Calculated globulin is clinically useful as a screening test for antibody deficiency in Turkish adult patients

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Abstract

Backgrounds: Heterogeneous clinical features of antibody deficiency (AD) may cause diagnostic delays. Calculated globulin (CG) (total protein minus albumin) has been proposed as a screening test to prevent morbidity due to diagnostic delays in AD. Our aim is to validate CG as a screening test in AD in Turkish adult patients by comparing its role with gamma globulin analysis in protein electrophoresis. *Methods:* Fifty serum samples were randomly collected for each level of CG from 1.5 to 2.5 mg/dl and tested for serum IgG, IgA, IgM levels and protein electrophoresis. Cut-off values predicting low IgG levels were calculated for electrophoretically determined gamma globulin and CG. Additionally, the data of 47 patients followed up in our clinic with a diagnosis of primary antibody deficiency (PAD) were retrospectively analyzed. *Results:* A total of 550 adult patients were included in the study. The CG value predicting patients with IgG <600 mg/dl as a screening test was determined as <2.0 with 83.8% sensitivity and 74.9% specificity. The gamma globulin value which predicted patients with the same IgG value of 89.0% sensitivity and 89.4% specificity was determined as <0.7. In the retrospective analysis, 37 of 47 patients (78.7%) with PAD had a CG value of <2.0 at the time of the diagnosis and all 13 patients (%100) whose gamma globulin values were measured at the time of the diagnosis had a gamma globulin value of <0.7. *Conclusion:* The determined CG cut-off value of <2.0 can be used as a screening test in Turkish adult patients.

Title Page

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Running Title: Calculated globulin to screen for antibody deficiencies

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ABSTRACT

Backgrounds: Heterogeneous clinical features of antibody deficiency (AD) may cause diagnostic delays. Calculated globulin (CG) (total protein minus albumin) has been proposed as a screening test to prevent morbidity due to diagnostic delays in AD.

Our aim is to validate CG as a screening test in AD in Turkish adult patients by comparing its role with gamma globulin analysis in protein electrophoresis.

Methods: Fifty serum samples were randomly collected for each level of CG from 1.5 to 2.5 mg/dl and tested for serum IgG, IgA, IgM levels and protein electrophoresis. Cut-off values predicting low IgG levels were calculated for electrophoretically determined gamma globulin and CG. Additionally, the data of 47 patients followed up in our clinic with a diagnosis of primary antibody deficiency (PAD) were retrospectively analyzed.

Results: A total of 550 adult patients were included in the study. The CG value predicting patients with IgG <600 mg/dl as a screening test was determined as <2.0 with 83.8% sensitivity and 74.9% specificity. The gamma globulin value which predicted patients with the same IgG value of 89.0% sensitivity and 89.4% specificity was determined as <0.7. In the retrospective analysis, 37 of 47 patients (78.7%) with PAD had a CG value of <2.0 at the time of the diagnosis and all 13 patients (%100) whose gamma globulin values were measured at the time of the diagnosis had a gamma globulin value of <0.7.

Conclusion: The determined CG cut-off value of <2.0 can be used as a screening test in Turkish adult patients.

Keywords: *Antibody deficiency, Calculated globulin, Immune deficiency, Immunoglobulin, Screening test*

INTRODUCTION

Antibody deficiency (AD), a subset of immunodeficiencies, is classified as primary or secondary in etiology[1]. Secondary antibody deficiencies (SAD) occur more frequently than primary antibody deficiencies (PAD)[1,2]. The most common severe PAD is common variable immunodeficiency (CVID), which accounts for the majority of all adult symptomatic primary immunodeficiency (PID) cases on the European Society for Immun-

odeficiencies (ESID) registry (*http://www.esid.org*)[2-4]. SAD is often related to the underlying conditions or treatments particularly those targeting B cells.

Clinical features of PAD are heterogeneous. Patients may present not only with recurrent infections but also with manifestations of autoimmunity, autoinflammation, lymphoproliferation, granulomas, allergy or malignancy. Furthermore, although PAD may present at any age, it is often incorrectly felt to be a childhood disease. Therefore, the accurate diagnosis can be overlooked or delayed[5,6]. Diagnostic delays can lead to recurrent infections and irreversible organ damage[2]. Also, patients with a wide number of different diagnoses in a range of different specialist clinics are receiving immunosuppressive treatments may suffer from SAD[7] and their diagnoses may also be delayed. Focusing on control of the underlying autoimmune, inflammatory or malignant disease by clinicians may result in the oversight of developing SAD and possible serious infectious complications and recurrent severe infections can lead to delay in treatment as well. Accordingly, a screening test may be valuable in highlighting a hitherto undiagnosed antibody deficiency.

Jolles S. et al. defined the calculated globulin (CG) method to avoid these diagnostic delays. CG is simply obtained by subtracting serum albumin from serum total protein which is commonly used as part of liver function tests and routine check-up tests in daily clinical practice. Since it contains the gamma fraction including immunoglobulins, low CG is also a reflection of a possible low serum IgG level. In their study, Jolles S. et al. showed that CG can be used as a screening test for patients with PAD and SAD[2]. Subsequently, Pecoraro A. et al. performed the Italian version of the CG study[8]. Following this, Piza C. et al. showed that CG and gamma globulin obtained from protein electrophoresis can be used as a screening test for AD in children[9].

In this study, we aimed to validate the CG method as a screening test for AD in Turkish adult patients by comparing its role with gamma globulin analysis in protein electrophoresis. In addition, we aimed to investigate possible relationships between CG and clinical features of the patients.

METHOD

Patient Recruitment and Study Design

The study was initiated after obtaining ethics committee approval (2019/1455) from the ethics committee of Istanbul University, Istanbul Faculty of Medicine. Patients were recruited from all adult patient clinics in Istanbul Faculty of Medicine Hospital which is the biggest tertiary university hospital in Istanbul, following approval from heads of departments. A simple application of computation which automatically subtracts serum albumin from serum total protein level was integrated to the hospital computer operating system. It computes the CG value in all patients whose serum total protein and albumin levels were measured due to any reason in the central biochemistry laboratory. Technicians working in the biochemistry laboratory informed the Immunology and Allergic Diseases Division daily about all the patients with a CG value between 1.5-2.5 g/dL for further evaluation. The patients were given detailed information about the study by phone and asked if they wished to become a study participant. Patients who agreed to participate in the study and signed the written informed consent forms were invited to the Immunology and Allergic Diseases outpatient clinic to provide further clinical information. Inpatients who gave consent to participate in the study were visited on the wards. Patients who did not give informed consent, those on intravenous fluids replacement and those under the age of 18 were excluded from the study. Further analyses of IgG, IgM, IgA levels and protein electrophoresis were performed on the study participants' remaining serum samples which were kept in the biochemistry laboratory. If there were no remaining suitable serum samples for further analysis, patients were asked for donation of venous blood samples. The patients who did not have remaining suitable serum samples and did not want to donate serum samples were excluded from the study.

Fifty patients were planned to be included for each level of CG at 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4 and 2.5 g/dL. Therefore, in order to reach 550 volunteers, 686 patients were interviewed. Since 82 of these patients did not give informed consent after receiving detailed study information, they were excluded. Fifty-four patients were also excluded from the study since their blood samples were unsuitable for laboratory tests (Figure 1).

Age, gender and clinical features, comprising of clinics which follow-up the patients, current diseases, drugs used, frequency of antibiotic use, need for parenteral antibiotics, infections in the medical history and infection frequency of the total 550 patients were recorded in the assessment forms.

Laboratory Measurements:

Total protein (Total protein Gen2), albumin (Albumin Gen2) and immunoglobulin (Tina-quant Gen2 IgA, Tina-quant Gen2 IgM, Tina-quant Gen2 IgG) test kits were purchased from Roche Diagnostics. Measurements were performed using the Cobas 8000 c702 autoanalyzer (Roche Diagnostics, GmbH). Bromocresol green (BCG) method was used for albumin measurement. Total protein was analyzed by biuret method and Immunoglobulins were analyzed by immunoturbidimetric method. The gamma globulin fraction was directly obtained by protein electrophoresis which was performed by Capillarys 3 Octa automated capillary electrophoresis system (Sebia, Paris, France).

Retrospective analysis of the data of common variable immunodeficiency (CVID) patients

In PAD patients, serum total protein, albumin, IgG levels and gamma fraction in protein electrophoresis which were obtained at the time of the diagnosis, were retrospectively evaluated from their medical records. Detailed clinical and laboratory information of forty-seven patients from 2015 and onwards were analyzed. Four of the patients were diagnosed with IgG subgroup deficiency, 2 with Good syndrome and the remaining 41 with CVID. The ability of the CG and electrophoretic gamma globulin cut-off values to predict low serum IgG levels was evaluated. Patients who did not have simultaneous CG and IgG values at the time of the diagnosis, were not included in the retrospective analysis.

Statistical analysis

All analyses were performed with the IBM Statistical Package for the Social Science version 25.0 (SPSS Inc., Chicago, IL, USA) for MacOS. Descriptive data were given as percentages and as median (IQR 25-75). The Spearman's correlation test was used to confirm the correlations between CG versus (vs.) serum immunoglobulins and gamma globulin vs. serum immunoglobulins. The power of correlation was defined as very weak if r <0.2, weak if r=0.2–0.4, moderate if r=0.4–0.6, strong if r=0.6–0.8 and very strong if r>0.8.

Receiver operating characteristic (ROC) curve analyses were performed to verify if CG and gamma globulin were independent discriminative values to detect subjects with IgG serum levels lower than 600, 500, 400 and 300 mg/dL. The accuracy of the obtained discriminant value was interpreted based on the area under curve (AUC). The Youden's index was calculated to confirm the discriminant score, defined as the highest value observed for the following operation: sensitivity + specificity – 1[10]. Since CG was designed as a screening test, the cut-off value which has a sensitivity of >80% was accepted as the most appropriate among the values with the highest Youden's index[10,11].

The chi-square test was applied to compare the frequency of different clinical characteristics between the CG value of <2.0 and [?]2.0. Significant variables were further evaluated using the logistic regression analysis. The results were assessed at a significant level of p<0.05 and a 95% confidence interval (CI).

RESULTS

Demographic and clinical features of the study participants

A total of 550 adult patients were included in the study. The median (IQR 25-75) age was 53 (40-63.25) and the gender distribution was almost equal (male:52.9%). Hematology (27.7%) and nephrology (27.7%) were the most common clinical specialties where these patients were followed-up. The most common diagnosis was hypertension (49.2%) which was followed by chronic renal failure (33.5%), diabetes (24.1%), nephrotic syndrome (20.9%), multiple myeloma (19.4%) and renal transplantation (17.8%), respectively. Regarding the drug usage, corticosteroid (55.0%) and other immunosuppressive drugs (55.8%) were prominent compared to other treatments. The IgG value of 191 patients was lower than 600 mg/dL. Distribution of the clinics, patient diagnosis and concomitant treatment according to different IgG levels are shown in Table 1.

The correlation analyses between CG vs. serum immunoglobulins and gamma fraction vs. immunoglobins

The analyses between CG vs. serum IgG and CG vs. serum total immunoglobulin (IgG + IgA + IgM) revealed strong correlations ($p<0.001$, $r=0.67$; $p<0.001$, $r=0.70$; respectively). Gamma globulin revealed very strong correlations with serum IgG and serum total immunoglobulin levels ($p<0.001$, $r=0.89$; $p<0.001$, $r=0.89$; respectively) (Figure 2).

ROC curve analysis and cut-off values

ROC curve analyses verified that CG and gamma globulin were independent discriminative values to detect IgG levels lower than 600 mg/dL, 500 mg/dL, 400 mg/dL and 300 mg/dL. For CG, AUC (95% CI) values were 0.84 (0.80-0.87) to detect IgG<600mg/dL ($p<0.001$), 0.84 (0.80-0.87) to detect IgG<500 mg/dL ($p<0.001$), 0.82 (0.78-0.86) to detect IgG<400 mg/dL and 0.82 (0.77-0.88) to detect IgG<300 mg/dL ($p<0.001$). For gamma globulin, AUC (95% CI) values were 0.94 (0.92-0.96) to detect IgG<600mg/dL ($p<0.001$), 0.95 (0.92-0.97) to detect IgG<500mg/dL ($p<0.001$), 0.95 (0.92-0.98) to detect IgG<400mg/dL ($p<0.001$) and 0.96 (0.94-0.99) to detect IgG<300mg/dL ($p<0.001$) (Figure 3).

Sensitivity and specificity of each CG and gamma globulin values against each of the IgG levels were calculated through the analysis of the ROC curves as shown in Table 2. Since we considered IgG <600 mg/dl as the limit for hypogammaglobulinemia, the CG value predicting patients with IgG <600 mg/dl as a screening test was determined as <2.0 with 83.8% sensitivity and 74.9% specificity. The gamma globulin value which predicted the same IgG value of the patients with 89.0% sensitivity and 89.4% specificity was determined as <0.7.

When the patients with a CG cut-off value of <2.0 were evaluated according to the features of suggestive immunodeficiency such as the presence of infections and recurrent parenteral antibiotic usage, univariate analysis showed that the frequency of pneumonia, presence of infections with opportunistic microorganisms and the frequency of parenteral antibiotic usage were higher in these patients with CG less than 2 g/dL than the ones with CG higher than 2 g/dL ($p=0.003$, $p=0.003$ and $p<0.001$, respectively) (Table 3A). Multivariate analysis of the model designed according to univariate analysis showed that the frequency of parenteral antibiotic usage was higher in patients with a CG cut-off value of <2.0 than the others ($p=0.025$) (Table 3B).

When the patients whose IgA and IgM values lower than the reference values (70-400 mg/dL, 40-230 mg/dL, respectively) were compared, the number of patients with CG <2.0 was significantly higher than those with CG [?]2.0 ($p<0.001$, $p<0.001$, respectively) and similarly, the number of patients with gamma globulin <0.7 was significantly higher than those with gamma globulin [?]0.7 ($p<0.001$, $p<0.001$, respectively). In patients with CG <2.0, 78.1% of them had an IgA value of <70 mg/dL and 64.8% of them had an IgM value of <40 mg/dL; and in patients with gamma globulin <0.7, 87.1% of them had an IgA value of <70 mg/dL and 79.2% of them had an IgM value of <40 mg/dl.

Retrospective analysis of the data of patients with primary antibody deficiency

The data of 47 patients followed up in our clinic with the diagnosis of PAD were retrospectively analyzed. It was observed that 37 of 47 patients (78.7%) had a CG value of <2.0 at the time of the diagnosis and all 13 patients (%100) whose gamma globulin values were measured at the time of the diagnosis had a gamma globulin value of <0.7.

DISCUSSION

The current study involving a large number of patients showed that a CG cut-off value of <2.0 mg/dl had a sensitivity of 83.8% and a specificity of 74.9% in order to detect AD in Turkish adult patients. Our results also showed that CG is useful to detect low IgG levels in parallel to low gamma globulin values in protein electrophoresis. Furthermore, its usefulness was confirmed in identifying almost 80% of adult PAD patients tested.

Since CG is easily accessible, simple, and inexpensive, it is suitable for usage as a screening test[12,13]. Furthermore, it is frequently used as part of routine laboratory testing in patients. It has a high potential to facilitate early diagnosis of PAD or SAD because of its widespread use, high sensitivity and specificity. The use of CG as a screening test in AD was first suggested by Jolles S. et al[2]. In their study, CG was obtained by subtracting albumin, which is measured by both BCG and bromocresol purple (BCP) methods, from total protein. They defined different cut-off values for these two methods[2]. Similar to the study of Pecoraro A. et al. [8], the Italian version of this study, we measured albumin only with the BCG method in our study.

In the study of Jolles S. et al., a CG value of <1.8 obtained by using the BCG method, predicted a sensitivity of 66% and a specificity of 78% for IgG levels lower than 500 mg/dL[2]. In the current study, we accepted the IgG value of 600 mg/dL as a limit for hypogammaglobulinemia[14]. Therefore, the aim of our CG cut-off value is to predict patients with a IgG value lower than 600 mg/dL. In addition, we also calculated cut-off values for different IgG levels. The most appropriate CG cut-off value for predicting patients with IgG <500 mg/dl was <1.9 which showed a sensitivity of 82.7% and a specificity of 79.3%. Our study revealed better sensitivity and specificity but with consistent results when compared to the original study by Jolles S. et al[2].

In the study of Pecoraro, A. et al., a CG value of <1.9 predicted a sensitivity of 70.0% and a specificity of 75.0% for IgG values lower than 600 mg/dL[8]. In our study, the most appropriate CG cut-off value, which we selected as <2.0, had a sensitivity of 83.8% and a specificity of 74.9% for predicting patients with this IgG value. Since CG value of 1.9 with a sensitivity of 75.4% and a specificity of 84.4% was also an appropriate cut-off value according to Youden's index, we could have selected it similar to Pecoraro et al. However, for screening tests, since it is recommended to select a sensitivity higher than 80.0%[11], we accepted the cut-off value of <2.0 as the most appropriate.

CG contains other globulins such as alpha 1, alpha 2, beta 1 and beta 2 as well as gamma globulins which are mostly formed by immunoglobulins[15,16]. Considering the presence of proteins in alpha and beta bands which can act as acute phase reactants, the CG value may remain high despite the presence of low gamma globulin value in cases with chronic disease associated with inflammation and or infection[15-17]. In addition, the gamma globulin level might be normal while the CG value is low due to the conditions that can decrease the production or increase the catabolism of the alpha and beta bands[15]. In order to observe this difference, we also measured the gamma globulin values of the patients participating in our study, unlike previous studies performed on adults. Gamma globulin cut-off value of <0.7 which best predicted IgG levels lower than 600 mg/dL, had a higher sensitivity and specificity than the CG cut-off value of <2.0 as expected. In the study of Piza C. et al. conducted on pediatric and adolescent patients, in parallel with our study, it was stated that gamma globulin had a higher sensitivity and specificity than CG and it would be appropriate to use it as a screening test like CG[9]. However, since CG is obtained in clinics much more frequently and cheaper than gamma globulin, it has the potential to screen a larger number of patients.

In our study, we were able to analyze the distribution of the clinical characteristics of the patients according to the cut-off value of CG. We observed that patients with a CG cut-off value of <2.0 had a significantly higher rate of recurrent parenteral antibiotic use than the others (p=0.025). To date, the CG has been defined as a screening test by the previous studies to predict the possibility of AD. In the current study, we have extended this to demonstrate the clinical implications, importance and utility CG.

The most frequent source clinics of the patients with low IgG values were nephrology and hematology. Excluding diseases such as hypertension and diabetes, which were similar in the whole patient population and not directly related to antibody deficiency, the patients were mostly diagnosed as having chronic renal disease, nephrotic syndrome, multiple myeloma and renal transplantation. In the study of Jolles S. et al., the hematology clinic was the biggest source for the patients with IgG values lower than 400 mg/dL[2]. However, nephrology was the biggest source for this patient group in our study. We attribute this result to the fact that Istanbul Faculty of Medicine has a large renal transplantation center because patients can receive intensive immunosuppression especially in the early stages of renal transplantation. Not surprisingly, apart from antihypertensives and statins, the most frequent medications were corticosteroids, chemotherapeutics and other immunosuppressive drugs.

The retrospective analyses of the data of 47 patients with primary AD (IgG<600) followed in our clinic showed that 78.7% had a CG value of <2.0 at the time of diagnosis. Pecoraro A. et al. found this rate to be 97.3% (37/38) in a similar analysis performed in their study[8]. This difference may be due to the clinical conditions of the patients such as the presence of infection and acute phase elevation at the time of admission. Furthermore, in all the patients whose protein electrophoresis were measured at the time of the diagnosis, gamma globulin level was less than 0.7 supporting this possibility.

Besides infections, the wide range of heterogeneous clinical noninfectious presentations of CVID may cause delays in diagnosis[2,18]. These delays may result in ongoing recurrent infections and irreversible organ damage[2]. However, early diagnosis which can be achieved by using the CG method might prevent chronic and irreversible complications associated with AD in this patient group[19,20]. On the other hand, hematological diseases including B cell-associated lymphoproliferative disease, chronic lymphocytic leukemia, multiple myeloma; diseases associated with impaired lymphoid circulation or increased immunoglobulin catabolism; treatments including mofetil mycophenolate, cyclophosphamide, corticosteroids, antiepileptics, rituximab and chemotherapeutics can all lead to SAD. CG represents an unbiased screening approach and could prevent complications that may occur due to delayed diagnosis, not only in PAD but also in SAD[7,8].

A potential limitation of our study is that the homogeneity of the patient population could have been affected due to the fact that Istanbul Faculty of Medicine is a tertiary hospital which is considered as a reference center for many diseases. Most of the people whose biochemical analyses were performed in our hospital were ill and there were almost not any healthy people who came just for a check-up. Therefore, numbers of less acutely unwell patients such as those seen most often in primary care may be less well represented in our study.

In conclusion, the current study indicated that a CG level of less than 2.0 mg/dl should be a warning sign for undetected AD in our population. Therefore, given the various manifestations of antibody deficiency and the potential presentations to both primary care and different secondary care services, low CG can alert clinicians for possible AD to prevent diagnostic delay and optimize therapy. CG screening represents a cheap and simple means of detecting primary and secondary antibody deficiency across a range of healthcare settings and we have established cut-off values for both CG and electrophoretic gamma globulin in out Turkish population.

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Table 1*:* Distribution of the clinics, patient diagnosis and concomitant treatment according to IgG levels

	IgG[?]600 (n=359)	**IgG<600 (n=191)**	**IgG<500 (n=139)**	**IgG<400 (n=89)**	**IgG<300 (n=51)**
Clinics n (%)	61(17.0)	53(27.7)	40(28.8)	30(33.7)	20(39.2)
Nephrology	32(8.9)	53(27.7)	42(30.2)	26(29.2)	15(29.4) 5(9.8)
Haematology	86(24.0)	21(11.0)	14(10.1)	9(10.1) 5(5.6)	3(5.9) 3(5.9)
General	17(4.7) 18(5.0)	15(7.9) 9(4.7)	10(7.2) 8(5.8)	5(5.6) 0(0.0)	0(0.0) 0(0.0)
practice	39(10.9) 2(0.6)	8(4.2) 7(3.7)	2(1.4) 6(4.3)	5(5.6) 3(3.4)	2(3.9) 1(2.0)
Rheumatology	9(2.5) 20(5.6)	5(2.6) 4(2.1)	3(2.2) 4(2.9)	3(3.4) 0(0.0)	0(0.0) 1(2.0)
Pulmonology	15(4.2) 3(0.8)	4(2.1) 3(1.6)	2(1.4) 3(2.2)	2(2.2) 0(0.0)	0(0.0) 0(0.0)
Cardiology	17(4.7) 27(7.5)	2(1.0) 1(0.5)	1(0.7) 1(0.7)	0(0.0) 1(1.1)	1(2.0) 0(0.0)
Oncology	0(0.0) 8(2.2)	2(1.0) 1(0.5)	2(1.4) 0(0.0)	0(0.0) 0(0.0)	0(0.0) 0(0.0)
Neurology	0(0.0) 3(0.8)	1(0.5) 1(0.5)	1(0.7) 0(0.0)	0(0.0) 0(0.0)	0(0.0)
Gastroenterol-	2(0.6)	1(0.5)	0(0.0)		
ogy Surgery					
Emergency					
Allergy-					
immunology					
Endocrinology					
Psychiatry					
Infection					
diseases ENT					
Orthopaedics					
Gynaecology					

	IgG[?]600 (n=359)	**IgG<600 (n=191)**	**IgG<500 (n=139)**	**IgG<400 (n=89)**	**IgG<300 (n=51)**
Diagnosis n	75(20.9)	46(24.1)	36(25.9)	19(21.3)	8(15.7)
(%) Diabetes	134(37.3)	94(49.2)	71(51.1)	47(52.8)	26(51.0)
Hypertension	46(12.8)	22(11.5)	17(12.2) 8(5.8)	11(12.4) 4(4.5)	6(11.0) 2(3.9)
Ischemic heart	16(4.5)	10(5.2)	47(33.8)	36(40.4)	23(45.1)
disease	62(17.3)	64(33.5)	24(17.3)	18(20.2)	11(21.6)
Congestive	50(13.9)	34(17.8)	31(22.3) 1(0.7)	25(28.1) 1(1.1)	16(31.4) 1(2.0)
heart failure	18(5.0) 18(5.0)	40(20.9) 2(1.0)	0(0.0) 15(10.8)	0(0.0) 7(6.7)	0(0.0) 4(7.8)
Chronic renal	7(1.9) 28(7.8)	1(0.5) 22(11.5)	0(0.0) 12(8.6)	0(0.0) 8(9.0)	0(0.0) 1(2.0)
failure Renal	3(0.8) 25(7.0)	0(0.0) 15(7.9)	8(5.8) 30(21.6)	5(5.6) 22(24.7)	0(0.0) 15(29.4)
transplanta-	6(1.7) 5(1.4)	9(4.7) 37(19.4)	4(2.9) 1(0.7)	3(3.4) 0(0.0)	2(3.9) 0(0.0)
tion Nephrotic	4(1.1) 3(0.8)	6(3.1) 1(0.5)	1(0.7) 7(5.0)	1(1.1) 5(5.6)	1(2.0) 4(7.8)
syndrome	3(0.8) 0(0.0)	1(0.5) 11(5.8)	2(1.4) 1(0.7)	0(0.0) 1(1.1)	0(0.0) 1(2.0)
Chronic liver	2(0.6) 1(0.3)	3(1.6) 1(0.5)	11(7.9) 2(1.4)	7(7.9) 2(2.2)	4(7.8) 1(2.0)
disease Liver	25(7.0) 8(2.2)	14(7.3) 3(1.6)	1(0.7) 0(0.0)	1(1.1) (0.0)	0(0.0) 0(0.0)
transplanta-	4(1.1) 1(0.3)	1(0.5) 0(0.0)	1(0.7) 4(2.9)	1(1.1) 2(2.2)	1(2.0) 1(2.0)
tion	1(0.3) 4(1.1)	1(0.5) 5(2.6)	2(1.4) 2(1.4)	2(2.2) 1(1.1)	1(2.0) 1(2.0)
Connective	2(0.6) 1(0.3)	2(1.0) 2(1.0)			
tissue disorder					
Sarcoidosis					
Solid					
malignancy					
Lymphoma					
Multiple					
myeloma CLL					
CML Acute					
leukemia Bone					
marrow					
transplant					
MDS Primary					
amyloidosis					
Hypothy-					
roidism					
Hyperthy-					
roidism					
Inflammatory					
bowel disease					
Celiac disease					
HIV Epilepsy					
Multiple					
sclerosis					
Atopic					
dermatitis					

	IgG[?]600 (n=359)	**IgG<600 (n=191)**	**IgG<500 (n=139)**	**IgG<400 (n=89)**	**IgG<300 (n=51)**
Treatment n (%)	22(6.1) 1(0.3) 9(2.5) 7(1.9)	46(24.1) 3(1.6) 13(6.8) 1(0.5)	39(28.1) 2(1.4) 12(8.6) 1(0.7)	28(31.5) 0(0.0) 9(10.1) 0(0.0)	14(27.5) 0(0.0) 5(9.8) 0(0.0)
Chemotherapy	93(25.9) 7(1.9)	105(55.0)	80(57.6) 4(2.9)	53(59.6) 3(3.4)	29(56.9) 1(2.0)
Anti TNF	15(4.2)	5(2.6) 5(2.6)	4(2.9) 22(15.8)	3(3.4) 14(15.7)	3(5.9) 8(15.7)
Rituximab	49(13.6) 3(0.8)	34(17.8)	21(15.1) 1(0.7)	16(18.0) 0(0.0)	10(19.6) 0(0.0)
Methotrexate	4(1.1) 56(15.6)	24(12.6) 3(1.6)	26(18.7)	19(21.3)	11(21.6)
Systemic corticosteroid	66(18.4) 46(12.8)	33(17.3) 46(24.1)	33(23.7) 20(14.4)	24(27.0) 15(16.9) 8(9.0)	16(31.4) 7(13.7) 3(5.9)
Tyrosine kinase inhibitor	65(18.1) 24(6.7) 6(1.7) 102(28.4)	24(12.6) 25(13.1) 19(9.9) 9(4.7) 106(55.8)	17(12.2) 16(11.5) 6(4.3) 86(61.9)	9(10.1) 4(4.5) 59(66.3)	3(5.9) 1(2.0) 35(68.6)
Azathioprine					
Mycophenolate					
Bortezomib					
Sulfasalazine					
Statin ACEi					
ARB OAD					
Insulin					
Anti-epileptics					
Immunosuppressive agents					

ACEi: Angiotensin Converting Enzyme inhibitor, ARB: Angiotensin Receptor Blocker, CLL: Chronic Lymphocytic Leukemia, CML: Chronic Myelocytic Leukemia, ENT: Ear Nose Throat, HIV: Human Immunodeficiency Virus, MDS: Myelodysplastic Syndrome, OAD: Oral Antidiabetic, TNF: Tumor Necrosis Factor

Table 2: CG cut-off values and IgG levels

	IgG < 600	**IgG < 600**	**IgG < 500**	**IgG <**
	Sensitivity	Specificity	Sensitivity	Specifi
Calculated globulin cut-off	Calculated globulin cut-off	Calculated globulin cut-off	Calculated globulin cut-off	Calcula
< 1.6	19,4	96,4	22,3	95,4
< 1.7	38,2	92,5	44,6	90,8
< 1.8	58,1	89,1	66,2	85,9
< 1.9	75,4	84,4	82,7	79,3
< 2.0	83,8	74,9	87,1	68,6
< 2.1	89,5	64,1	92,1	58,2
< 2.2	92,7	51,8	93,5	46,5
< 2.3	94,2	38,7	95,0	34,8
< 2.4	97,4	26,5	98,6	23,8
< 2.5	98,4	13,1	98,6	11,7
Gamma globulin cut-off	Gamma globulin cut-off	Gamma globulin cut-off	Gamma globulin cut-off	Gamm
< 0.2	2,6	100	3,6	100
< 0.3	6,3	100	8,6	100
< 0.4	18,8	100	25,9	100
< 0.5	41,9	100	56,8	99,8

	IgG < 600	**IgG < 600**	**IgG < 500**	**IgG <**
< 0.6	67,5	99,2	84,9	96,6
< 0.7	89,0	89,4	94,2	81,3
< 0.8	94,8	71,9	95,7	63,7
< 0.9	97,9	51,5	97,8	45,3
< 1.0	99,0	29,5	98,6	25,8
< 1.1	99,0	11,1	98,6	9,7
< 1.2	99,5	3,9	99,3	3,4
< 1.3	99,5	1,7	99,3	1,5
< 1.4	99,5	0,6	99,3	0,5
< 1.5	99,5	0,3	99,3	0,2
< 1.7	99,5	0	99,3	0

Table 3: Analysis of the features suggestive of immunodeficiency depending on the CG value

A: Univariate analysis

	CG value <2.0 n (%)	**CG value [?]2.0 n (%)**	**p value**
[?]2 otitis in one year	13 (59.1)	9 (40.9)	NS
[?]2 sinusitis in one year without allergy	20 (43.5)	26 (56.5)	NS
[?]1 pneumonia in one year	24 (68.6)	11 (31.4)	**0.003**
Recurrent parenteral antibiotic usage	22 (81.5)	5 (18.5)	**<0.001**
Presence of infections with opportunistic organisms	16 (76.2)	5 (23.8)	**0.003**
Recurrent viral infections	2 (66.7)	1 (33.3)	NS
Chronic diarrhea with weight loss	2 (28.6)	5 (71.4)	NS
Family history of primary immunodeficiency	1 (100.0)	0 (0.0)	NS
B: Multivariate analysis	**B: Multivariate analysis p value**	**B: Multivariate analysis OR**	**B: Multivariate analysis 95% CI**
[?]1 pneumonia in one year	NS	1.6	0.7 – 3.8
Recurrent parenteral antibiotic usage	**0.018**	**3.6**	**1.2 – 10.7**
Presence of infections with opportunistic organisms	NS	2.4	0.8 – 7.2

CG: Calculated globulin, CI: Confidence interval, NS: Non-significant, OR: Odds ratio

Figure legends:

Figure 1: Flow-chart of patient recruitment process

Figure 2: The correlation analyses between CG versus (vs.) serum IgG (mg/dl) (A) and CG vs. total immunoglobulin (mg/dl) (B). The correlation analyses between gamma globulin vs. total immunoglobin (mg/dl) (C) and gamma globulin vs. serum IgG (mg/dl) (D).

Figure 3: ROC curve analyses to verify if CG (A) and gamma globulin (B) were independent discriminative values to detect subjects with IgG serum levels lower than 600, 500, 400 and 300 mg/dl. AUC: Area under curve, CI: Confidence interval

Figure 1

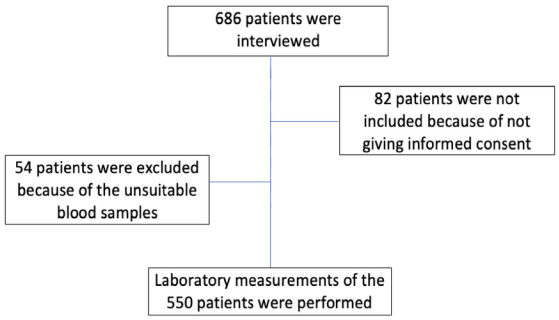

Figure 2

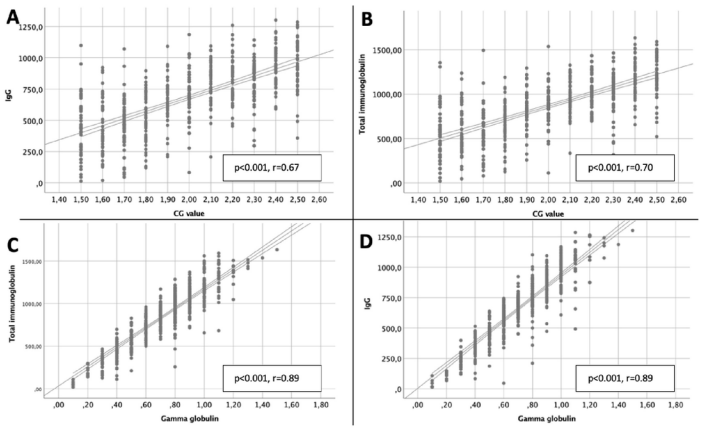

Figure 3