

Nine hundred twenty-one (921) consecutive patients were recruited. The inclusion criteria were age ≥ 18 years old and the provision of informed consent. Eight hundred sixty-six (866) CG samples and 862 Gamma samples were analyzed.

See Supplementary Fig. 1 for sample inclusion flowchart.

We analyzed the associations between IgG and CG measurements and associations between IgG and Gamma measurements using linear regression models. Characteristic receiver operation (ROC) curves were constructed to identify discriminant CG and Gamma cutoff values between patients with levels below the lower limit of the adult reference range (< 6 g/L).

The accuracy of the discriminant cutoff values was verified by the analysis of the area under the ROC curve (AUC) and its respective confidence interval (95% CI); sensitivity tests (true positive rate – correct identification of patients with an IgG level below the reference); and specificity tests (true negative rate – correct identification of patients with normal IgG levels). The accuracy of the obtained discriminant cutoff values was interpreted based on the AUC and classified as “perfect” (AUC = 1), “exceptional” ($0.9 \leq$ AUC < 1), “excellent” ($0.8 \leq$ AUC < 0.9), “acceptable” ($0.7 \leq$ AUC < 0.8) and “poor” (AUC < 0.7), taking into account that the AUC is not significantly different from that obtained by chance for values of AUC ≤ 0.5. The most accurate discriminant cutoff value was defined as the one with the highest Youden index, defined as the highest value observed for the following operation: sensitivity + specificity – 1 [50–52].

All analyses were conducted in GraphPad Prism 9 (GraphPad Software, California, USA), adopting a significance level (α) of 5% ($P < 0.05$).

Results

Table 1 shows the clinical and demographic characteristics of all 886 participants. There was a predominance of females (71.2%), and cutaneous allergies represented the most frequent diagnosis.

Table 1
Clinical and demographic characteristics

	All	Females	Males
Sex - n (%)	886	631 (71.2)	255 (28.8)
Age - median (range)	42 (16–101)	42 (18–101)	41 (16–89)
IgG (mg/dL) (SD)	11.38 (3.12)	11.45 (2.99)	11.15 (3.52)
Gamma globulin mg/dL) (SD)	11.10 (2.89)	11.04 (3.19)	10.25 (3.83)
Calculated globulin mg/dL) (SD)	28.99 (4.12)	28.81 (5.72)	27.27 (6.62)
Diagnoses (n)			
primary immunodeficiencies	83	48	35
cutaneous allergies	463	353	110
respiratory allergies	224	150	74
others	74	52	22
healthy	18	14	4
recurrent respiratory tract infections	13	10	3

Both CG and Gamma were significantly and positively correlated with IgG values (Fig. 1). The CG values could explain 46% of the IgG variance, and Gamma, 79%.

Based on the AUC, the accuracy of discriminant cutoff values between patients with IgG levels below the reference range (n = 29 for both CG and Gamma analyses) and patients with normal IgG levels was classified as exceptional for diagnoses made on the basis of both CG (Fig. 2A) and Gamma (Fig. 2B). Table 2 presents other parameters related to the analysis.

Table 2
CG and Gamma values as functions of IgG

	AUC	p value	Cutoff value (g/L)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
Calculated Globin	0.948 (0.908–0.988)	< 0.001	24	86.2 (69.4–94.5)	92.0 (90.0-93.6)
Gamma globulin	0.998 (0.995-1.000)	< 0.001	7.15	100 (88.3–100)	96.8 (95.3–97.8)

Discussion

Reducing diagnostic delay remains a central challenge for patients with PADs and SADs and is critical to avoiding morbidity and mortality. Antibody deficiencies are pathologies that exhibit highly variable clinical manifestations; affect patients at any age; and may present as autoimmune, inflammatory, allergic, or malignant diseases where the underlying diagnosis is easily overlooked [1, 2, 5, 17, 18].

Antibody deficiencies are a growing phenomenon for several reasons. Newly identified monogenic defects are being added in the classification of inborn errors of immunity with a speed not seen before, many of them affecting antibody production or function[3]. Furthermore, the growth in new therapies for malignancies, organ transplantation, and autoimmune diseases, many targeting B-cell function and survival, is rapid and contributes to the increased development of iatrogenic SADs [18–22,53,54]. These patients are at increased risk of infection, and diagnostic delay can result in irreversible end-organ damage and even death [55, 56].

Important actions have been made to shorten the diagnostic delay in antibody deficiencies; however, the majority of these, apart from screening, require that immunodeficiency is considered in the differential diagnosis. These include successful initiatives from the ESID40 and JMF [57] and other medical societies. Strategies utilizing parameters from routinely ordered laboratory tests that are altered by changes in immunoglobulin levels potentially provide greater reach, as they do not require the requestor to consider antibody deficiency [39]. These laboratory tests can be used as opportunistic screening tests (that is, when an opportunity arises), as in the case of laboratory tests requested by health-care providers for diverse health conditions. In contrast to population screening, opportunistic tests using routine laboratory test parameters have substantial advantages, as they are better able to handle the issues of the broad age range and varied clinical presentations of antibody deficiencies [1, 2]. Calculated globulin values, utilizing different laboratory methods, have been shown to correlate well with IgG values. Jolles et al. [41] described CG as a screening method in Wales, using the Architect Biuret method for total protein calculation and the bromocresol green method for albumin. The authors chose a cutoff value of CG < 18 g/L, with a sensitivity of 0.82 and a specificity of 0.71 for IgG < 3 g/L. Holding et al. [45] showed the results of an extensive screening program in England using a rate biuret method or total protein and bromocresol purple for albumin. With a cutoff value for CG < 18 g/L, they found a positive predictive value of 8.6% (7–11%) for IgG < 3 g/L. Pecoraro et al. [43], using the same methods as Jolles et al., chose a cutoff value of 19 g/L to detect IgG levels below 6 g/L, with a sensitivity of 70% and a specificity of 75%.

Protein electrophoresis is a low-cost laboratory test ordered as a routine evaluation for a variety of disorders, especially oncologic and autoimmune diseases [46,47] and its results may automatically provide information on CG or Gamma. Our work shows that both CG and Gamma values, obtained from serum protein electrophoresis, can serve as correlates of IgG levels and may be used as screening methods for detecting antibody deficiency in adults, using a cutoff value of 24 g/L for CG and 7.15 g/L for Gamma to detect IgG levels below 6 g/L. In a previous study, we have shown that CG and the Gamma globulin fraction are excellent correlates for IgG values in children and adolescents, using different cutoff values according to age [58]. The sensitivity and specificity of the tests are expected to be much lower for

detecting other primary antibody deficiencies, such as IgA or IgM deficiencies. IgG corresponds to approximately 75% of normal immunoglobulins, and IgA and IgM levels are 4–5 times and 7–10 times lower, respectively [59]. For the same reason, selective IgG subclass or specific antibody deficiencies are not suitable conditions for screening using CG or Gamma values. The definitive diagnosis should be made using specific tests, such as the determination of immunoglobulin levels, followed by B- and T-cell analysis, vaccine response studies, and genomic tests [23].

The calculated globulin and Gamma globulin fractions used as screening tools for the detection of IgG antibody deficiency follow all the rules proposed by Wilson and Jungner [60] and most of the revised rules proposed by Dobrow et al. [61]. The tests are low-cost, readily available, and regularly performed to diagnose or follow-up other diseases or as a part of baseline investigations and drug monitoring.

In conclusion, CG and Gamma can be used to screen for antibody deficiency in adults. They should be widely applicable, especially in resource-poor settings, allowing earlier diagnosis and better outcomes for patients with hitherto undetected antibody deficiencies. Further studies in various health-care settings will help refine cutoff values and test accuracy, enabling low-cost screening for many more people.

Abbreviations

CG – calculated globulin

IgG – immunoglobulin

Gamma – gamma globulin

PAD – primary antibody deficiency

PID – primary immune deficiency

LFT – liver function test

SAD – secondary antibody deficiency

Declarations

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DISCLOSURE OF CONFLICTS OF INTEREST

CFSTP, CSA, DS and ACN declare no conflict of interests.

SJ has received consulting fees, speaker fees and research grants from CSL Behring, Takeda and Octapharma. He is a member of the UCB Pharma Data Safety and Management Board.

DATA AVAILABILITY

All data regarding the findings are available upon request to the corresponding author.

AUTHORSHIP CONTRIBUTIONS

Study conception and design were executed by CFSTP, SJ and ACN. Data collection was implemented by CFSTP, CSA and DS. All authors performed data analysis, manuscript review and final manuscript approval.

ETHICS APPROVAL

The University of São Paulo and Federal University of São Paulo Ethics Committees approved the protocol (approval numbers 3.340.392 and 3.499.511, respectively) according to the Brazilian Ministry of Health and the Declaration of Helsinki rules and regulations.

CONSENT TO PARTICIPATE

Written informed consent was obtained before the inclusion of participants and blood collection.

CONSENT TO PUBLISH

All authors have reviewed the final manuscript and consented to publication.

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Figures

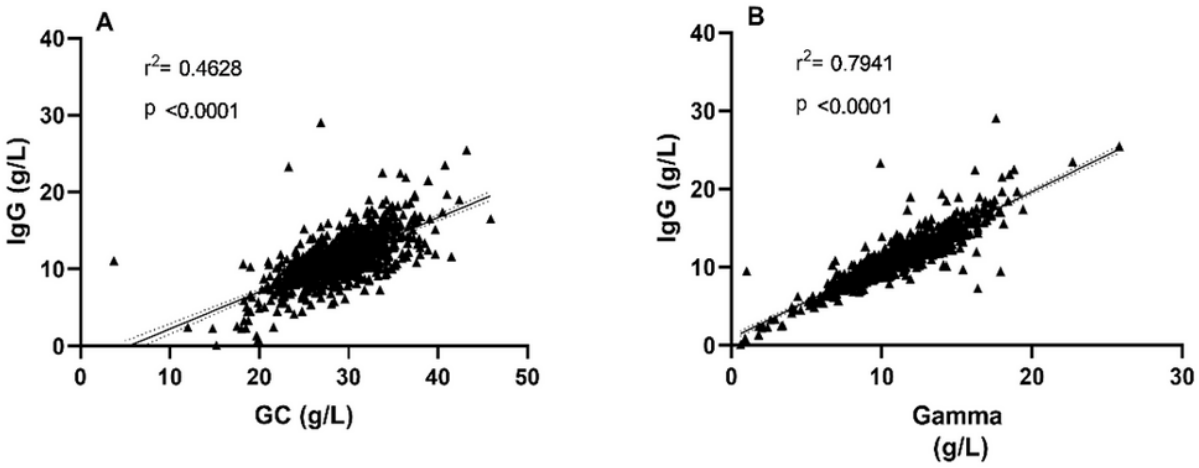

Figure 1

Correlation between IgG values, CG and Gamma

(A) – Calculated Globin. (B) – Gamma globulin

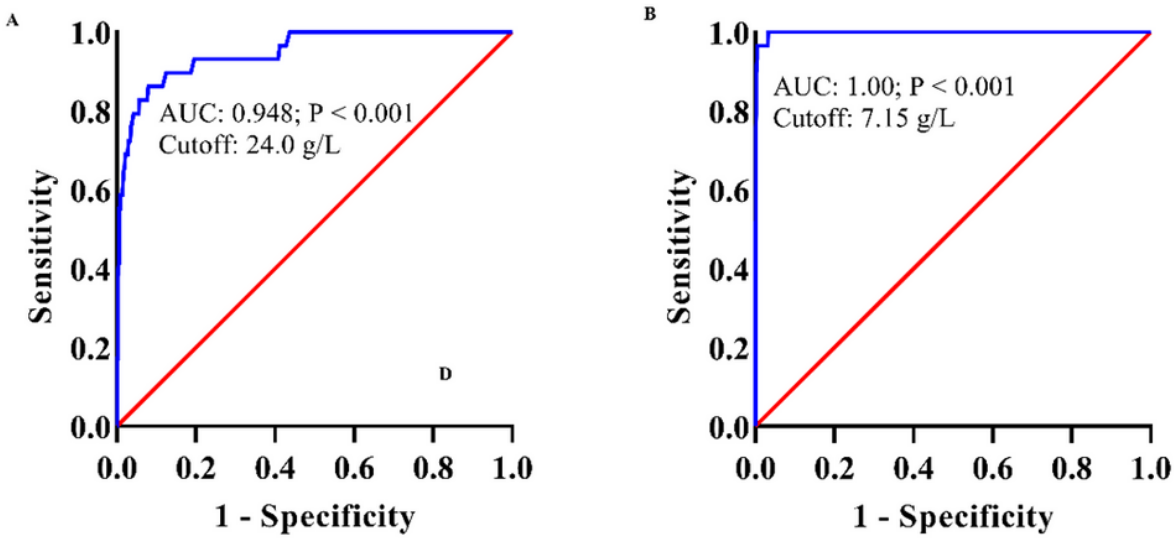

Figure 2

Receiver operator curves

Receiver operator curves for CG (A) and Gamma (B) for samples with normal (≥ 6 g/L) and low (<6 g/L) IgG values.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigure1Sampleflow.pptx