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ORIGINAL ARTICLE

Relationship Between the Age and Sex of the Patient With the Results of the Indirect Immunofluorescence Test in Patients With Bullous Dermatoses

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Abstract

Background/Aim: Autoimmune bullous diseases are characterised by the production of autoantibodies to epidermal or subepidermal adhesive proteins. The aim of this study was to determine the relationship between age and sex of patients with the results of indirect immunofluorescence test in patients with newly diagnosed bullous dermatoses.
Methods: The investigation presents a retrospective study of newly diagnosed patients with autoimmune bullous diseases at the Clinic for Skin and Venereal Diseases of the University Clinical Centre in Banja Luka in the period 2016-2021. In addition to demographic data, the results of an indirect immunofluorescence test in two titres (≥ 1:10 and ≥ 1:100) were analysed.
Results: In this study, almost the same number of patients with pemphigus (45.2 %) and pemphigoid (54.8 %) was found. There were more women than men in the total sample (p = 0.049). The average age of subjects with pemphigoid was higher than that of patients with pemphigus (p = 0.001). 48.2 % of patients with pemphigus and 51.8 % of patients with pemphigoid had a positive indirect immunofluorescence test. A positive test for epidermal intercellular substance in both sexes at a titre ≥ 1:100 is higher than a titre ≥ 1:10 (p = 0.029). Patients with autoantibody titres ≥ 1:100 to desmoglein-1 were statistically significantly older than patients with titres ≥ 1:10 (p = 0.047).
Conclusion: Number of patients with pemphigus and pemphigoid were similar, with no difference in sex distribution between the two groups of patients, but patients with pemphigoid were older than patients with pemphigus. The difference between high and low autoantibody titres in both sexes was found only in the group of pemphigus on epidermal intercellular substance and desmoglein-1.

Key words: Pemphigus; Pemphigoid; Indirect immunofluorescence test.

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Introduction

Autoimmune bullous diseases are characterised by the loss of tolerance to structural skin proteins, forming as a result autoantibodies to epidermal or subepidermal adhesive proteins.[1] Pemphigus group represents a disease of autoimmune nature which is characterised by the appearance of blisters caused by acantholysis. This group includes *pemphigus vulgaris, foliaceus, fogo salvagem*, drug-induced pemphigus and paraneoplastic pemphigus. The most common types of this group are *pemphigus vulgaris* and *pemphigus foliaceus*. *Pemphigus vulgaris* is more common in Europe and North America, representing 60-90 % of pemphigus cases, whereas *pemphigus foliaceus* is more common in South America and North Africa where endemic

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pemphigus foliaceus is also present. Other clinical forms of pemphigus are less common.

In European countries, pemphigus most often occurs in the age groups between 50 and 60 years, without preference regarding sex.[1, 2] The incidence of *bullous pemphigoid* in Europe is increasing and is associated with aging, in patients with diabetes or malignancy, but it can also be triggered by medication. It most often occurs in people older than 70 years of age. It is more common in women. The mortality rate increases with each decade of life.[3] In pemphigus, IgG autoantibodies are directed against desmoglein-1 and 3, which are part of the intercellular cadherin family, responsible for maintaining intercellular adherence in the epidermis and epithelium of the oral mucosa.

Pemphigus foliaceus is characterised by the presence of antibodies to desmoglein-1, leading to loss of keratinocyte adhesion. Additionally, it is considered that the formation of blisters in pemphigus occurs because of increased presence of proinflammatory mediators or other mechanisms, such as activation of specific muscarinic receptors expressed on keratinocytes, abnormalities in intercellular signalling, or activation of apoptosis. The group of autoimmune subepidermal diseases is characterised by the production of circulating autoantibodies against structural proteins of the basement membrane zone.

Bullous pemphigoid is characterised by the production of autoantibodies directed against BP180 and BP230.[4] Clinically, *pemphigus vulgaris* initially appears on the oral mucosa where it can remain localised for a long time, but also precede skin changes in the form of blisters on unaltered or erythematous skin. After bursting of blisters, painful erosions occur that epithelialise *ad integrum* followed by residual post-inflammatory hyperpigmented macules, which can persist for a long time.[5] The appearance of blisters in *bullous pemphigoid* is preceded by a prodromal phase, with appearance of urticarial, purple, eczema, targetoid, nodular or lichenoid changes. Blisters in *bullous pemphigoid* have a tense covering of various sizes, often causing a subjective feeling of itching. After the rupture of blisters, erosions occur and they epithelialise without scarring. Mucous membranes can be affected in up to 30 % of cases.[6]

The diagnosis of pemphigus and pemphigoid diseases is confirmed based on medical history, clinical manifestations, pathohistological findings, direct immunofluorescence test (DIF) findings and using indirect immunofluorescence (IIF) method, which determines the level of circulating autoantibodies. Monkey oesophagus is most often used as substrate. The enzyme-linked immunosorbent assays (ELISA) are a more sensitive method for measuring antibodies to desmoglein-1 and 3.[7]

The aim of the paper was to determine the relationship between the results of the indirect immunofluorescence test and the age and gender in newly diagnosed patients with bullous dermatoses.

Methods

This research was conducted as a retrospective study that included all newly diagnosed patients suffering from autoimmune bullous diseases treated at the Clinic for Skin and Venereal Diseases of the University Clinical Centre in Banja Luka in the period from 2016 to 2021. The study included a total of 93 patients of both sexes. The patients were divided into two groups. The first group consisted of newly diagnosed patients from the pemphigus group, and the second group consisted of newly diagnosed patients from the pemphigoid group. In all patients, in addition to demographic data, the data on the indirect IIF immunofluorescence test performed in two titres, ≥ 10 and ≥ 100, with the EUROIMMUN immunofluorescence test *Dermatology Mosaic 7* were included. In accordance with the recommendation of the manufacturer EUROIMMUN, in these patients the dilution of samples in titres ≥ 1:10 and ≥ 1:100 from human serum or plasma and electron microscopy were performed. BIOCHIP Dermatology Mosaic 7 contains 6 fields that require fluorescence microscopy. In the first field, in the case of a positive reaction, antibodies to the desmosomes of spinous cells react with surface antigens of keratinocytes. Oesophageal tissue gives granular, reticulated fluorescence in the intercellular substance of the entire spinous layer. In the second field, if the serum contains autoantibodies to the epidermal basement membrane, fine linear dyeing occurs between the

basement layer and the connective tissue. In the third, fourth and sixth field, if the serum contains autoantibodies desmogelin-1, desmogelin-3 and BP230gC, smooth, fine, granular cytoplasmic fluorescence is visualised, partly with cell membrane fluorescence, on transfected cells of the appropriate array substrate. The nucleus of cells is not dyed or only slightly fluoresces. The fifth field contains BP 2180-NC16A if the serum antibodies are specific for BP180 fluorescent green diamond-shaped fields as opposed to the dark field.

The statistical analysis was performed using the SPSS software package (Statistical Product and Service Solutions) 20. The results were described by average values ($\bar{X}$) and standard deviations (SD) for continuous variables, and incidence and percentages (%) for categorical variables. Differences between average values of variables in groups were analysed using the independent samples t-test, whereas the differences between frequencies of individual groups of patients were tested using Pearson's Chi-squared test. The Pearson correlation coefficient (r) was used to calculate the correlation between the two continuous variables. Binary Logistic Regression was used to examine the effect of gender and age on predicting IIF test results among the study population. P values lower than 0.05 were considered statistically significant.

Results

In the results, the IIF test values in 93 patients of both sexes was monitored. There were 45.2 % of patients with pemphigus and 54.8 % of patients with pemphigoid. Of the total number of patients, 60.2 % were women and 39.8 % were men. The number of affected women was statistically significantly higher than the number of men (p = 0.049) in the total patient population. In the group of patients with pemphigus, 48.2 % were women and 40.5 % were men. In the group of patients with *bullous pemphigoid*, 51.8 % were women and 59.5 % were men. There was no statistically significant difference between the sexes in patients with pemphigus and pemphigoid, (χ (1) = 0.530, p = 0.467).

The average age of the total number of patients in our study was 70.88 ± 14.22 years, in the group of patients with pemphigus 65.64 ± 15.06 years, and in the group of patients with pemphigoid 75.20 ± 11.99 years. The difference in the age of the patients between the two groups of patients was statistically significant, t (91) = -3,405, p = 0.001.

The average age of patients with a negative IIF test was 73.11 ± 14.76 years and are on average 3.70 years older than patients with a positive test. However, this age difference was not statistically significant (t (91) = 1,231, p = 0.222, p > 0.05. The Pearson correlation also indicates that there is a weak, positive correlation between age and IIF test results, but that it is not statistically significant (r = 0.128, n = 93, p = 0.222). The result of the independent t-test indicated that patients with newly diagnosed pemphigus and negative IIF test have an average age of 71.19 ± 16.71 years and are on average 8.96 years older than patients with positive test (62.23 ± 13.06). Pearson correlation indicated that in patients with pemphigus there is a moderate, positive correlation between age and IIF test results, which was not statistically significant (r = 0.292, n = 42, p = 0.060).

The result of the independent t-test indicated that patients with newly diagnosed pemphigoid with a negative IIF test had an average age of 74.57 ± 13.22 years and were on average 1.06 years younger than patients with a positive test (75.63 ± 11.27). However, this difference was not statistically significant, t (49) = -0.308, p = 0.759, p > 0.05. The Pearson correlation indicated that in patients with pemphigoid there was a weak, negative correlation between age and IIF test results, but not statistically significant (r = -0.044, n = 51, p = 0.759).

The number of patients with a positive IIF test was 56 (48.2 % with pemphigus and 51.8 % with pemphigoid). The average age of patients with a positive IIF test was 69.41 ± 13.79. The results of the independent t-test indicated that patients with a positive test belonging to the pemphigus group are on average 13.4 years younger than patients with pemphigoid, which was a statistically significant difference (t (54) = -4,123, p = 0.000). The Pearson correlation indicated that in patients who were positive for the IIF test there was an extremely strong, positive, statistically significant correlation between age and test positivity (r = 0.489, n = 56, p = 0.000) (Table 1).

Table 1: General patients' characteristics and immunofluorescence test results

Variables	All patients	(Group I) Pemphigus	(Group II) Pemphigoid	*p*-value
Gender, N (%)	93 (100.0)	42 (45.2)	51 (54.8)	0.350*
Male	37 (39.8)	15 (40.5)	22 (59.5)	0.467*
Female	56 (60.2)	27 (48.2)	29 (51.8)	
Age (years) $\bar{X}$ ± SD	70.88 ± 14.22	65.64 ± 15.06	75.20 ± 11.99	0.001**
Negative IIFT, n (%)	37 (100.0)	15 (40.5)	22 (59.5)	0.249*
($\bar{X}$ ± SD)	73.11 ± 14.76	71.19 ± 16.81	74.57 ± 13.22	0.497**
Positive IIFT, n (%)	56 (100.0)	27 (48.2)	29 (51.8)	0.789*
($\bar{X}$ ± SD)	(69.41 ± 13.79)	62.23 ± 13.06	75.63 ± 11.27	0.000**

*N (%) – number (percentage); $\bar{X}$ – average; SD – standard deviation; IIFT – indirect immunofluorescent test; * Chi-square test; ** Independent samples t-test*

Table 2: Relationship between autoantibody titres with the patients' gender and age in the pemphigus group

Autoantibodies	Antibody titre	N	Female N (%)	Male N (%)	*P*	Age $\bar{X}$ ± SD	*P**
EIcS	≥ :10	7	7 (100.0)	-	0.029	58.71 ± 9.21	0.668
	≥ 1:100	15	8 (53.3)	7 (46.7)		61.33 ± 14.50	
Dsg1	≥ 1:10	4	1 (25.0)	3 (75.0)	0.071	56.00 ± 16.55	0.047
	≥ 1:100	9	7 (77.8)	2 (22.2)		69.67 ± 12.61	
Dsg3	≥ 1:10	11	6 (54.5)	5 (45.5)	0.582	58.55 ± 6.19	0.128
	≥ 1:100	9	6 (66.7)	3 (33.3)		60.89 ± 18.26	

N (%) – number (percentage); $\bar{X}$ – average; SD – standard deviation; EIcS - Epidermal intercellular substance; Dsg – desmoglein; P – Chi-squared test; P – Independent t-test*

Table 3: Relationship between autoantibody titres with the patients' gender and age in the pemphigoid group

Autoantibodies	Antibody titre	N	Female N (%)	Male N (%)	*P*	Age $\bar{X}$ ± SD	*P**
EBM	≥ 1:10	13	7 (53.8)	6 (46.2)	0.785	72.54 ± 13.06	0.162
	≥ 1:100	17	10 (58.8)	7 (41.2)		78.29 ± 8.92	
BP180	≥ 1:10	7	3 (42.9)	4 (57.1)	0.629	73.29 ± 15.13	0.576
	≥ 1:100	11	6 (54.5)	5 (45.5)		76.91 ± 11.79	
BP230	≥ 1:10	5	4 (80.0)	1 (20.0)	-	69.80 ± 15.52	-
	≥ 1:100	-	-	-			

N – number (percentage); $\bar{X}$ – average; SD – standard deviation; EBM – epidermal basement membrane; BP – bullous pemphigoid; P – Chi-squared test; P – Independent t-test*

After using the Chi-square test, it was determined that there was a statistically significant difference in the frequency of patients according to gender and the titre of autoantibodies to epidermal intercellular substance (EicS). The number of patients of both sexes with a titre ≥ 1:100 was statistically significantly higher compared to patients of both sexes with a result ≥ 1:10 (p = 0.029). Using the same test, no statistically significant differences in the frequency of patients by sex and titre of autoantibodies to desmoglein-1 were confirmed (Dsg1), (p = 0.071), as well as between patients by sex and titre of antibodies to desmoglein-3 (Dsg3), (p = 0.582). After using an independent t-test, the age difference between positive patients with an autoantibody titre with a titre ≥ 1:10 and a titre ≥ 1:100 was examined. It was determined that the age differences were statistically significant only in patients positive for Dsg1, with patients with autoantibody titres ≥ 1:100 statistically significantly older (69.67 years) than patients with titres ≥ 1:10 (56.00 years), (p = 0.047). In other cases, no statistically significant difference in the patients' age and titre height to EIcs (p = 0.668) and to Dsg3 (p = 0.128) was found. In both cases, patients with a titre ≥ 1:100 were older, but this difference was not statistically significant (Table 2).

In patients positive for epidermal basement membrane (EBM), there were no statistically significant differences in the frequency of patients according to gender and autoantibody titre values (p = 0.785). The same was found in BP180-positive patients (p = 0.629). In the case of BP230, it was not possible to use Chi-squared test because there were no patients with a titre ≥ 1:100. Using an independent t-test, differences in average age between positive patients with different titre values were analysed. In cases with positive autoantibodies to EBM and BP180, patients with a titre ≥ 1:100 were older, but this difference was not statistically significantly greater than in patients with a titre ≥ 1:10 (Table 3).

Discussion

This research confirmed that an indirect immunofluorescence test was performed on 93 patients with bullous dermatoses over a five-year period. This test quantitatively determines the level of circulating autoantibodies in patients and it is important in the diagnosis, differential diagnosis of autoimmune bullous diseases and monitoring the results of administered therapy. There were 45.2 % of patients suffering from pemphigus and 54.8 % of patients diagnosed with pemphigoid. Out of the total number of patients, 60.3 % were women and 39.8 % were men. The number of women was statistically significantly higher compared to the number of men (p = 0.049) in the total patient population. In the group of patients diagnosed with pemphigus there were 48.2 % women and 40.5 % men, whereas in the group of patients diagnosed with pemphigoids there were 51.8 % women, and 59.5 % men. The results showed no statistically significant difference between the sexes in newly diagnosed patients in both groups (p = 0.467).

The study of Arbache et al included 421 patients, 277 patients in the pemphigus group and 144 patients in the subepidermal autoimmune disease group. In serology, elevated titres have been reported for *vulgar* and *pemphigus foliaceus* that correlate with earlier stages of the disease or active disease. The results confirmed the importance of direct and indirect immunofluorescence in the diagnosis of autoimmune bullous diseases. As for IIFT, lower values were found for *bullous pemphigoid*. This deviation may be a consequence of technical problems, especially in terms of the nature of the substrate used.[8]

Kutlubay and his associates analysed 346 new cases of patients with autoimmune bullous dermatoses in the period from 2003 to 2019, and the research included 151 men and 195 women. The average age of patients was 54.4 years and the average duration of the disease was 85.6 months. The most common diagnoses were *pemphigus vulgaris* 70 %, *bullous pemphigoid* 13 %, and *pemphigus foliaceus* 6 %, and *pemphigus vulgaris* mostly appeared in women in a ratio of 1:4. Patients with *bullous pemphigoid*, with an average age of 75.6 years, were present in a lower percentage compared to *vulgar pemphigus*, and the highest incidence rates of pemphigoid in both sexes were registered in the age groups 70 to 79, especially in people over 80 years of age, which is in accordance with the data from literature, as well as the results of the present research.[9]

The average age of patients in this study in both groups was 70.88 ± 14.22. The average age of patients suffering from pemphigus was higher than the average age of patients diagnosed with pemphigoid (p = 0.001). This study included a higher percentage of women compared to men in both groups of patients. According to the data of similar studies, pemphigus occurs more often in women, and when it comes to age, it is most often diagnosed between the ages of 50 and 60 in European countries, whereas in other countries worldwide, it occurs between the ages of 30 and 50. *Bullous pemphigoid* occurs in the age of 70 and older, as it did in the patients presented in this study. Although the overall incidence is slightly higher in women, after 80 years it is more common in men, according to Alpsoy et al.[10] In the study of Milinković and his associates in Serbia, *pemphigus vulgaris* appears in a higher percentage in patients who are 80 years old or older and is more common in women.[11]

In Schmidt et al, *pemphigus vulgaris* occurs in 70-80 % cases, *pemphigus foliaceus* in 20 %, paraneoplastic pemphigus in 5 % and IgA pemphigus in 1-3 % of all described bullous autoimmune diseases. In Germany, their prevalence is around 40,000 cases across the country, and their incidence is around 20 new cases per million inhabitants per year.[12]

In the study of De and his associates, a total of 268 cases of autoimmune bullous diseases were

registered at the Immunobullous Disease Clinic, where 50 patients (18.7 %) were subepidermal, and 20 of them (40 %) were patients with *bullous pemphigoid* with an average age of 59 (33- 80) years, and the ratio of men to women was 1.2:1. *Bullous pemphigoid* is the most common subepidermal autoimmune bullous disease in India as well as in Western Europe, although the proportion appears to be lower, at least in their group of patients, with the disease starting earlier in life. In the present study, patients with pemphigoid are older than 59 years, opposite to the results indicated in De's paper.[13]

In this study, the number of patients with positive IIFT was 56, with and the average age of patients was 69.41 ± 13.79 years. Patients from the pemphigus group were on average 13.4 years younger than patients belonging to the pemphigoid group, which is a statistically significant difference ($p < 0.05$). Following the analysis of IIFT and patients' gender in these results, a statistically significant connection in both groups of patients was not found ($p < 0.05$).

Creedin and Bergman carried out a retrospective cohort study that included 770 patients in whom IIFT was performed for the presence of intercellular autoantibodies in the monkey oesophagus in the period 2000-2017. 176 patients were diagnosed with *pemphigus vulgaris*, and 29 patients with *pemphigus foliaceus*. The sensitivity of this immunoassay was significantly higher for the *pemphigus vulgaris* diagnosis ($p = 0.018$). Among female patients, 65.9 % had *pemphigus vulgaris* and 34.5 % had *pemphigus foliaceus*, which is in line with the results of this study in which the predominance of female patients in the group with pemphigus vulgaris was determined.[14] The Pearson correlation indicated that in both groups of patients with positive IIFT in this study, there was an extremely strong, positive correlation with the patients' age ($p = 0.000$).

The retrospective study of Askin and associates included 320 patients with autoimmune bullous diseases and 70 % of them had a positive IIFT result for *pemphigus vulgaris*. Another result of the study showed that the disease is more common in women and has a peak incidence in the fifth decade, but that there was no statistically significant difference between age and sex of patients with IIFT results.[15]

The results indicate a statistically significant difference in patient frequency by sex and autoantibody titre to EicS. The number of patients of both sexes with titre ≥ 1:100 was statistically significantly higher compared to patients of both sexes with ≥ 1:10 ($p = 0.029$). The average age of the patients was 58.71 ± 9.21 in patients with a titre ≥ 1:10 and 61.33 ± 14.50 with a titre ≥ 1:100. These results are in accordance with the results of a study conducted by Hashimoto and associates, which included about 5,000 cases. The study included 30 patients with IgG and IgA autoantibodies to the intercellular substance, among which clinically, there was no prevalence between men and women, and average patients' age was 55.6 years.[16]

These results did not show any statistically significant difference in the age and sex of patients according to the titre of autoantibodies to desmoglein-1 ($p = 0.071$) and desmoglein-3 ($p = 0.582$). Delavarian and associates conducted a study that included 19 patients diagnosed with *pemphigus vulgaris*, 9 men and 10 women, with an average age of 47.26 ± 15.11, opposite to the patients presented in this study, whose average age was significantly higher (65.64 ± 15.06). Among 19 patients with pemphigus, desmoglein-3 positive autoantibodies were present in 95 % of patients and desmoglein-1 positive in 63 % of patients.[17]

There were not any statistically significant differences according to the sex and age of the patients and the values of autoantibody titres in patients in this study who tested positive for EBM and BP180. In the case of autoantibodies to BP230, there were not any patients with a titre of ≥ 1:100.

The study of Muhammed N and associates included 30 patients with a *bullous pemphigoid* diagnosis, the patients' average age was 56.33 ± 13.69. Women were present in a higher percentage than men. Autoantibody titres to BP 180 and 230 were performed in patients. Patients with higher autoantibody titres to BP180 had more severe disease, whereas autoantibody titres to BP230 had no correlation with the disease severity, and this study had no patients with autoantibodies to BP230 at titres ≥ 1:100. The patients were older, and there was no predominance according to gender.[18]

Conclusion

Number of patients with pemphigus and pemphigoid were similar, with no difference in sex distribution between the two groups of patients, but patients with pemphigoid were older than patients with pemphigus. The difference between high and low autoantibody titres in both sexes was found only in the group of pemphigus on epidermal intercellular substance and desmoglein-1.

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Conflict of interest

None.

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