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RESEARCH

Levels of Natural Antibodies Before and After Immunoglobulin Replacement Treatment Affect the Clinical Phenotype in Common Variable Immunodeficiency

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Abstract

Natural antibodies (NAbs) occurring in individuals without prior exposure to specific antigens, provide direct first barrier protection against pathogens, and exert immunoregulation thus actively contributing to the maintenance of immune homeostasis, controlling inflammatory processes and preventing autoimmunity. Common variable immunodeficiency (CVID) is a heterogeneous group of disorders characterized by a compromised immune function that brings into focus the role of NAbs. Our aim was to explore whether NAb levels could serve as potential key indicators in CVID for monitoring disease progression and predicting outcomes. In this study, we analyzed a Hellenic cohort of 56 patients with CVID (31 newly diagnosed and 25 under immunoglobulin replacement therapy-IgRT) and 33 healthy controls, for total Ig levels and serum IgM and IgG NAb levels against five informative target-antigens of NAbs, namely, actin, DNA, carbonic anhydrase, $F(ab')_2$ fragments of human IgG and TriNitroPhenyl. In addition, follow-up pre- and post- IgRT samples were analyzed in ten (10) patients of our cohort. Results showed that Ig-treated patients exhibited significantly lower IgM NAb levels than untreated patients and healthy controls against all panel antigens. In the follow-up samples, pre-treatment IgM NAb levels negatively correlated with total serum IgM. This imbalance was only partially restored after IgRT, with a significant decrease in IgM NAb levels observed in nine out of ten patients. Moreover, post-treatment patients with recurrent infections presented significantly lower IgM NAb levels, a reduction also observed in patients with bronchiectasis independently of treatment status. On the contrary, post-treatment patients with enteropathy had significantly higher IgM NAb levels against all panel antigens, an increase also noted in patients with autoimmune diseases. Regarding IgG NAbs, replacement therapy restored levels to those of healthy controls. In conclusion, impaired NAb levels are found in CVID patients, particularly related to certain phenotypes. Moreover, the significant decrease in IgM NAb levels after IgRT suggests a potential association with disease course and complications. The results suggest that administration of human IgM NAbs may be an effective combinatorial treatment in selected patients. Further research is needed to understand the functional roles of NAbs in CVID and its complex clinical phenotypes.

Keywords CVID · Clinical phenotype · Immunoglobulin Replacement Treatment (IgRT) · Natural Antibodies (NAbs) · IgM · IgG

Introduction

Common variable immunodeficiency (CVID) is a heterogeneous group of disorders characterized by permanent and sustained hypogammaglobulinemia, an inadequate or lacking response to immunization, and a wide spectrum of clinical manifestations, including recurrent infections, autoimmunity, granulomas formation, benign lymphoproliferation and an increased incidence of lymphomas and cancer [1, 2]. The golden standard of treatment in CVID is immunoglobulin replacement therapy (IgRT), intravenously (IVIG)

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or subcutaneously (SCIG), along with antibiotics for the treatment of infections. The management of non-infectious manifestations of CVID remains a challenge for clinicians [3]. Moreover, even following appropriate IgRT and the successful trough IgG levels in the bloodstream, there are still patients who are unable to eradicate infection or who display severe complications, including bronchiectasis [4, 5].

Natural antibodies (NAbs) are germline encoded antibodies present in individuals without prior exposure to specific antigens [6]. NAbs are mainly produced by B1 cells and are mostly of IgM isotype but also of IgG, IgA and IgE isotypes [7]. NAbs are polyreactive, targeting a variety of self and foreign antigens and this property is directly linked to their numerous biological functions [8]. NAbs are considered part of the innate immune system. They play crucial roles in first line host defense by providing immediate protection against pathogens, as well as in clearance of apoptotic cells and immune complexes, and thereby have a major role in maintaining tissue homeostasis and preventing excessive inflammation [9]. Moreover, NAbs serve as important components of the immune system that contribute to immunoregulation and shape the development and function of B and T cells [10, 11]. Despite their potential significance in the pathogenesis of primary antibody deficiency (PAD), the involvement of NAbs in CVID patients is understudied. Lower IgG NAb levels to isohemagglutinins have been previously documented in CVID patients [12], and significant alterations in marginal zone B cells and B1 cells have been reported, yet with contradictory results [13].

To determine whether fluctuations or discrepancies in NAb levels might contribute to the pathogenesis and/or the varied clinical phenotype of CVID, here we investigated NAb levels in CVID patients, before and after treatment initiation (i.e., IVIg replacement) and correlated them with clinical manifestations. We analyzed 56 CVID patients (31 newly-diagnosed, 25 under IgRT), and 33 healthy controls for total Ig levels, and IgM and IgG NAb levels against five target antigens which share informative features associated with NAb recognition and their regulatory functions. Specifically, the target antigens were G-actin, a cytoskeletal component and common target of NAbs implicated in various human diseases [14, 15], DNA, a known target of autoantibodies-markers of systemic autoimmune diseases [16], carbonic anhydrase (CA), an evolutionarily conserved autoantigen associated with red blood cells which frequently mediates autoimmune manifestations [17], $F(ab')_2$ fragments of human IgG, which are major components of NAb-mediated immunoregulatory network via Fab-Fab interactions [18], and TriNitroPhenyl (TNP), a surrogate measure to assess polyreactivity of NAbs [19]. Follow-up analysis of pre- and post-IgRT samples was also performed in ten patients. Data of CVID clinical manifestations for each patient were analyzed together with NAb levels in univariate and multivariate models to reveal potential associations.

Materials and Methods

Patients and Samples

Subjects enrolled in the study are shown in Table 1. Among them, 31 CVID patients were evaluated before the initiation of IgRT, 25 CVID patients were already under IgRT, and 33 were healthy matched controls. All subjects were analyzed in parallel. In addition, 10 newly diagnosed patients were evaluated in follow-up pre- and post IgRT samples. The diagnosis of the disease was based on standard criteria including (a) low serum levels of IgG, IgA and/or IgM, more than two standard deviations below the normal mean for the age; (b) absence of isohemagglutinins and poor responses to vaccines (especially the polysaccharide ones); and (c) an exclusion of other defined causes of hypogammaglobulinemia and/or other types of Inborn Errors of Immunity [20]. The study was approved by the Ethics Committee of the General University Hospital of Larissa in Thessaly (6/18.3.2015), and an informed consent was obtained from all patients.

CVID complications such as frequent recurrent infections (3-4 attacks per year), autoimmunity, bronchiectasis, lymphoproliferation, enteropathy, atopy, cancer and granulomatosis were identified and recorded by the clinician at the time of diagnosis (Table 2). Enteropathy includes chronic diarrhea, malabsorption, and inflammatory bowel disease-like symptoms.

ELISA Measurements

IgM and IgG NAb levels in patient sera were measured by indirect in-house ELISA: i.e., NAbs against DNA from calf thymus (D1501, Sigma Aldrich), CA from human erythrocytes (C3934, Sigma Aldrich), as well as TNP-BSA, human

Table 1 Patients and controls enrolled in the study

Subjects	*n*	Age (yrs±SD)	Sex (Male/Female)	IgRT Duration (mo±SD)
Total	56	43±16	24/32	40±77
Newly diagnosed	31	43±18	12/19	
Follow-up:	10		4/8	
Pre-treatment		40±14		
Post-treatment		44±14		29±25
Under long term IgRT	25	42±15	12/13	94±107
Healthy Controls	33	38±10	10/23	

Table 2 CVID related patients' characteristics

Characteristics	Patients (*n*,%)
Recurrent Infections	52 (85.7%)
Autoimmunity	39 (69.6%)
Bronchiectasis	15 (26.8%)
Lymphoproliferation	34 (60.7%)
Atopy and/or drug allergy	14 (25%)
Enteropathy	11 (19.6%)
Cancer	10 (19.7%)
Granulomatosis	8 (14.3%)

G-actin and IgG-F(ab´)$_2$ fragments prepared as previously described [21, 22]. Antigens were immobilized on high-binding, flat-bottomed 96-well MaxiSorp plates (Nunc, Denmark) at 10μg/ml for actin, DNA and TNP-BSA, 5 μg/ml for CA and 2 μg/ml for F(ab´)$_2$ fragments.

Briefly, plates were coated overnight at 4°C in carbonate-bicarbonate buffer 0,1M pH 9,6. Plates were thoroughly washed with PBS, and then saturated for 1 h at 37 °C with PBS containing 1% BSA. Patient sera diluted 1/50 in PBS containing 0.1% Tween and 1% BSA (PBS-T-BSA) were incubated independently with each panel antigen overnight at 4 °C. Plates were washed and incubated with secondary antibodies (goat anti-human -μ, and -γ chain) conjugated with alkaline phosphatase diluted at optimum predefined concentration in PBS-T-BSA for 2 h at 37°C. After thorough wash, antibody binding to immobilized antigen was assessed with the addition of the enzyme's soluble chromogenic substrate p-nitrophenyl phosphate disodium hexahydrate (pNPP) (Sigma-Aldrich) and optical density (OD) of colored reaction product was measured at 405 nm (620 nm reference) using a spectrophotometer (TECAN Spark Control Magellan V2.2, Grödig/Salzburg, Austria). Values were converted to arbitrary units by the equation AU=OD*1000. All experiments were run in duplicate and three positive control sera (previously defined as reference samples) were always added in each plate for inter-assay normalization (<15%).

Statistical Analysis

The d'Agostino-Pearson omnibus normality test was used to assess the normality of the data distribution. For non-parametric two independent group comparisons the Mann–Whitney U test was used. The non-parametric (ANOVA) Friedman test was used to detect differences between groups. The non-parametric Spearman correlation matrix was employed to assess the strength and direction of monotonic relationships between total serum IgM and IgM NAb levels. In all cases, the significance level was set at 5%, the tests were two sided and a result was considered significant if the estimated *p*-value was less than the significance level ($p<0.05$). Statistical analysis was performed and graphs were made in GraphPad Prism version 9.0.0 (GraphPad Software, San Diego, California USA).

Results

IgM NAb Levels among CVID Patients and Healthy Controls

Among the 56 patient sera, five newly diagnosed patients and six patients under IgRT had undetectable serum IgM and were excluded from this analysis. Therefore, levels of serum IgM NAbs against G-actin, DNA, CA, F(ab´)$_2$ and TNP were estimated in 26 newly diagnosed patients and 19 patients under treatment, as well as in 33 healthy individuals, as shown in Fig. 1. We found significantly lower NAb levels in Ig-treated patients compared to newly diagnosed patients ($p<0.01$). Moreover, for all antigens tested, NAb levels were significantly lower in both newly diagnosed and treated patients compared to healthy controls, reaffirming the deficiency of antibody production within these patients.

Association of Serum IgM NAb Levels with Total IgM in Follow-Up Samples

Lower total serum IgM was found in IgRT-patients when compared to newly diagnosed patients ($p<0.01$) and healthy controls ($p<0.001$). In order to evaluate the relationship of total serum IgM with NAb levels, we analyzed ten (10) patients of our cohort with paired samples (pre- and post-treatment) and with detectable levels of total IgM. Figure 2 illustrates IgM NAb levels of the panel antigens for pre- and post- IgRT patient samples. Eight (8) out of the ten patients exhibited a significant decrease in IgM NAb levels for all panel antigens ($p<0.05$). The study revealed two outliers, one 47 year-old male, with significantly increased IgM NAb levels for all panel antigens, and one 49 year-old female, with increased anti-CA and anti-TNP IgM NAb levels post-treatment. Both were autoimmune patients, with co-existing clinical manifestations such as lymphoproliferation and enteropathy in the first case, and recurrent infections and brochiectasis in the second, while neither of them had cancer. Interestingly, these two patients had no significant variations in total serum IgM in the pre- and post- treatment samples.

To evaluate the relationship of total serum IgM with IgM NAb levels, we performed a correlation analysis and Fig. 3 represents a correlation matrix graph with given spearman values (r) (see also supplementary material – Table S1). Levels of IgM NAbs against all studied antigens were negatively correlated with total serum IgM in pre-treatment

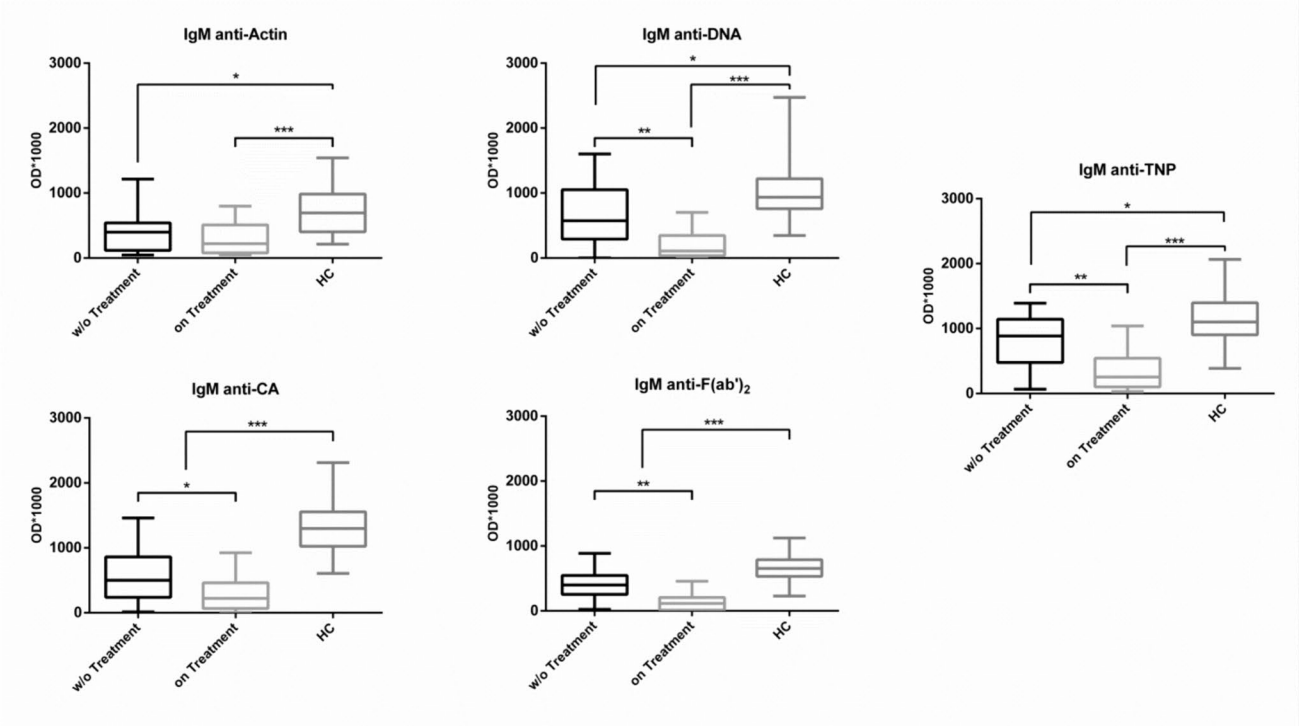

Fig. 1 IgM NAb levels in serum of CVID patients and healthy controls. IgM NAb levels against actin, DNA, CA, F(ab´)$_2$ fragments and TNP were measured for CVID patients without any treatment (n=26), CVID patients on IgRT (n=19) and healthy controls (n=33). *p<0.05. **p<0.01, ***p<0.001

samples (Fig. 3A), whereas this imbalance was only partially restored post- treatment (Fig. 3B). After IVIg therapy, levels of anti-TNP IgM NAbs were positively correlated with total serum IgM (r=0.49), anti-G-actin, anti-CA, and anti-F(ab´)$_2$ NAbs showed intermediate correlation, while anti-DNA IgM NAbs remained negatively correlated with total serum IgM (r=-0.36).

Association of IgM NAb Levels with CVID-Related Clinical Complications

We analyzed the levels of IgM NAbs in relationship to CVID clinical complications such as the existence of frequent infections, bronchiectasis, autoimmunity, enteropathy, atopy, granulomatosis and cancer. Table 3 presents the significant differences in NAb levels targeting G-actin, DNA, CA, F(ab´)$_2$ and TNP among patients with different CVID related complications. We found that after the initiation of IgRT, patients with low incidence of infections had significantly higher levels of NAbs against all antigens tested, when compared to those patients with high incidence of infections. Interestingly, we found significantly decreased levels of NAbs in patients with bronchiectasis when compared to those without, independently of treatment status. For CVID patients with enteropathy, we found increased levels of NAbs against all panel antigens after treatment initiation, when compared to those without enteropathy. For CVID patients with autoimmunity, we found significantly increased levels of NAbs against actin, CA and TNP after treatment initiation, when compared to patients without any autoimmune disorder. No significant differences in NAb levels were found in patients exhibiting lymphoproliferation, granulomatosis or cancer. When demographic data (e.g. age and sex) and CVID related phenotypes were analyzed together in multivariate linear regression models (see also supplementary material – Table S2), a correlation between anti-CA IgM NAb levels and brochiectasis and granulomatosis was found in patients at diagnosis. Additionally, for patients on IgRT, the correlation of IgM NAb levels with enteropathy was confirmed and evident for all panel antigens while anti-G-actin and anti-CA IgM NAb levels were correlated with recurrent infections.

IgG NAb Levels in CVID Patients Compared to Healthy Controls

Levels of serum IgG NAbs against G-actin, DNA, CA, F(ab´)$_2$ and TNP were estimated in 24 newly diagnosed patients with detectable serum IgG, 25 patients under IgRT and 33 healthy individuals, as shown in Fig. 4. We found

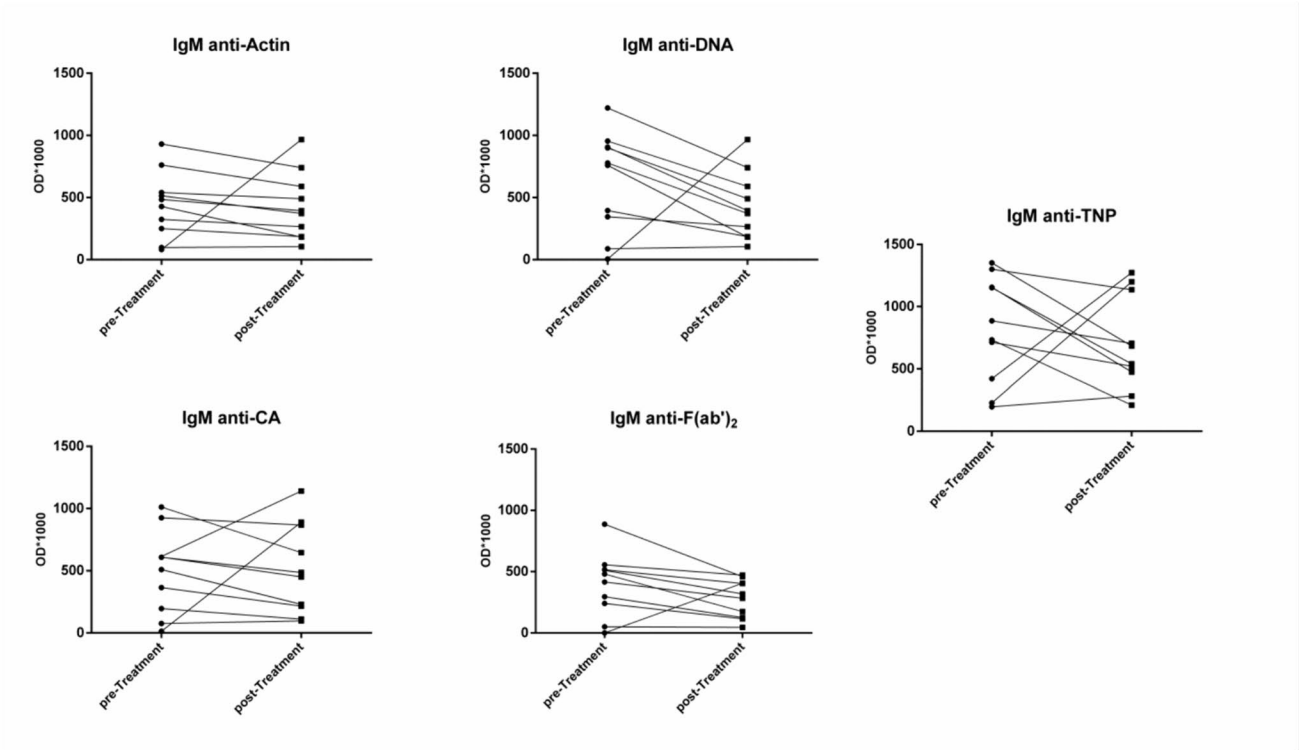

Fig. 2 IgM NAb levels among CVID patients and healthy controls. Levels of IgM NAbs against actin, DNA, CA, F(ab′)$_2$ fragments and TNP were measured in ten (10) CVID patients with follow-up pre- and post- IgRT samples. Every dot in the plot represents an individual patient in the left column and a line is connecting the dot of the same individual in the right column

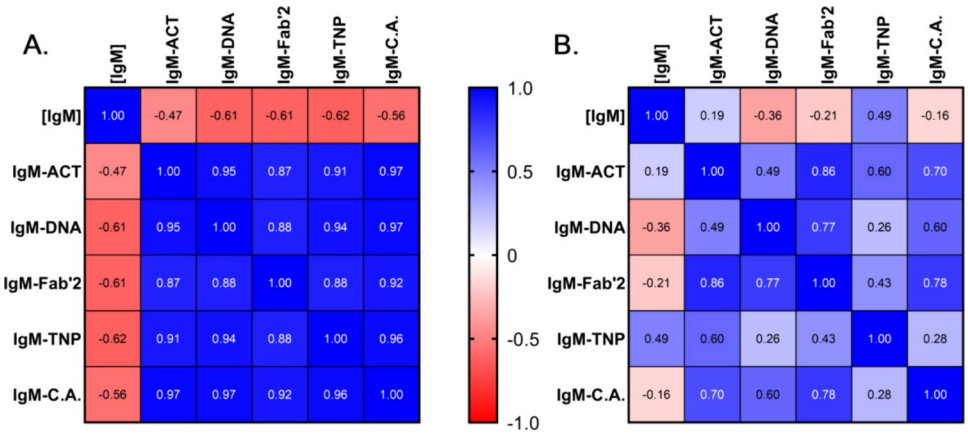

Fig. 3 Spearman correlation matrix for total IgM concentration and IgM NAb levels. **A**: Total serum IgM of CVID patients pre-treatment correlated to the respective values of NAb levels. **B**: Total serum IgM of CVID patients post- IgRT correlated to the respective values of NAb levels. The same patients were analyzed (n=10) before and after the initiation of the IVIg replacement therapy

higher NAb levels in treated patients compared to newly diagnosed patients. Anti-F(ab')$_2$ NAb levels were significantly higher in treated patients compared to healthy controls (p<0.01), reflecting the polyvalency of IVIg administration.

Discussion

Underlying immune mechanisms in CVID are yet not fully understood, despite considerable progress in understanding other primary immunodeficiency diseases at the molecular level [23]. The diverse clinical manifestations of CVID suggest that various defects in immune regulation may converge upon a common pathway, resulting in reduced levels of immunoglobulins [24]. The term CVID, currently used

Table 3 Analysis of IgM NAb levels in relation to different CVID-related clinical complications

Table 3	Infections			Brochiectasis			Enteropathy			Autoimmunity		
Ag	All patients	Patients at Diagnosis	Patients on Treatment	All patients	Patients at Diagnosis	Patients on Treatment	All patients	Patients at Diagnosis	Patients on Treatment	All patients	Patients at Diagnosis	Patients on Treatment
Actin	↓ **(*p*=0,048)**	↓ NS	↓ **(*p*<0.001)**	↓ **(*p*=0,011)**	↓ NS	↓ NS	↑ **(*p*=0,030)**	↑ NS	↑ **(*p*=0.001)**	↑ NS	↑ NS	↑ **(*p*=0,011)**
DNA	↓ NS	↓ NS	↓ **(*p*=0.008)**	↓ **(*p*=0,001)**	↓ **(*p*=0,002)**	↓ NS	↑ **(*p*=0,042)**	↑ NS	↑ **(*p*=0.033)**	↑ NS	↑ NS	↑ NS
CA	↓ NS	↓ NS	↓ **(*p*<0.001)**	↓ NS	↓ NS	↓ **(*p*<0.010)**	↑ **(*p*=0,047)**	↑ NS	↑ **(*p*=0.005)**	↑ NS	↑ NS	↑ **(*p*=0,005)**
$F(ab')_2$	↓ NS	↓ NS	↓ **(*p*=0.001)**	↓ **(*p*=0,002)**	↓ NS	↓ **(*p*<0.034)**	↑ **(*p*=0,012)**	↑ NS	↑ **(*p*<0.001)**	↑ NS	↑ NS	↑ NS
TNP	↓ **(*p*=0,029**	↓ NS	↓ **(*p*=0.002)**	↓**(*p*<0,001**	↓ NS	↓ NS	↑ NS	↑ NS	↑ **(*p*=0.010)**	↑ NS	↑ NS	↑ **(*p*=0,006)**

Levels of NAbs against G-actin, DNA, CA, $F(ab')_2$, and TNP were compared among patients with or without common CVID complications such as incidence of high infections, brochiectasis, enteropathy and autoimmunity. Arrows show the increase (↑) or decrease (↓) in NAb levels among patients with different CVID complications compared to those without, and *p* values are given in the cases of significant differences

to describe this condition, is likely to encompass a range of disorders that share the common feature of deficient B cell differentiation, impaired immunoglobulin production and dysregulated immune responses [25, 26]. Failure of the immune system in PADs is associated with recurrent infections, autoimmunity, and cancer.

Insufficient responses to glycan-based vaccines or low titers of isohemagglutinins, i.e. NAbs to polysaccharide blood group antigens, are characteristic and diagnostic features of CVID [10]. Unfortunately, there are few studies highlighting the importance of identifying other NAbs as informative markers for CVID patients, and clinical assessments are often based on reactivity to a limited antigen panel. Previously, an altered human carbohydrate-specific IgG NAb repertoire was described in CVID patients involving significant qualitative glycan-recognition defect profiles for Gala- and GalNAc-reactivity [27]. Additionally, impaired recognition of microbial, self-antigens, and tumor-associated carbohydrate antigens may also occur in these patients. Therefore, NAb repertoire analysis could serve as a valuable tool to understand the extent and clinical implications of immune system failure in individual patients, and provide valuable insights for clinical decision-making. Here, we selected representative target antigens to assess natural autoimmunity in CVID patients and normal individuals, as previously described [28]. We select proteins like actin and CA, nucleic acids like DNA, and haptens like TNP, to comprehensively analyze the measurement and variability of NAbs in serum. NAbs that bind to actin, CA and DNA are considered to be part of the autoreactive NAb repertoire, and can be used as a measure of internal immune regulation [9]. On the other hand, subjects are not normally exposed to TNP, and it can be used as a measure of polyreactivity and exogenous recognition. Moreover, *in vitro* measurements of autoreactive NAbs to $F(ab')_2$ fragments of human IgG, known to participate in immunoregulatory mechanisms *in vivo,* enabled us to detect changes in the dynamics of the antibody (Fab-Fab) mediated network [29]. Even so, given the myriad potential targets of NAbs, defining a minimal set of panel antigens that reflect natural autoimmunity in humans is challenging.

Disturbances in NAb levels may compromise immune responses to pathogens, increasing susceptibility to autoimmune manifestations and promoting cancer development [30]. This study along with other observations [12], suggest that the severity and course of CVID in patients may be impacted by serum levels of NAbs, with lower levels being associated with more severe disease progression, increased susceptibility to infections, and potential complications. Acute lung infections can cause pneumonia, and long-term lung infections can cause a chronic form of bronchitis known as bronchiectasis, which is characterized by thickened airway walls colonized by bacteria. IgM NAbs targeting surface

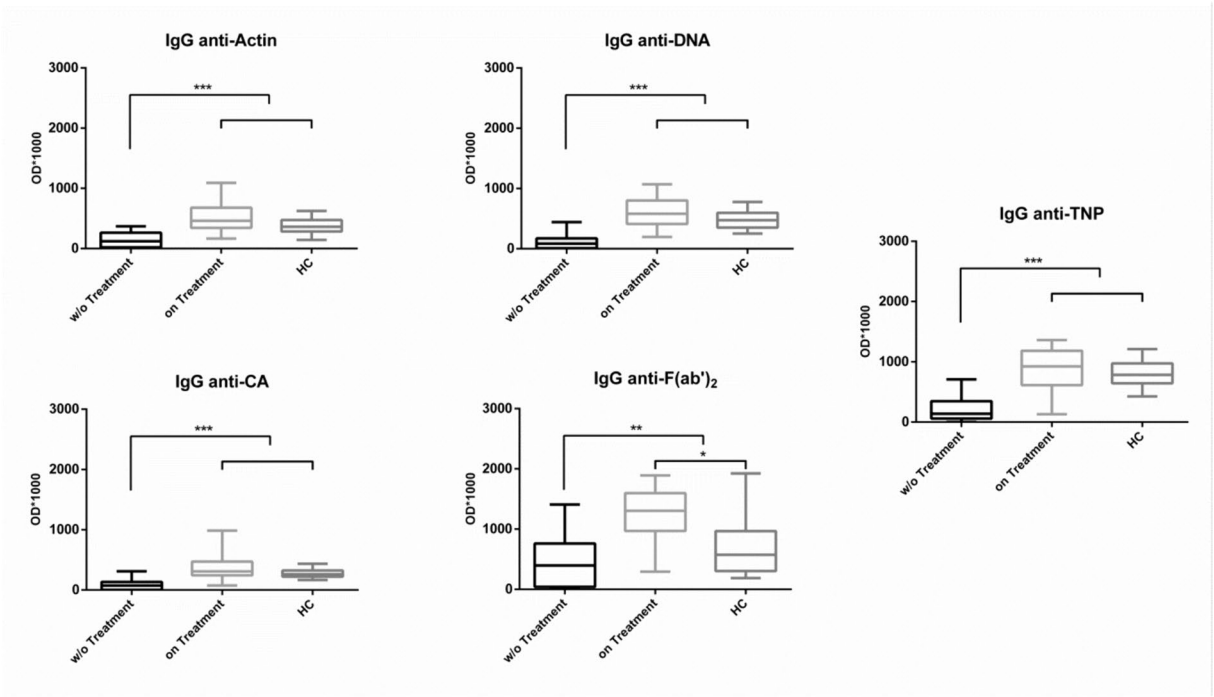

Fig. 4 IgG NAb levels in CVID patients and healthy controls. IgG NAb levels against G-actin, DNA, CA, F(ab´)$_2$ fragments and TNP were measured for CVID patients without any treatment (n=24), CVID patients on IgRT (n=25) and healthy controls (n=33). *p<0.05. **p<0.01, ***p<0.001

carbohydrates on pathogens are conserved across many species, including fish and mammals [31, 32]. Moreover, Rita Carsetti et al., have previously described an inverse correlation between the frequency of IgM memory B cells and susceptibility to bacterial lower respiratory tract infections, and together with anti-pneumococcal polysaccharide (anti-PnPS) IgM NAb levels can effectively discriminate low or high-risk patients for recurrent infections caused by trapped bacteria and low or high risk of bronchiectasis [33]. Considering the importance of IgM NAbs as key players in first-line defense and pathogen elimination, our results are consistent with the hypothesis that lower IgM NAb levels lead to loss of protection and susceptibility to infection. Most importantly we found that following IVIg replacement therapy, patients who still experienced recurrent infections had significantly lower IgM NAb levels compared to those with lower incidence of infection, a finding confirmed by linear regression analysis for NAbs targeting G-actin and CA. Further, patients with bronchiectasis generally exhibited lower IgM NAb levels regardless of treatment status.

Another possible complication associated with CVID is enteropathy, which may result in symptoms such as chronic diarrhea, malabsorption, weight loss, and abdominal pain. It is known that enteropathy in CVID patients is a result of dysregulated immune responses in the gastrointestinal tract. The specific mechanisms linking NAbs to CVID-associated enteropathy have not been fully elucidated. Previous studies show that levels of secretory IgM in IgA PAD patients are increased as a possible regulatory feedback mechanism involved in mucosal homeostasis and host-microbial interaction [34]. In this study, patients with enteropathy had significantly increased levels of IgM NAb against all panel antigens, and a strong correlation of IgM NAb levels and enteropathy was confirmed for patients on treatment by regression analysis. Considering that enteropathy may emerge independently of infection, IgRT is expected to have limited effect on treated patients although improved clinical outcomes have been observed in some cases [35]. Given the importance of secretory IgM in gut immune homeostasis, our preliminary data makes it challenging to determine whether NAb levels have a direct effect on the enteropathy phenotype and this important finding should be further explored including a larger number of patients [36].

Autoimmune and chronic inflammatory disorders result from a complex interplay of genetic and environmental factors, making it difficult to distinguish specific defects that cause dysregulation of apoptotic cell clearance. Furthermore, despite the observed protective role of higher levels of certain IgM antibodies against various disease manifestations, individuals with autoimmune diseases (autoimmune patients) may still exhibit an overall increase in these beneficial autoantibodies compared to healthy controls [37]. It has been suggested that

certain specificities within the pool of circulating IgM might undergo an increase as part of a positive feedback mechanism, indicative of a compensatory effort to resolve inflammation and enhance the clearance of apoptotic cells. This is in line with our results that autoimmune patients had increased levels of NAbs against all panel antigens following IVIg replacement therapy, suggesting that this positive feedback loop was activated by or after initiation of therapy.

It is well known that polyvalent IVIg preparations consisting of pooled IgG antibodies derived from thousands of donors, contain a wide range of specificities, making them very effective in immune replacement therapies [38]. In PAD patients, different IVIg preparations are used at different doses for diverse clinical phenotype and patient profiles. In general, high-dose IVIg elicits a more potent immunosuppressive and anti-inflammatory response compared to lower doses [39]. Although research has shed light on various mechanisms of IVIg's mode of action, these effects are difficult to generalize and must be considered on a case-by-case basis for each specific disease. A decrease in B-1 cells and IgM immunoglobulins after IVIg replacement treatment in selected CVID patients has been previously described, and to our knowledge the current study is one of the few studies to report a decrease in IgM NAb levels in these patients after IgRT [40]. Considering that in healthy state the vast majority of circulating IgM antibodies are NAbs, the negative correlation of IgM NAb levels with total IgM concentration before IgRT in our follow up analysis of CVID patients is indicative of immune dysregulation in these patients [41]. Furthermore, IgM NAb levels were still not correlated with total IgM concentration after IgRT, indicating a potential treatment inefficiency in terms of restoring IgM NAb levels. Additionally, two out of ten patients showed increased NAb levels after IgRT, without profound changes in total IgM concentration. This could be attributed either to the onset of an infection occurring at the time of sampling or as an outcome of IgRT, emphasizing once more the variability of CVID patients as a group of disorders and the multivalent mode of IgRT effects. Taking all together, decreased IgM NAb levels may affect the clinical phenotype and the course of CVID. This is already a matter of intensive research in order to achieve an effective treatment for all patients with PAD [42].

The dysregulated production of NAbs in CVID implicates these antibodies in several aspects of immune regulation, development of adaptive immune system and the continuous regulation of the adult B cell repertoire. Our study highlights the importance of NAbs in patients with CVID, where serum levels vary among the spectrum of CVID clinical phenotypes and have potential utility in diagnosis and treatment. The significant decrease in IgM NAb levels after IgRT, suggests a potential association with disease course and complications, and that administration of IgM-enriched preparations might enhance the effectiveness of IgRT in selected patients, as has been proposed also by others [43]. Further research is needed to understand the functional roles of NAbs in CVID and its complexity with respect to clinical phenotypes.

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Institutional Review Board Statement The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the University General Hospital of Larissa (29268/16-07-2019).

Author Contribution Conceptualization, P.L. and A.G.; methodology, I.S., P.L.; software, I.S., G.T.; validation, M.S and A.G.; formal analysis, I.S, G.T.; investigation, I.S.; resources, F.K., M.S., P.L.; data curation, I.S., G.T., F.K.; writing original draft preparation, I.S., G.T.; writing—review and editing, P.L., M.S., A.G.; visualization, I.S., G.T.; supervision, P.L.; project administration, All authors reviewed the manuscript and have agreed to the published version of the manuscript.

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Data Availability The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Informed Consent Statement Informed consent was obtained from all subjects involved in the study.

Competing Interests The authors declare no competing interests.

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