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Original study article

Evaluation of the effectiveness of combined therapy with intravenous immunoglobulin and plasmapheresis in patients with steroid-resistant form of pemphigus based on the cytokine and chemokine profiles assessment

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ABSTRACT

BACKGROUND: Pemphigus is a serious life-threatening disease characterized by the formation of IgG autoantibodies against the cell membranes, leading to the formation of intraepidermal blisters.

AIM: To evaluate the effectiveness of combined therapy with intravenous immunoglobulin and plasma exchange for steroid-resistant patients with pemphigus based on the cytokine, chemokine and granulysin profiles investigation.

MATERIALS AND METHODS: The group of patients receiving systemic glucocorticoid monotherapy (Group 1; control group) consisted of 26 patients with pemphigus vulgaris. The group of steroid-resistant patients (Group 2; main group) who received combined therapy with systemic glucocorticoid, intravenous immunoglobulin (IVIg), and plasmapheresis included 15 people. The presence of steroid resistance was assessed by Murrell consensus (2008). All pemphigus patients received the initial dose of systemic glucocorticoids of 80–100 mg/day with subsequent slow tapering, according to guidelines. The treatment protocol for combined therapy included four sessions of discrete plasma exchange per week every other day. Immediately after completion of the plasma exchange cycle, IVIg was added to the ongoing treatment, with a total dose of 2 g/kg per cycle. The levels of IL-4, IL-10, IL-15, TNF-α, chemokines CXCL8, CCL11, and granulysin were assessed via ELISA method.

RESULTS: We observed some discrepancies in cytokine profiles in both groups of patients. In patients who received combined therapy, there was a statistically significant decrease in the levels of IL-4, IL-15, TNF-α compared to those in patients on systemic glucocorticoid monotherapy — IL-4, IL-15 TNF-α ($p < 0.01$). Notably, that the level of CCL11 in serum of steroid-resistant patients before the IVIg therapy was significantly higher (Me=51 pg/ml) compared to systemic glucocorticoid monotherapy group (Me=10 pg/ml; $p < 0.01$). The level of granulysin after the treatment with IVIg and plasma exchange in group 2 was also significantly lower (Me=0 ng/ml) compared to the group of control (Me=2700 ng/ml respectively; $p < 0.01$).

CONCLUSION: We found a trend towards higher serum levels of IL-4, IL-15, and CCL11 in steroid-resistant pemphigus patients who received combined therapy with IVIg and plasma exchange compared to the control group. Moreover, these cytokines can be considered as the potential biomarkers for refractory disease course, and might be used as therapeutic targets in the future. It should be also noted that the prolonged remission of patients receiving combined therapy with systemic glucocorticoid, IVIg, and plasma exchange, was on average two years.

Keywords: cytokine profile; chemokines; granulysin; pemphigus; steroid resistance; intravenous immunoglobulin; plasmapheresis.

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Оригинальное исследование

Оценка эффективности комбинированной терапии плазмаферезом и человеческим иммуноглобулином больных стероидрезистентной формой пузырчатки на основании исследования цитокинового и хемокинового профиля

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АННОТАЦИЯ

Обоснование. Акантолитическая пузырчатка представляет собой тяжёлое жизнеугрожающее заболевание, в основе которого лежит формирование аутоантител класса IgG к мембранам клеток шиповатого слоя с последующим формированием интраэпидермальных пузырей.

Цель исследования — оценить эффективность комбинированной терапии человеческим иммуноглобулином и плазмаферезом стероидрезистентной акантолитической пузырчатки на основании исследования цитокинового и хемокинового профиля, а также уровня гранулизина в сыворотке.

Материалы и методы. В контрольной группе (группа 1; *n*=26) пациенты с вульгарной пузырчаткой получали монотерапию системными глюкокортикоидами, в группе стероидрезистентных больных (группа 2; основная; *n*=15) — комбинированную терапию системными глюкокортикоидами, человеческим иммуноглобулином (IVIg) и плазмаферезом. Наличие стероидной резистентности оценивали с помощью критериев Murrell. Все больные пузырчаткой получали начальную дозу системных глюкокортикоидов 80–100 мг/сут с последующим медленным снижением, согласно клиническим рекомендациям. Протокол лечения комбинированной терапии включал четыре сеанса дискретного плазмафереза в неделю через день. Сразу по завершении цикла плазмафереза к проводимому лечению добавлялся человеческий иммуноглобулин, общая доза которого составляла 2 г/кг за цикл. Концентрация IL-4, IL-10, IL-15, TNF-α, хемокинов CXCL8, CCL11 и гранулизина оценивалась с помощью метода иммуноферментного анализа.

Результаты. У пациентов основной группы (комбинированная терапия) наблюдалось статистически значимое снижение уровня цитокинов IL-4, IL-15, TNF-α по сравнению с профилем контрольной группы (p <0,01). Следует также отметить, что уровень CCL11 в сыворотке стероидрезистентных больных до начала лечения человеческим иммуноглобулином и плазмаферезом был статистически значимо выше (Me=51 пг/мл), чем у пациентов группы монотерапии системными глюкокортикоидами (Me=10 пг/мл; p <0,01). Уровень гранулизина после лечения в основной группе (Me=0 нг/мл) был также статистически значимо ниже по сравнению с группой контроля (Me=2700 нг/мл; p <0,01).

Заключение. Обнаружена тенденция к более высокой концентрации IL-4, IL-15 и CCL11 в сыворотке стероидрезистентных больных акантолитической пузырчаткой, получавших комбинированную терапию, по сравнению с группой контроля, что подтверждает иммуномодулирующее действие человеческого иммуноглобулина. Кроме того, данные цитокины можно рассматривать с точки зрения потенциальных биомаркеров более рефрактерного течения акантолитической пузырчатки, а также их использования в качестве терапевтической мишени в будущем. Следует отметить и достижение более длительной ремиссии этими пациентами, которая составила в среднем 2,5 года.

Ключевые слова: цитокиновый профиль; хемокины; гранулизин; пузырчатка; стероидная резистентность; внутривенный иммуноглобулин; плазмаферез.

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BACKGROUND

Acantholytic pemphigus (AP) is a severe, life-threatening disease of the skin and mucous membranes. It is characterized by the formation of immunoglobulin G (IgG) autoantibodies against the intercellular substance of the spinous and granular layers of the epidermis, which subsequently leads to the formation of intraepidermal vesicles. Activation of B cell immunity in pemphigus is initiated by genetic factors, in particular by the association with human leukocyte antigens (HLA) of classes I and II histocompatibility [1].

Systemic glucocorticoids (SGCs) are the first-line drugs for the treatment of AP. The use of these drugs since the 1950s of the 20th century has allowed to reduce the mortality to an average of 30%, which reached 75% in the "presteroid era." The average remission in such patients, according to recent reports, is approximately six years. However, some patients with AP exhibit low sensitivity to high-dose therapy with SGCs, as evidenced by the absence of a pronounced positive therapeutic effect, frequent relapses, and complications. This phenomenon has been termed "steroid resistance" in the scientific literature [2].

In 2008, Murrell et al. [3] presented clinical criteria for non-response of patients with pemphigus to SGCs. These criteria include the following:

1) emergence of new rashes, accompanied by the expansion of preexisting lesions;
2) absence of healing of existing rashes despite three weeks of treatment with SGCs at a daily dosage of 1.5 mg/kg, either as monotherapy or in combination with adjuvant drugs.

Adjuvant drugs, particularly immunosuppressive medications such as rituximab, cyclophosphamide, azathioprine, cyclosporine, methotrexate, mycophenolate mofetil, and plasmapheresis, are frequently used in combination with SGCs to enhance the efficacy of treatment regimens. These drugs reduce the need for high daily doses of SGCs, thereby mitigating side effects and allowing more active SGC dose reduction [1, 4].

Human or intravenous immunoglobulin (IVIG) may also be used as adjuvant therapy in patients with AP, especially the resistant form, according to studies and clinical guidelines. In addition, IVIG is known to have steroid-sparing effects. The mechanism of IVIG action is complex and includes the regulation of Fc receptors, the interaction of cytokines, and the differentiation and function of both T and B cells. For example, Keskin et al. [5] assessed the serum levels of interleukins (IL) 1b, 6, 8, 4, 10, and IFN-γ during IVIG therapy. The level of cytokines was shown to decrease more rapidly in patients undergoing combination therapy with SGCs and IVIG compared with monotherapy with SGCs. However, the impact of combination therapy involving plasmapheresis and IVIG on the cytokine and chemokine profile, as well as the serum level of granulysin in patients who are insensitive to SGCs, remains to be elucidated.

The study aimed to evaluate the cytokine and chemokine profile in patients with AP who were treated with a combination of IVIG and plasmapheresis.

MATERIALS AND METHODS

Study design

It was a prospective cohort study.

The primary endpoint was to determine differences in the levels of cytokines IL-4, IL-10, IL-15, tumor necrosis factor α (TNF-α), chemokines (CXCL8 and CCL11), and granulysin between patients with AP who were administered SGC monotherapy and those who received combination therapy involving IVIG, plasmapheresis, and SGCs.

Intermediate endpoints encompassed the assessment of the level of cutaneous lesions (Pemphigus Disease Area Index, PDAI) prior to, during, and following combination therapy involving IVIG and plasmapheresis.

Eligibility criteria

Inclusion criteria: histologically and immunohistochemically confirmed diagnosis of pemphigus (Fig. 1); patients aged 18 years and older.

Exclusion criteria: patient's refusal to participate in the study.

Study setting

The study was conducted at the Department of Skin and Venereal Diseases named after V.A. Rakhmanov of the Federal State Autonomous Educational Institution of Higher Education Sechenov First Moscow State Medical University of the Ministry of Health of Russia (Sechenov University), Department of Molecular Pharmacology and Radiobiology named after Academician P.V. Sergeev of the Federal State Autonomous Educational Institution of Higher Education Pirogov Russian National Research Medical University, and Blood Center of the Federal State Autonomous Educational Institution of Higher Education Sechenov First Moscow State Medical University of the Ministry of Health of Russia.

Study duration

The study was conducted between 2019 and 2023.

Intervention description

All patients diagnosed with pemphigus received an initial dosage of 80–100 mg/day of SGCs, followed by a gradual reduction in accordance with established clinical guidelines. The treatment protocol encompassed four sessions of discrete plasmapheresis per week, administered on alternate days. After the completion of each plasmapheresis cycle, IVIG was introduced as an adjunctive treatment, with a total dosage of 2 g/kg per cycle. This dosage was then divided into five equal doses and administered intravenously over five consecutive days.

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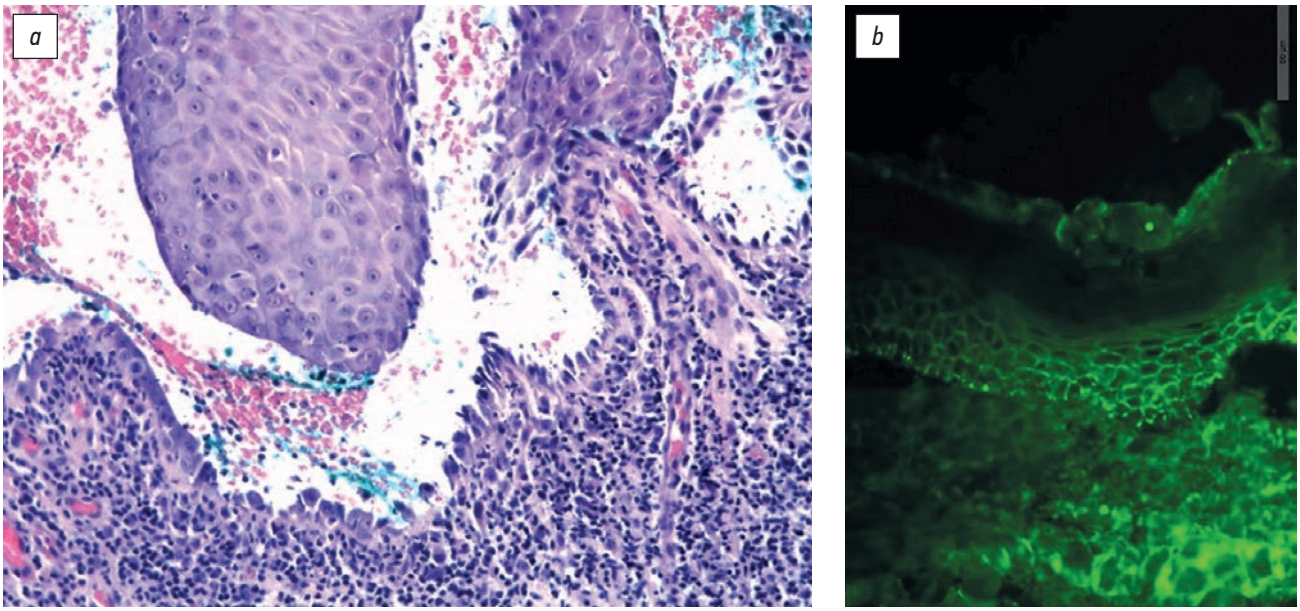

Fig. 1. Suprabasal acantholysis in pemphigus vulgaris (*a*) and IgG fixation in stratum spinosum (*b*).

Findings

Main study outcome. The study revealed no fatal outcomes or severe complications in patients with AP. The main study outcome was an assessment of differences in the serum levels of IL-4, IL-10, IL-15, TNF-α, CXCL8, CCL11, and granulysin in patients with AP undergoing monotherapy with SGCs and combination therapy with SGCs, IVIG, and plasmapheresis. The endpoints were analyzed based on the consensus of Murrell et al. (2008) [3].

The late endpoints of the disease course were as follows:

1) Complete remission defined as the absence of new rashes and the growth of old lesions in the absence of systemic therapy with SGCs for at least two months;
2) Complete remission achieved during therapy with minimal doses of SGCs (⩽10 mg/day) as evidenced by the absence of new rashes for at least two months;
3) Partial remission observed following the complete discontinuation of SGCs for at least two months with persistent rashes that healed within one week;
4) Partial remission during minimal therapy with SGCs (⩽10 mg/day) and topical steroids with persistent rashes and the appearance of new rashes that healed within one week [3].

Subgroup analysis

The cohort of patients administered monotherapy with SGCs (Group 1; control group) comprised 26 patients diagnosed with AP; of these, four were male and 22 were female, with an average age of 53 years. The group of steroid-resistant subjects (Group 2; study group) receiving combination therapy with SGCs, IVIG, and plasmapheresis included 15 patients. Of these, three were male and 12 were female, with an average age of 52 years. The group of healthy donors comprised 43 individuals, including 19 males and 24 females, with an average age of 51 years. Consequently, the groups were comparable in age and sex. The presence of steroid resistance was evaluated using the consensus criteria established by Murrell et al. (2008) [3].

Four (26.7%) patients in the study group received three cycles of IVIG and plasmapheresis, and 11 (73.3%) patients received 5 cycles each. The mean daily dose of SGCs was 85 mg. Methods for registration of outcomes

A blood sample was collected from all patients and placed in a tube containing ethylenediaminetetraacetic acid (EDTA). Levels of the cytokines TNF-α, IL-4, IL-15, IL-10, granulysin, and the chemokines CXCL8 and CCL11 were determined by cytometric bead array and enzyme-linked immunosorbent assay (ELISA).

The analysis of cytokines was conducted using an assay system comprising immunoassay kits and panels (Millipore MILLIPLEX Human Cytokine Panel I Premixed 7 Plex, HCYTOMAG60K07). Serum samples were subjected to incubation with antibody-coated granules at 4 °C. Subsequent incubation steps involved the addition of biotin-labeled antibodies directed against human cytokines, followed by incubation with streptavidin and phycoerythrin. The samples were analyzed using a Luminex 200 instrument (USA) with xPOTENT software. Standard concentration curves of recombinant human cytokines were used to convert fluorescence units to concentrations (pg/mL). The 5-parameter logistic method or the spline curve approximation method was employed to calculate cytokine concentrations in serum samples.

Ethical review

The study was approved by the Ethics Committee of the Sechenov University (Protocol No. 03-22 dated February 03, 2022).

Statistical analysis

Contemporary universal non-parametric (randomization and reversal) algorithms were used to construct confidence intervals (CIs) and statistical comparisons using bootstrap and Monte Carlo methods.

For the CIs, a compact form of recording was employed, wherein the lower and upper limits of the interval were designated as subscripts to the left and right of the point estimate, respectively.

A statistical description of the quantitative indicators was conducted, including the estimation of mean and median values with 95% CI. Additionally, the distribution was assessed for its conformity to a normal law. The standard deviation and coefficient of variation around the mean were also calculated.

A quantitative comparison of two groups was conducted using the parametric Student's t-test for independent samples and the non-parametric Mann–Whitney U-test. The null hypothesis for the Student's *t*-test for each parameter was defined as no difference in mean values between Groups 1 and 2, whereas the alternative hypothesis was defined as the difference in mean values. The null hypothesis for the Mann–Whitney U-test posited that the distributions of the corresponding attribute (and, consequently, the median values) in the groups with the presence/absence of a certain categorical attribute were equivalent. The alternative hypothesis stipulated that the distributions of the attribute (and median values) were different. If the null hypothesis was rejected, a relationship was confirmed between a given quantitative factor and a binary indicator (presence/absence of a given attribute). For parametric criteria, the mean difference with 95% CI was demonstrated, whereas for non-parametric criteria, the Hodges-Lehman median difference with 95% CI was exhibited. A Cohen's or Hedges' standardized difference effect (for groups of <16 subjects) or a biserial correlation coefficient was calculated from the comparison of both groups. The observed *p*-value was sample size dependent, whereas the standardized effect was not. The effect was interpreted according to the lower limit of the CI.

RESULTS

Participant characteristics

The study included patients diagnosed with AP (*n*=41), with a mean age of 52 years. Of these patients, 26 received SGC monotherapy, and 15 patients who were steroid-resistant received SGCs, human IVIG, and plasmapheresis. There were 80% of females and 20% of males (Fig. 2). AP was mild in 20% of patients, moderate in 40%, and severe in 40% (Fig. 3). The duration of the disease ranged from 1 to 13 years.

In Group 2 (study group), the prior adjuvant therapy consisted of azathioprine (53%), methotrexate and rituximab (7% each), and 33% of patients received no additional therapy (Fig. 4).

In Group 2, 20%, 40% and 40% of patients exhibited one, two and three exacerbations of AP per year, respectively (Figs. 5, 6). Four patients received three cycles of combination therapy, which included IVIG and discrete plasmapheresis, while 11 patients received five cycles (Fig. 7).

The group of healthy donors included 43 individuals, of whom 19 were male and 24 were female.

During therapy, a statistically significant decrease in the PDAI by 98% (p <0.001) was observed in patients (Fig. 8).

The mean daily dose of SGCs after the fifth cycle was 11.6 mg.

Primary findings

There were some differences in cytokine profile in patients of both groups during therapy. Specifically, patients who received combination therapy demonstrated a statistically significant decrease in the levels of cytokines IL-4 (Me=4 pg/mL), IL-15 (Me=3 pg/mL), and TNF-α (Me=3 pg/mL).

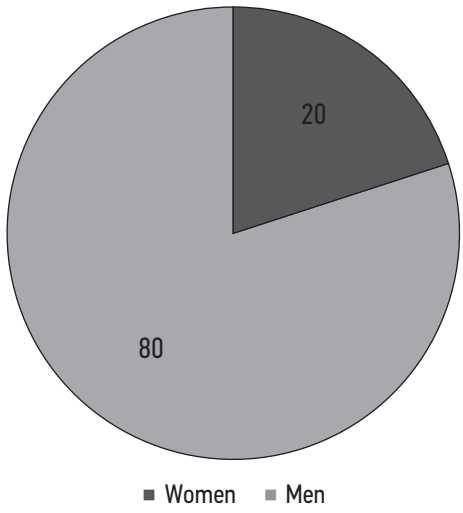

Fig. 2. Distribution of patients by gender (%).

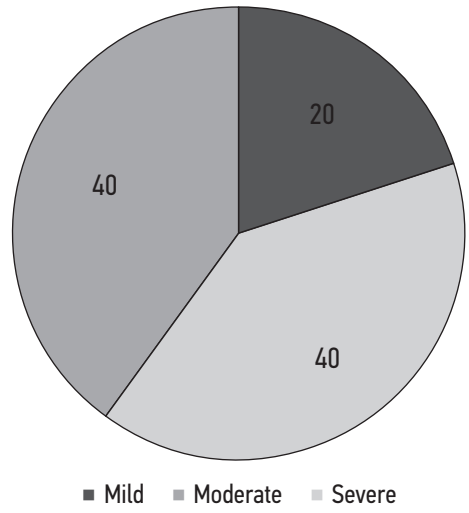

Fig. 3. Distribution of patients according to the disease severity (%).

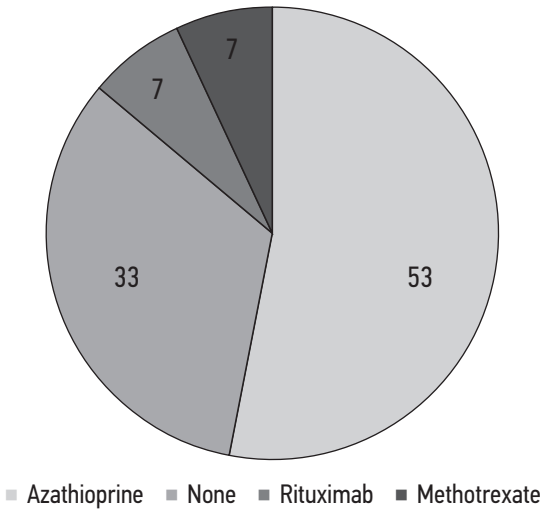

Fig. 4. Previous adjuvant therapy in steroid-resistant group of patients (%).

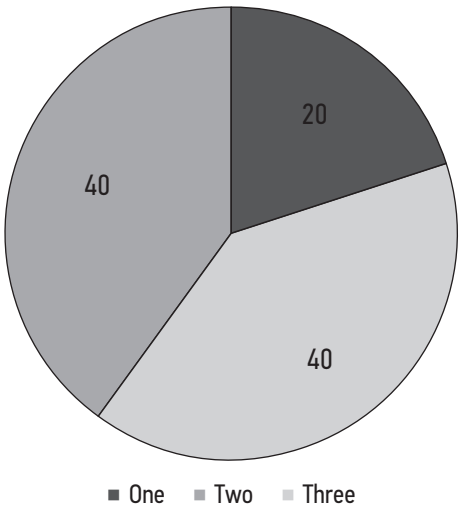

Fig. 5. Distribution of steroid-resistant patients according to the frequency of annual exacerbations (%).

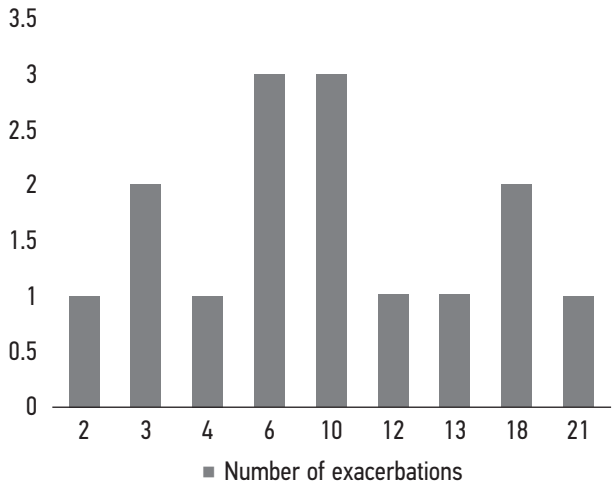

Fig. 6. Distribution of steroid-resistant patients by frequency of annual exacerbations from disease onset.

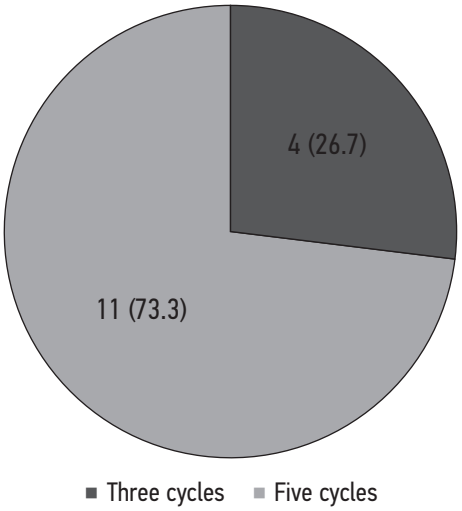

Fig. 7. Number of cycles of intravenous immunoglobulin and plasmapheresis (%).

Conversely, the SGC monotherapy group exhibited higher levels of these cytokines, with IL-4 (Me=13 pg/mL), IL-15 (Me=14 pg/mL), and TNF-α (Me=10 pg/mL) (p <0.01) (Fig. 9). Notably, IL-10 levels were significantly higher in patients with AP in both groups compared with healthy donors; however, there were no differences in IL-10 between Groups 1 and 2 (p >0.01).

In addition, no statistically significant results were observed for serum CXCL8 levels in patients in either group (Fig. 10). In Group 2 (study group), there was a significant decrease in serum CCL11 levels (Me=3 pg/mL) compared with the control group (Me=15 pg/mL). In addition, the serum CCL11 level was statistically significantly higher in steroid-resistant patients before treatment with IVIG and plasmapheresis (Me=51 pg/mL) compared with patients receiving monotherapy with SGCs (Me=10 pg/mL; p <0.01). A notable finding was the statistically significant decrease in serum granulysin levels in patients with AP following combination IVIG therapy and plasmapheresis (Me=0 ng/mL) compared with patients receiving monotherapy with SGCs (Me=2700 ng/mL, respectively). Furthermore, the group of healthy donors exhibited lower levels of all parameters, including IL10 (Me=3 pg/mL), IL15 (Me=5 pg/mL), IL4 (Me=4 pg/mL), TNF-α (Me=7 pg/mL), CCL11 (Me=13 pg/mL), CXCL8 (Me=22 pg/mL), and granulysin (Me=1000 ng/mL) than both groups of patients before treatment (p <0.01) (Fig. 11).

During the IVIG therapy and plasmapheresis, complete remission was observed in one patient following complete withdrawal of SGCs. Furthermore, complete remission with

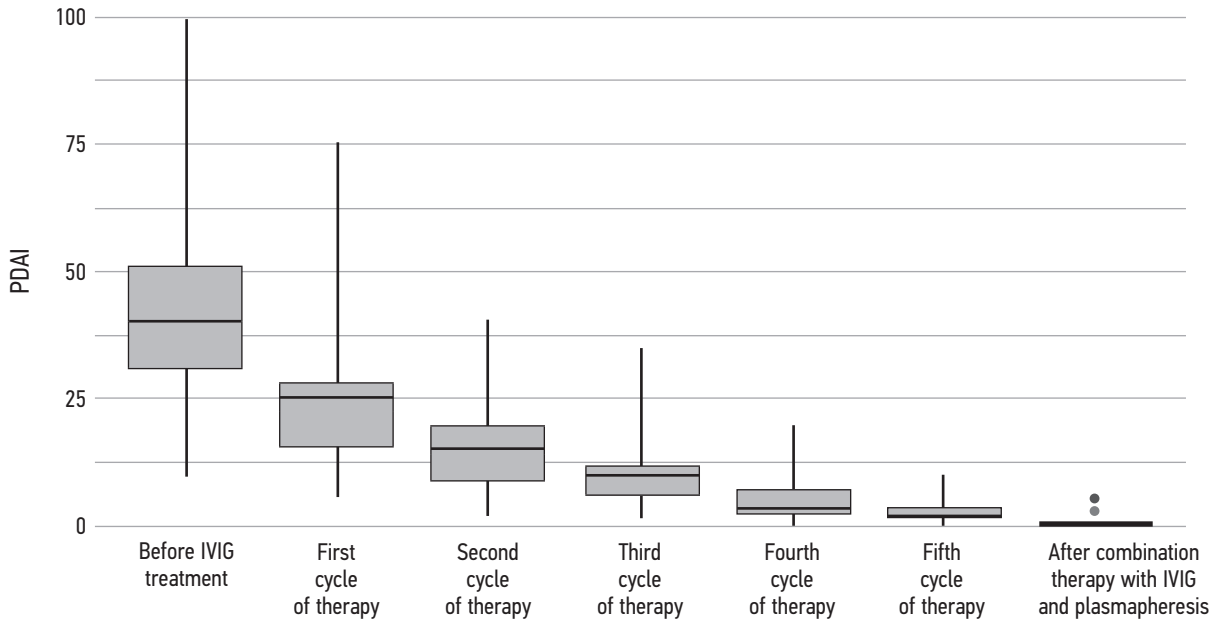

Fig. 8. The changes of PDAI score during the combined therapy with systemic glucocorticoids, intravenous immunoglobulin and plasmapheresis.

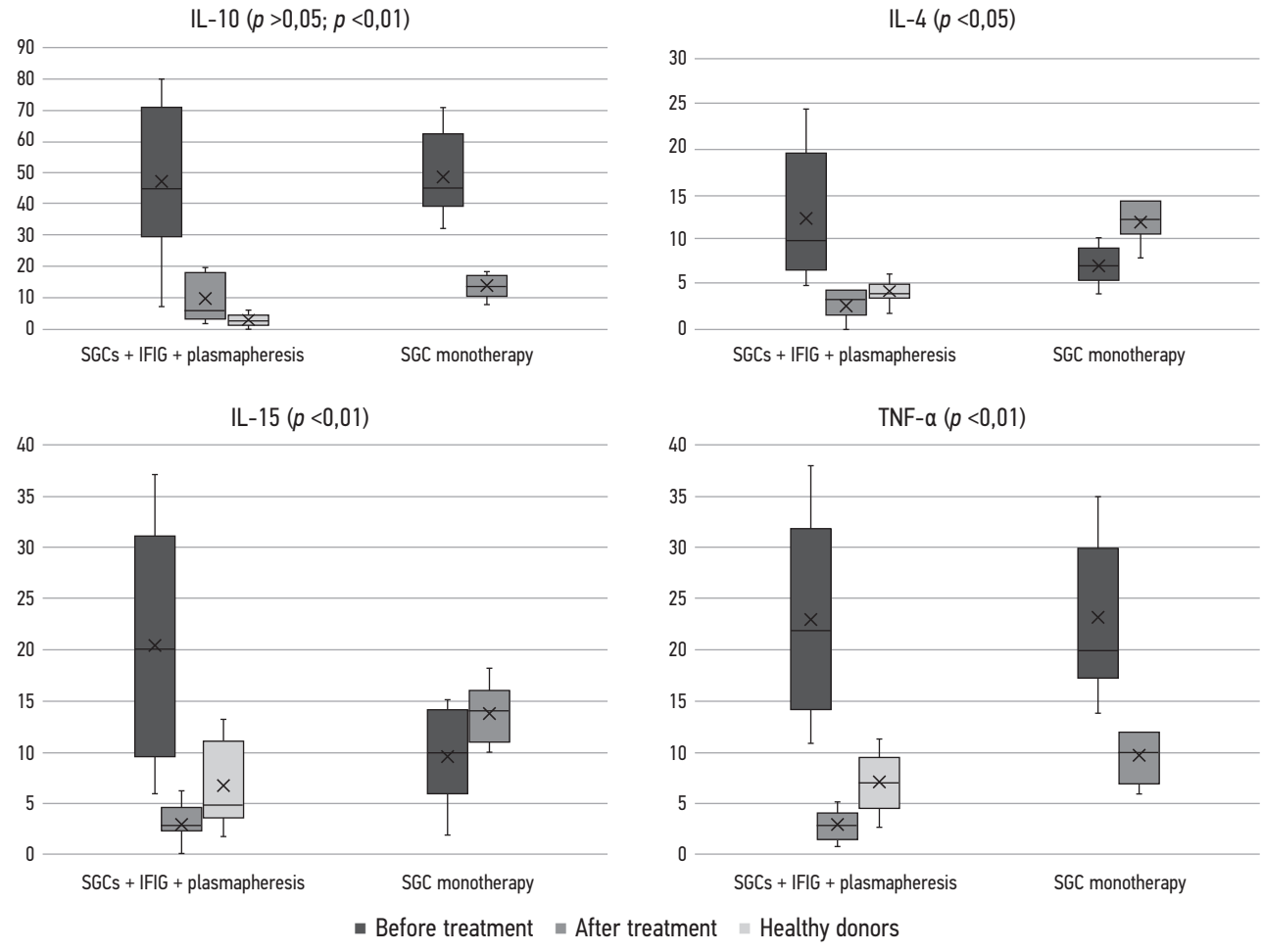

Fig. 9. Cytokine levels in serum of steroid-resistant and steroid-sensitive patients.

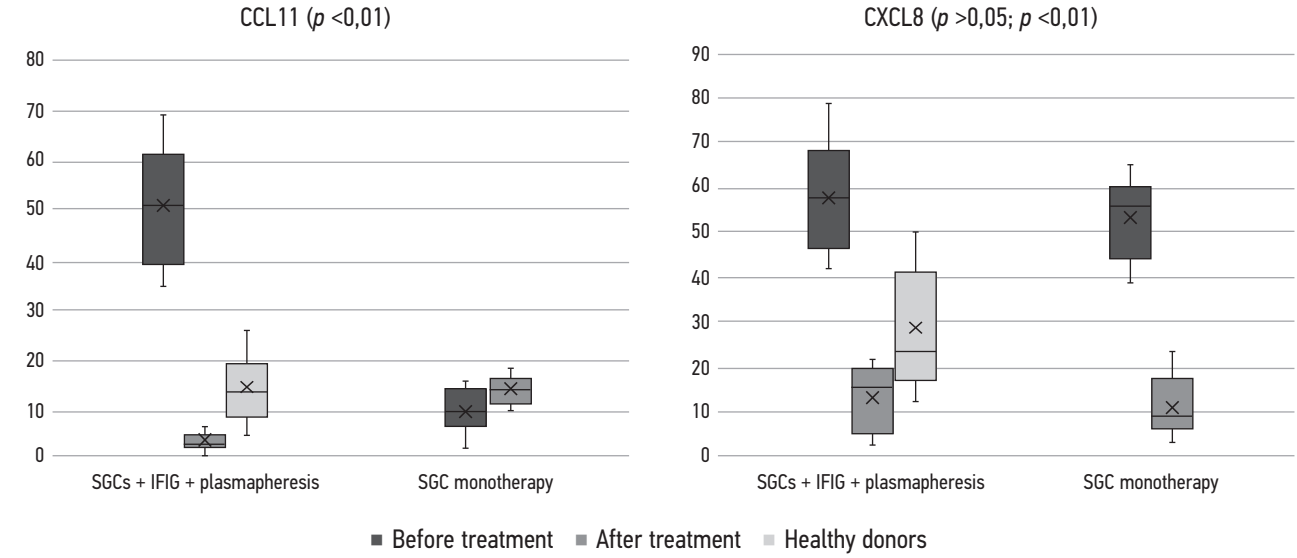

Fig. 10. Chemokine levels in serum of steroid-resistant and steroid-sensitive patients.

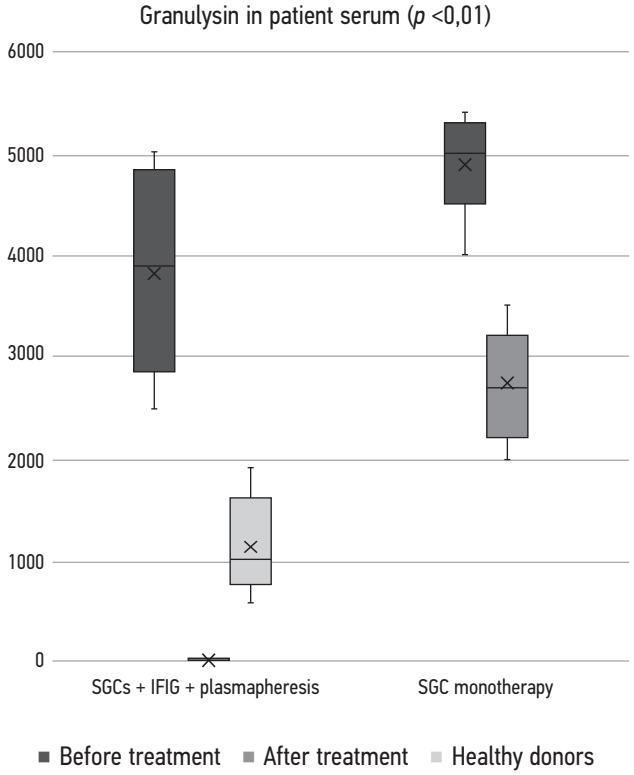

Fig. 11. Granulysin level in serum of steroid-resistant and steroid-sensitive patients.

minimal dose of SGCs was observed in 12 patients (Fig. 12), and partial remission with minimal dose of SGCs (10 mg/day) was observed in two patients (Figs. 13, 14). Remission lasted two years in five (33.3%) patients, three years in four (26.7%) patients, and one year in three (20%) patients (Fig. 15). One patient each (6.7%) had remission periods of 0.5, 1.5, and 4 years (Fig. 12).

DISCUSSION

According to several studies, IVIG may regulate the cytokine profile in the serum of patients with autoimmune dermatoses. Thus, this study showed a statistically significant decrease in the levels of proinflammatory cytokines IL-4, IL-10, IL-15, TNF-α, chemokine CCL11, and granulysin in patients receiving combination therapy with SGCs, IVIG, and plasmapheresis compared with the control group. Moreover, a statistically significant decrease in the patients' serum TNF-α levels was observed after combination therapy, suggesting that IVIG may influence the effector functions of immune cells [5].

Role of chemokines in the pathogenesis of acantholytic pemphigus

Certain chemokines are known to influence the enhancement of the immune response in pemphigus. The authors demonstrated that IL-4 could induce the production of the chemokine CCL11 by fibroblasts. This chemokine belongs to the CC-chemokine subfamily of eotoxins, which consists of eotoxin (CCL11), eotoxin-2 (CCL24), and eotoxin-3 (CCL26). *In vivo* and *in vitro* studies have demonstrated that CCL11 plays a direct role in the induction of eosinophil chemotaxis [6]. Günther et al. [6] investigated the levels of CCL11 in the skin, vesicular fluid, and serum of patients with bullous pemphigoid (BP) to determine its role in eosinophil recruitment in this disease. The researchers found higher concentrations of CCL11 in the serum of patients with BP compared with the control group and patients with AP. Additionally, the authors observed a correlation between CCL11 levels and the prevalence of rashes in patients with BP [6]. Notably, the highest levels of CCL11 were observed in patients with severe BP [6]. For example, CCL11 promoted the migration of eosinophils to the inflammation site by binding to the CCR3 receptor, which is expressed on the surface of

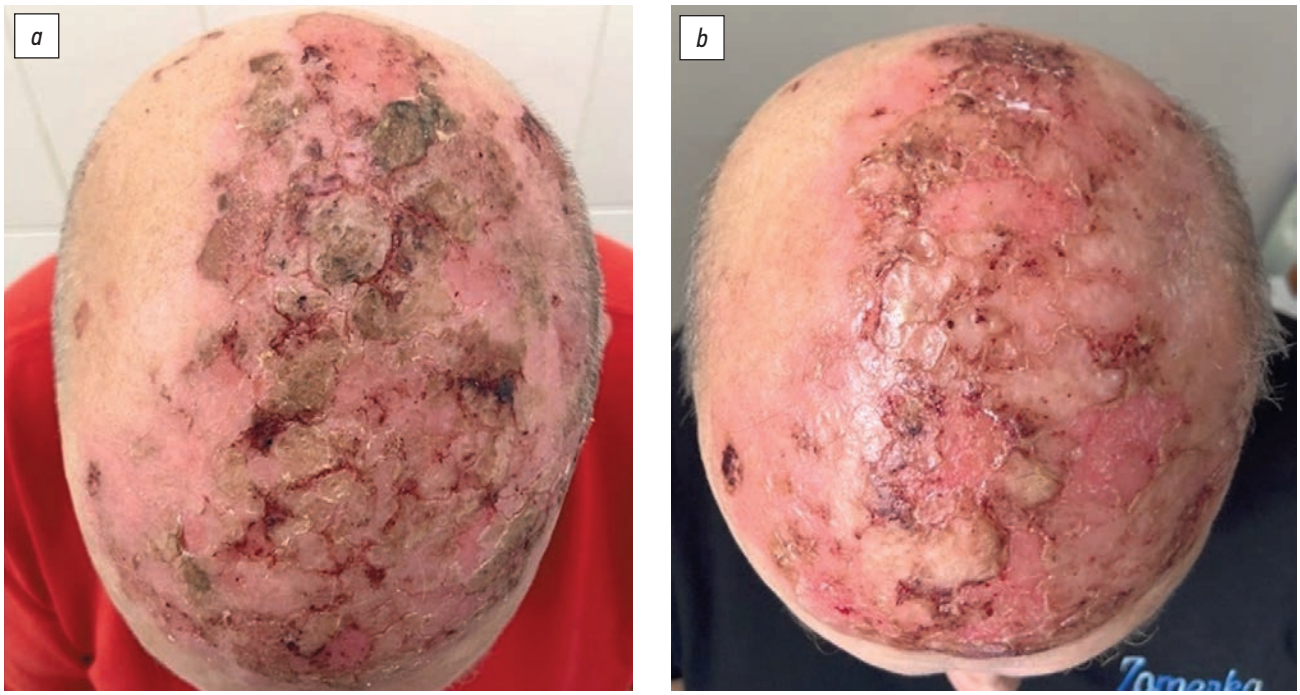

Fig. 12. Patient N., 59 years old, diagnosis of pemphigus foliaceus: before (*a*), against the background of (*b*) and after (*c*) treatment with systemic glucocorticoids, intravenous immunoglobulin and plasmapheresis (remission 2 years).

these cells. Additionally, studies have demonstrated that this chemokine induces eosinophil chemotaxis in both *in vitro* and *in vivo* settings in patients with BP [7]. High levels of CCL11 have been detected in the skin and vesicular fluid of patients with BP in other studies as well. Notably, CCL11 was found to be secreted not only by eosinophils but also by keratinocytes, fibroblasts, and macrophages [8].

Chemokines have also been studied in AP. For example, Timoteo et al. [9] found higher expression of the chemokine CXCL8 in the skin of patients with pemphigus. This finding was associated with the severity and activity of the disease. Furthermore, the chemokine was associated with a Th-17 cell response, which plays a direct role in the formation of acantholysis. Conversely, the expression levels of other chemokines, such as CXCL10, were found to be significantly lower in the same patients [9].

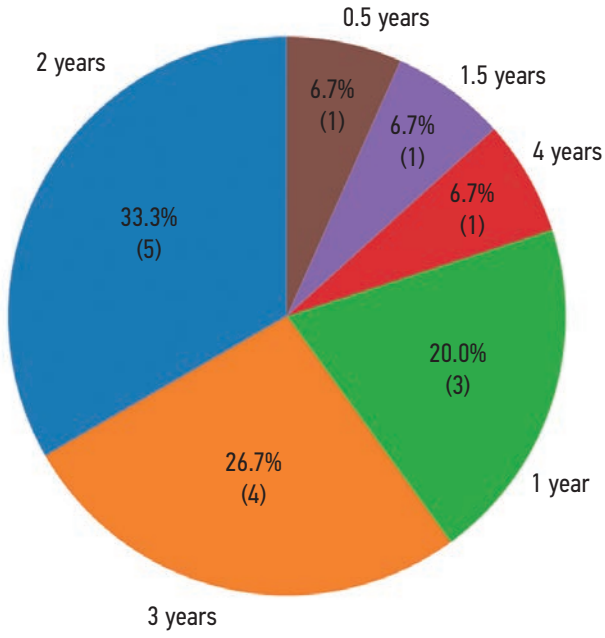

Fig. 13. Evaluation of remission duration in patients receiving combined therapy with intravenous immunoglobulin and plasmapheresis.

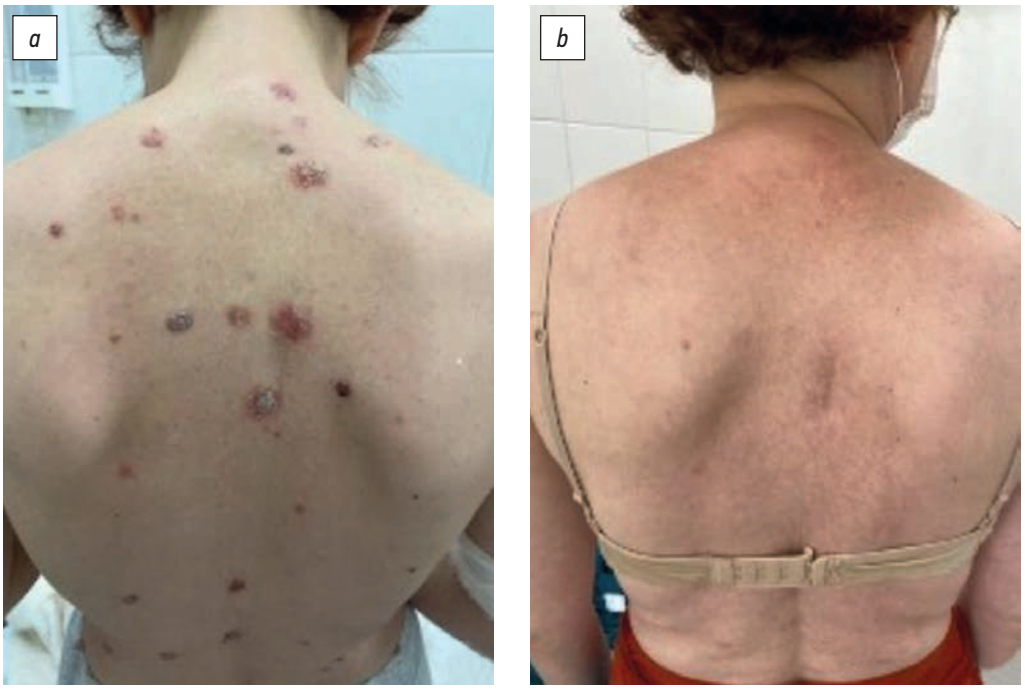

Fig. 14. Patient N., 63 years old, diagnosis pemphigus vulgaris, before (*a*) and after (*b*) the treatment with systemic glucocorticoids, intravenous immunoglobulin and plasmapheresis (remission 2 years).

The present study demonstrated the effect of human IVIG on the serum levels of chemokines CCL11 and CXCL8 in patients with AP before and after treatment. The CCL11 level was found to be statistically significantly higher in steroid-resistant patients receiving combination therapy with SGCs, IVIG, and plasmapheresis compared with the control group (SGC monotherapy). This suggests that CCL11 could be considered as a biomarker of steroid resistance (Fig. 10).

Role of cytokines in the pathogenesis of acantholytic pemphigus and mechanism of IVIG action

TNF-α plays an important role in the formation of acantholysis in patients with AP [10]. For example, Lee et al. [11], when studying the levels of TNF-α in patients with pemphigus in 1999, found its significant increase in both vesicular fluid and serum, thus confirming its "local" role in vesicle formation. Moreover, this mechanism has been demonstrated in patients with toxic epidermal necrolysis (TEN). Araujo et al. [12], when studying the factors contributing to keratinocyte death in the vesicular fluid of patients with TEN, found a simultaneous significant increase in TNF-α, INF-γ, and TRIAL in the vesicular fluid compared with the serum of these patients and controls. The authors concluded that this synergism induced apoptosis and keratinocyte death in these patients. Our data confirmed the increased serum level of TNF-α in patients with AP (Me=23 pg/mL and Me=20 pg/mL) compared with healthy donors (Me=7 pg/mL; $p < 0.01$). Furthermore, in the cohort of patients receiving combination therapy with SGCs, IVIG, and plasmapheresis, the post-treatment level of TNF-α was found to be statistically significantly lower vs. the control group, thereby substantiating the efficacy and immunomodulatory effect of IVIG in patients with AP refractory to SGCs [5].

In addition to binding to different immunoglobulin subtypes, IVIG has been reported to react with several membrane molecules of T cells, B cells, and monocytes. These molecules are relevant to the control of autoreactivity and the induction of autotolerance. IVIG contains antibodies to variable and constant regions of the human T cell receptor, cytokine receptors, CD5, CD4, HLA class molecules, chemokine receptors CCR5 and CD40, and Fas ligand. This extensive tropism to various lymphocyte molecules serves to underscore the immunomodulatory effect of IVIG. Furthermore, dendritic cells have been identified as a target of IVIG, according to several studies [13]. *In vitro*, IVIG has been shown to inhibit the differentiation and maturation of dendritic cells, as well as to eliminate their ability to secrete IL-12 during activation, while concomitantly enhancing IL-10 production. Additionally, IVIG could downregulate costimulatory molecules associated with the modulation of cytokine secretion, thereby leading to the inhibition of T cell proliferation [13].

HLA molecules and costimulatory signals transmitted by CD80 and CD86 cells have been demonstrated to play a role in the pathogenesis of autoimmune diseases [14]. These cells are involved in optimal antigen presentation and T cell activation. Therefore, a study of the suppression of the expression of these molecules by intravenous IVIG and its effect on B cell memory may reveal additional effects of this drug [5].

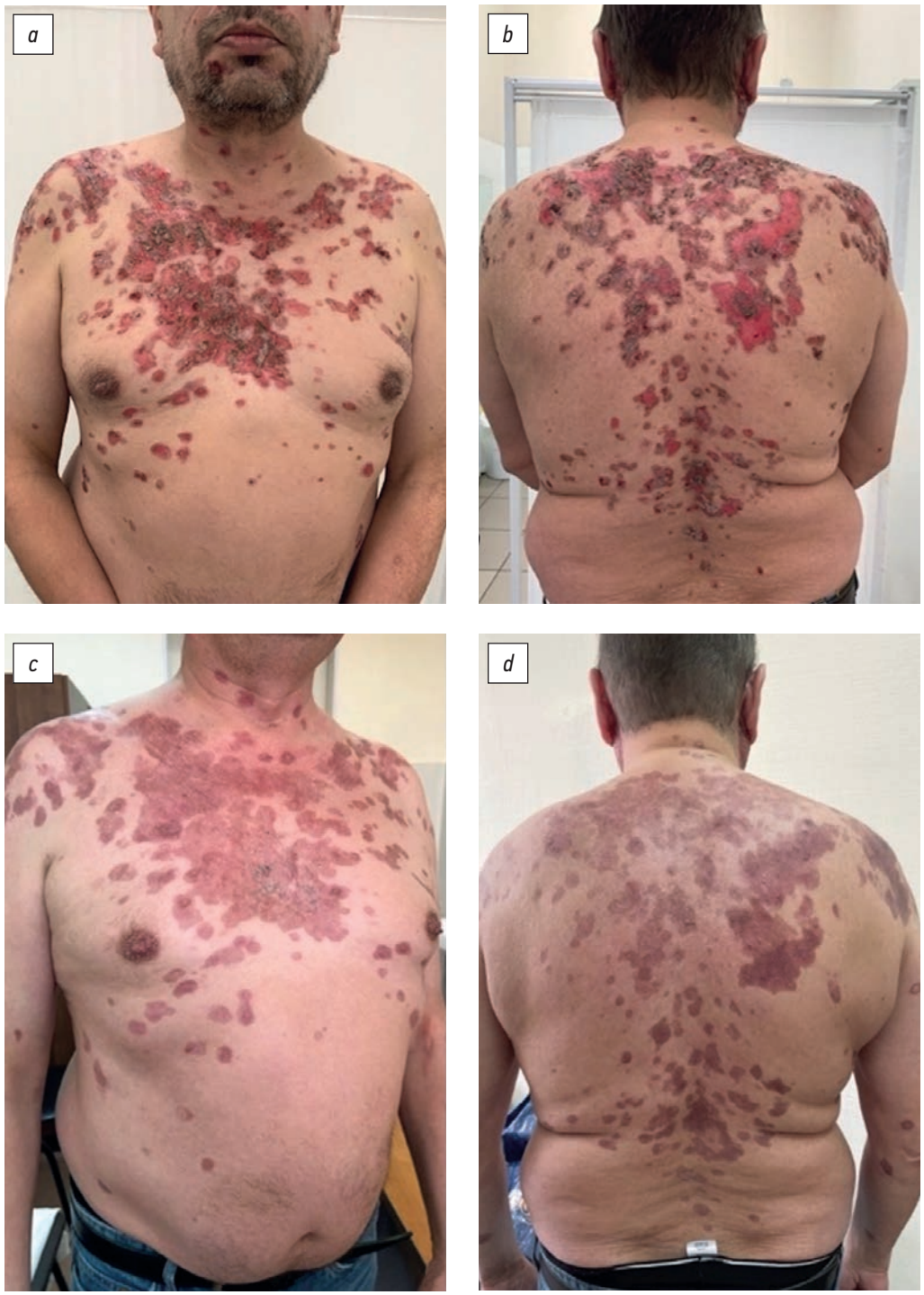

Fig. 15. Patient N., 53 years old, diagnosis pemphigus vulgaris: before (*a*, *b*) and on ongoing therapy (*c, d*) with systemic glucocorticoids, intravenous immunoglobulin and plasmapheresis (remission 1 year).

Experimental evidence has demonstrated that IL-10 plays a pivotal role in the cell-mediated dysregulation. IL-10 induces B-cell proliferation, as well as the differentiation and secretion of immunoglobulins [15]. This mechanism constitutes an integral component of the immune response in diseases such as AP and BP. Additionally, IL-10 has been shown to downregulate the production of cytokines by antigen-presenting cells [16]. However, IL-10 has been observed to play a role in down-regulation of inflammation locally in the skin. The investigation of IL-10 levels in serum and vesicular fluid of patients with AP and BP has been a subject of numerous studies [9, 15, 17–20]. Our study demonstrated that serum IL-10 levels were significantly higher in patients with AP in both groups (Me=45 pg/mL and Me=43 pg/mL) compared with healthy donors (Me=3 pg/mL; p <0.01). Subsequent analysis revealed no significant difference in IL-10 levels between the two groups of patients with AP. Consequently, IL-10 is not associated with steroid resistance (Fig. 9).

Role of granulysin in the pathogenesis of acantholytic pemphigus and other bullous dermatoses

Granulysin, a protein synthesized by cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells, has been identified as a direct causative agent of disseminated keratinocyte death in TEN [21]. Using an immunohistochemical approach, the researchers observed that the necrosis area in the epidermis of patients with TEN exhibited more intense staining for granulysin compared with the control group [21]. Granulysin expression has been observed to be predominantly present on CD8+ and CD56+ cells (CTLs and NK cells) in vesicular fluid [21]. The administration of granulysin (15-kDa) to mice has been shown to induce epidermal rejection, typical for TEN [21]. In addition, Chung et al. [21] found a correlation between the granulysin level in vesicular fluid and the clinical severity of Stevens-Johnson syndrome (overlapping syndrome) and TEN.

Therefore, granulysin may be regarded as a potential biomarker of the severity of other bullous dermatoses. However, the level of granulysin in blood, vesicular fluid, and skin in AP has not been studied. The present study evaluated serum granulysin levels in patients with AP in both groups, as well as in healthy donors. Interestingly, during combination therapy with SGCs, IVIG, and plasmapheresis, there was a statistically significant decrease in the granulysin level compared with the control group. This observation suggests the potential for IVIG to regulate cytotoxic T lymphocytes and NK cells in AP (Fig. 11). Further prospective studies involving a more extensive sample of patients are necessary to substantiate this hypothesis.

Study limitations

Due to the rarity of the presented dermatoses, a relatively small number of patients were recruited in this study, which may have caused a systematic selection bias. Therefore, multicenter studies on a large sample of patients (⩾1000 patients) are needed to confirm the results and eliminate potential errors.

CONCLUSION

The study evaluated the efficacy of combination therapy with IVIG and plasmapheresis in steroid-resistant patients with AP based on the study of serum cytokine and chemokine profile and granulysin levels. There was a trend for a higher serum level of IL-4, IL-15, and CCL11 in steroid-resistant patients with AP receiving combination therapy with IVIG and plasmapheresis compared with the control group. These cytokines may be regarded as potential biomarkers of a more refractory course of AP, as well as in the perspective of their future use as therapeutic targets.

In addition, the potential role of IVIG in the regulation of cytotoxic lymphocytes and NK cells in patients with AP should be considered. These cells synthesize granulysin, which is responsible for disseminated keratinocyte death, most commonly in patients with TEN. This is confirmed by the present study, which showed a significant decrease in serum granulysin levels in patients with AP after treatment with combination therapy including IVIG and plasmapheresis compared with the control group. The effect of combination therapy on the cytokine profile of patients with AP should also be considered. A statistically significant decrease in the levels of IL-4, IL-15, and TNF-α was observed in steroid-resistant patients during combination therapy compared with the control group. The data obtained confirm the immunomodulatory effect of IVIG. In addition, the increased levels of several cytokines, chemokines, and granulysin in this study may be related to the activation of different types of immune cells and keratinocytes in the pathogenesis of AP.

Consequently, the study demonstrated the superior efficacy of combination therapy involving SGCs, IVIG, and plasmapheresis in patients with the steroid-resistant form of AP. This efficacy was demonstrated not only by a significant reduction in IL-4, IL-15, TNF-α, chemokine CCL11 and granulysin levels, but also by the achievement of prolonged remissions in these severely ill patients.

ADDITIONAL INFORMATION

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Author contribution. Author made a substantial contribution to the conception of the work, acquisition, analysis, interpretation of data for the work, drafting and revising the work, final approval of the version to be published and agree to be accountable for all aspects of the work.

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