Presence of markers of different neoplasms in serum of patients suffering on autoimmunological bullous diseases

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Abstract
The aim of the study was to indicate serum cancer markers of internal organs increased level in blood of patients with bullous diseases with autoimmunological background (CEA, Ca125, tPSA). Levels of neoplasmatic markers were determined by ELISA method. Auto antibodies were determined by immunofluorescence IF. 50 patients (male – 19 and 31 female) were investigated. Patients were divided into 2 groups: with pemphigus (14 patients, male and female) and with pemphigoid (36 patients male and female). In serum obtained from all (100%) of female patients with pemphigoid auto antibodies IgG anti BMZ were present. In 44.5% presence of Ca125 was determined as well as presence of CEA (11.2%). Results obtained from serum of male pemphigoid patients were different. 66.7% have shown presence of auto antibodies as well as presence of tPSA but 100% of these patients shown negative CEA. 100% of female patients with pemphigus had autoantibodies present in investigated serum, but only 15.3% shown presence of Ca125 and another 15.3% – presence of CEA.

Immunofluorescence shown positive result in 100% of male patients with pemphigus with presence of CEA and negative result of tPSA. High level of autoantibodies and higher level of Ca125 were observed in blood of pemphigoid female patients. The correlation between presence of autoantibodies and higher levels of tPSA in blood of male pemphigoid patients were observed.

Key words: bullous diseases, pemphigus, pemphigoid, neoplasmatic markers.

Introduction
Pemphigus and its varieties, as well as pemphigoid are autoimmunological conditions, which in some cases may co-occur with neoplasms of internal organs, i.e. they may be their manifestations. In the case of pemphigus, the disease process consists in the occurrence of intraepidermal bullas which are formed when pathogenic autoantibodies bind with adhesive desmosomal particles. These particles form contacts between epidermal cells and multilayered mucosal epithelial cells [1-11].

Pemphigus vulgaris and pemphigus foliaceus are the most common forms of pemphigus. Pemphigus vulgaris antibodies attack mainly desmoglein III (cadherine 130 kDa) [1, 2], that is why bullas are clinically localized in the lower layer of the epithelium, whereas in the case of pemphigus foliaceus the antibodies attack desmoglein I, and thus the bullas are localized in the upper layer of epithelium [4, 10, 11]. Due to the above, acantholysis in pemphigus vulgaris occurs in the basal layer, and in pemphigus foliaceus in the corneous layer [13].

Paraneoplastic pemphigus is the most severe form of pemphigus. Clinical picture of the disease is very diverse [8], apart from lesions characteristic of pemphigus vulgaris it also affects the epithelium of pulmonary alveoli, urinary
young people and even if reported it never is a neoplastic because the disease is very seldom reported in children and organs and immunological system [12, 14-21]. There are contradictory reports on coexistence of pemphigoid paraneoplastic pemphigoid with neoplasms of the inner organs and which suggest only a connection with advanced age of the studied patients, because the disease is very seldom reported in children and even if reported it never is a neoplastic manifestation [14]. Due to significant development of immunological diagnostic test procedures it is possible to diagnose bullous diseases in their early stages and introduce early prophylaxis and oncological treatment.

The study aimed at showing increased incidence of internal organ neoplasms in patients with vesicle diseases of autoimmunological origin. Markers for internal organ neoplasms which are most prevalent in population CEA, Ca125 (females) and tPSA (males) have been selected. Immunoenzymatic method (ELISA) was applied to determine levels of neoplasm markers. Immunofluorescent tests were used to indicate the presence of autoantibodies in the serum of the studied patients.

**Material and Methods**

The study included 50 people aged 42-89 (19 males, aged 45-89 and 31 females, aged 44-82) who had the history of clinical changes suggesting occurrence of autoimmunological bullous disease. These were patients hospitalized and treated in the Department of Dermatology and The Hospital Outpatient Clinic of the Provincial Specialist Hospital No. 4 in Bytom.

Skin biopsy specimens were collected from all the patients and histopathologically evaluated. The patients were divided into two study groups: diagnosed with pemphigoid and pemphigus. The pemphigoid group included 36 (72%) patients, including 18 (36%) females and 18 (36%) males. The pemphigus group included 14 (28%) patients, including 13 (26%) females and 1 (2%) male. The mean age of the pemphigoid group females was 69 and 71 years for males, and the mean age of the pemphigus group was 65 and 60 years respectively. The analysis allowed for the most common coexisting inner organ diseases: arterial hypertension, renal failure, gastric and/or duodenal ulcer disease, diabetes, hyperthyroidism, anemia, ischemic heart disease, survived myocardial infarction.

The tested material was the serum derived from blood of the patients, taken in hospital conditions, with the patients' prior consent to the test. The tests excluded pregnant women, syphilitic patients, patients with other autoimmune diseases, patients with AIDS, patients diagnosed with neoplasms, patients with active tuberculosis, patients with phototoxic reactions, patients with drug-induced reactions and other congenital bullous diseases as well as with reactive diseases. The tested material was the serum derived from venous blood taken in the amount of 4.9 ml. The blood was taken by means of a sterile blood collection kit (disposable vacuum S-Monovette kit with Monovette needle produced by ARSTEND), from ulnar vein after prior disinfection of the needle insertion spot. The material was appropriately labeled and was transported in a S-Monovette syringe to the Department and Chair of Microbiology and Immunology of the Silesian Medical University in Zabrze-Rokitnica.

The blood was separated for 10 min in appropriate centrifuges at 1800-2000 rpm. After separation, the serum was delicately collected with an automatic pipette with sterile tips that were disposed of every time a sample was changed. The serum, which was appropriately labeled and protected, was stored until sufficient number of samples was collected. All blood collection principles were observed. Immunoenzymatic method (ELISA) was applied to determine levels of neoplasm markers Ca125 (females), tPSA (males) and CEA (all the patients). Kits including, anti Ca125, CEA, tPSA monoclonal antibodies were used, and their native antigens that were included in ready-to-use ELISA kits. The values determined in the serum samples (ng/ml) exceeding 5 for tPSA, 60 for CEA and 35 for Ca125 were regarded as positive.

**Protocol of the study**

The components were heated to the temperature of 22-28 C. Appropriate number of holes were determined on the kit microplate for the patients’ serum samples, standards and control tests. Each serum sample was examined at least twice. Serum samples and control tests were diluted with sample dilution fluid in the radio of 2:100. Standards were not diluted. Standards, diluted control fluids and serum samples were inserted into the microplate hole in the amount of 100 µl.

Blind trial for the microplate reading: 100 µl of diluted control sample without examining the serum sample was incubated for 30 min in room temperature. Examined serum samples in the amount of 100 µl in each microplate hole were incubated for 15 min in room temperature. Each hole was rinsed three times. Then, 50 µl of the so-called “stop-fluid” was added to each hole. Reading was carried out within 15 min. Absorption of the microplate holes’ content was measured by means of ca. 4500 nm wave length. Reading values of blind trial derived from optical density of the standard, control and examined samples were deducted.

Immunofluorescent method, with ready-to-use kits produced by Euroimmun (guinea pig esophagus and rat urinary bladder were the substrates) was used to determine...
the presence of anti-desmosomal and anti-EBM antibodies with the use of fluorescent microscope (Wrocław Euroimmun Company).

**Test protocol**

The kits were stored at temperature of 2 to 8°C. Prior to determination procedure, the components were heated to the temperature of 18 to 25°C. The tested serum samples were diluted with PBS Tweed in the ration of 1:100 and were stirred for at least 4 s in a stirrer (hematological). After obtaining a homogenous solution, 25 µl of the solution was placed on BIOCHIP and incubated in room temperature (18 to 25°C) for 30 min. Then, it was rinsed with PBS-Tweed and stored for 5 min in a glass cuvette. 20 µl of G fluorescein marked human immunoglobulin Ig conjugate was added to individual samples and incubated for 30 min (at temperature of 18 to 25°C). After incubation, BIOCHIP was rinsed again with PBS Tweed and buffer solution was added. After combining it with a medium on the slide (plate), and after applying microscopic cover glass immuno-fluorescence was read out by fluorescent microscope with reference to positive and negative control. Immuno-fluorescence intensity was marked in the scale of 1+ to 3+. If the results were positive, proper dilutions of examined serum samples were prepared, determining the titer for individual samples. The microscope was equipped with 488 nm filters, 510 nm color separator and 520 nm terminal filter.

**Results**

The tests produced the following results: the pemphigoid group included 36 (72%) patients, including 18 (36%) females and 18 (36%) males. The pemphigus group included 14 (28%) patients, including 13 (26%) females and 1 (2%) male. The mean age of the pemphigoid group females was 69 years and 71 years for males, and the mean age of the pemphigus group was 65 and 60 years respectively.

In 18 female patients (100%) out of 18 pemphigoid group female patients the diagnosis was confirmed by histopathological examination. Positive result of immunofluorescent examination was obtained in 12 patients (66.67%) of the patients, and negative result was obtained in 6 (33.33%) patients. In 2 (11.11%) cases the tier was 10, in 8 (44.44%) cases the tier was 80, and in 2 (11.11%) cases the tier was 320. When determining the level of neoplasm markers, positive result was obtained in 12 cases (66.67%) for tPSA, in all cases (100%) negative result was obtained for CEA. The most common co-existing internal organ diseases in this group include:

- prostate adenoma – 10 (55.56%) patients,
- arterial hypertension – 8 (44.44%) patients,
- survived myocardial infarction – 6 (33.33%) patients,
- renal failure – 2 (11.11%) patients.

In 13 (100%) female patients (out of 13 pemphigus group) the diagnosis was confirmed by histopathological examination. Positive result of immunofluorescent examination was obtained in 13 cases (100%) female patients. In 3 (23.08%) cases the tier was 320, in 10 (76.92%) cases the tier was 160. When determining the level of neoplasm markers, positive result was obtained in 2 cases (15.38%) for Ca125 and in 2 (15.38%) cases for CEA. The most common co-existing internal organ diseases in this group include:

- arterial hypertension – 4 (30.76%) patients,
- renal failure – 2 (15.38%) patients,
- diabetes – 2 (15.38%) patients,
- anaemia – 2 (15.38%) patients.

In 1 (100%) male patient out of 1 pemphigus group male patients with the diagnosis confirmed by histopathological examination, autoantibodies with the tier of 320, positive result for CEA antigen and negative result for tPSA were observed. No co-existing diseases were found.

**Discussion**

On the basis of the conducted tests, a distinct connection between increased autoantibody tier values in pemphigoid and increased Ca125 ovarian carcinoma marker values in the group of examined females and tPSA prostate carcinoma marker in the group of examined males were observed. These results confirm numerous cases on basis of which it was stated that pemphigoid-type skin lesions are often accompanied by carcinoma-type solid neoplasm of the internal organs [14, 22]. Available literature also provides reports indicating connection between co-existence of pemphigoid and lymphoreticular proliferations [3, 16] and Kaposi sarcoma [20].

The mean age in the group of examined pemphigoid female patients was 69, so it cannot be ruled out that increased prevalence of increased Ca125 marker values
may be caused by more frequent incidence of carcinomas in old age population [14].

In the group of examined males with pemphigoid a distinct interdependence between prevalence of increased IPSA values and autoantibodies characteristic for pemphigoid was observed. The mean age in this group was also high and equaled 71 years. Thus, in this case one should also allow for interdependence between age and carcinoma incidence.

Other causes explaining obtained results may include immunization phenomena. It is known that some of epithelial carcinomas of interior organs produce laminin-5, so the system may be immunized by this antigen [19], which may influence increased carcinoma incidence in patients with pemphigoid [12]. Pemphigoid-type skin lesions may also be the result of radiotherapy applied in treatment of other forms of carcinoma, mainly skin carcinoma. No such cases were observed in the examined group.

On the basis of tests conducted among patients with pemphigus no connection between co-existence of pemphigus and increased incidence carcinoma markers of which were determined was observed. It may be caused by small number of examined groups. Literature shows connection between incidence of pemphigus and neoplasms of internal organs, in particular immunological system neoplasms [21].

Early detection of increased levels of neoplasms markers in serum of patients with bullous diseases enables introduction of fast diagnostic procedures and oncological treatment. In is even more important in the case of solid neoplasms, because of fast diagnostic procedures and oncological treatment. In is in old age population [14].

References