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Article type: Original article

Received: October 27, 2019.

Accepted: December 5, 2019.

Published online: December 9, 2019.

ISSN: 1897-9483

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Original Article

Pernicious anemia and the presence of antibodies involved in the development of this disease and other autoimmune diseases

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Short Title: Pernicious anemia and antibodies

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Conflicts of interest: none declared
What’s new

Pernicious Anemia (PA) is an autoimmune disease in the pathogenesis of which autoantibodies against the Intrinsic Factor (IFAb) and Parietal Cells (APCA) are involved. In the paper, occurrence of IFAb and APCA in newly diagnosed PA patients, as well as their co-occurrence with antibodies involved in pathomechanism of some other (non-haematological) autoimmune diseases has been evaluated. An interesting issue of “autoimmunological alert”, i.e. predisposition of various autoimmunological diseases to co-exist, despite their different pathomechanisms and various tissues and organs being affected, has been introduced. The study has shown that simultaneous determination of IFAb and APCA in blood increases the possibility of the PA diagnosis. Also, screening assessment for connective tissue diseases and coeliac disease may be considered in PA patients, according to the observation that autoantibodies against nuclear components (ANA) are present in 16.1% of PA patients. Moreover, presence of IFAb or APCA increases possibility of occurrence of antiendomysial antibodies.
Abstract

Introduction: Pernicious Anemia (PA) is an autoimmune hematopoietic disease.

Objectives: The aim of the study was to determine the occurrence of autoantibodies in patients with PA participating in the pathogenesis of the disease as well as the development of other autoimmune disorders - Connective Tissue Diseases (CTD) and Celiac Disease (CD). We have also strived to document the potential usefulness of the specific diagnostic and screening tests in patients affected with PA.

Patients and methods: The study group consisted of 124 women and men with newly diagnosed PA and a control group (C) of 41 healthy people. Antibodies against intrinsic factor (IFAb), parietal cells (APCA), endomysium (EmA), and nuclear components (ANA) were determined in blood samples.

Results: Within the study group, the presence of antibodies involved in the pathogenesis of PA can be classified as 61.3% for IFAb or APCA, 46.0% for APCA, 30.6% for IFAb, 15.3% for IFAb and APCA. Statistical analysis shows that there is no significant difference in the occurrence of ANA and EmA between the PA and C groups. However, ANA was found in 16.1% of PA patients and in 4.9% of the controls. The occurrence of EmA in both groups is similar (3.2 vs. 2.4%), although, it has been shown that patients with IFAb or APCA are more prone to be EmA positive.

Conclusions: Simultaneous determination of IFAb and APCA significantly increases the possibility to confirm the diagnosis of PA. Also, screening assessment for CTD and CD may be considered in patients with PA.

Key words: antibodies; autoimmunity; intrinsic factor; parietal cell; pernicious anemia
INTRODUCTION

Pernicious Anemia (PA; Biermer’s disease, Addisonian Anemia) is an autoimmune disease in the pathogenesis of which autoantibodies against the Intrinsic Factor (IFAb) and Parietal Cells (APCA) are involved [1-16]. While IFAb interfere with absorption of Intrinsic Factor (IF) - vitamin B\textsubscript{12} complex in the terminal ileum, APCA are directed against gastric enzyme H\textsuperscript{+}/K\textsuperscript{+}-ATPase (the proton pump) [5, 9, 17]. In patients with diagnosed autoimmune disease, other disorders of the kind may develop with time or simultaneously, most likely as a result of general immune disturbances as well as cross-reactions between the antibodies [1, 14, 16, 18-26]. The afore-mentioned pathophysiological mechanism would justify expanding the diagnostic process in order to detect the possible dysfunction of other organs. This seems especially significant considering that treatment of the primary disease may interfere with the diagnosis of the subsequent disorder. In this context, the coexistence of immunological pathologies of the hematopoietic system and other autoimmune disorders could justify implementation of a wider diagnostic panel at the onset of the initial symptoms of the above-mentioned diseases [1].

The aim of the study was to determine occurrence of IFAb and APCA in patients diagnosed with PA, as well as co-occurrence of IFAb/APCA and antibodies involved in pathological mechanism of some other (non-haematological) autoimmune diseases. Our centre of interest encompasses a notion of an “autoimmunological alert”, i. e. predisposition of various autoimmunological diseases to co-exist, in spite of their different pathological mechanisms and various tissues, organs and systems being affected. The antibodies examined include those participating in the pathogenesis of PA as well as the development of other autoimmune disorders - Connective Tissue Diseases (CTD) and Coeliac Disease (CD). We have also strived to document the potential usefulness of the specific diagnostic and screening tests in patients affected with PA.
Characteristics of the antibodies tested
IFAb are specific for PA (98.6-100%) [9, 14]. IFAb sensitivity of 37-70% [9, 14, 18, 27-30] increasing up to 80% along with the duration of the disease [27] is reported. However, these antibodies are not present in all patients diagnosed with PA [12]. Both types of antibodies targeting IF are class IgG [14, 27]. In turn, the diagnostic sensitivity of APCA for PA is very high (80-97%), although their specificity (50-90.3%) is limited due to the number of other diseases in which these antibodies are present. APCA are detected in almost every case of PA but often also in the course of gastritis without anemia as well as in various autoimmune disorders [1, 4, 5, 9, 10, 14-18, 20, 24, 27-31]. The prevalence of APCA can precede the clinical symptoms of PA by years [9, 12, 22] and subsequently declines over time [12, 22, 28], possibly due to the loss of their target, i.e. the parietal cells along with the progression of autoimmune gastritis [1, 4, 5, 9, 10, 14-16, 22, 24, 27-29, 31], what could explain why some of the PA patients are APCA negative. Also, their prevalence in the general population rises with age [32, 33]. Most sources indicate that APCA do not occur in healthy people [4, 5, 9, 10, 14-16, 24, 27-29, 31], although according to some studies, APCA are present in 2.5-9% of the healthy population [34].

Anti-Nuclear Antibodies (ANA) play a role in the development and pathophysiological mechanism of CTD. Their clinical utility has long been well-established. For instance, they are relevant for diagnosis of conditions such as systemic lupus erythematosus, Sjogren's syndrome, scleroderma, dermatomyositis, rheumatoid arthritis, CREST syndrome, or mixed connective tissue disease. Among several antibodies described as participants in the development of CD, we were mostly interested in the role of Anti-Endomysium Antibodies (EmA). In children with suspicion of CD, presence of certain autoantibodies plays role in diagnostic process. First of all, the presence of anti-
transglutaminase antibodies (tTGA) is examined. In some cases, sufficiently high concentration of tTGA associated with presence of EmA allows to omit the diagnostic biopsy. Measurement of antibodies to deamidated gliadin peptides might also be of use [35].

Diagnostic criteria for adults allow several more possibilities. However, in this case as well serological testing for CD relies on tTGA as the first step. EmA may be used as a confirmatory test, particularly when tTGA has a low titer. The tTGA are the most sensitive for CD, whereas EmA are most specific [36, 37]. The above-mentioned antibodies are detected in the serum of CD patients [38] with high regularity. EmA are often detected even in the inactive stage of the autoimmune process and indicate a predisposition to the disease. However, antiendomysial IgG may occur in apparently healthy persons.

PATIENTS AND METHODS

The research project was approved by the Bioethical Commission of the Medical University of Silesia (Resolution No. KNW/0022/KB1/84/10). All subjects gave informed consent to participate in the study. The study group (PA) was made up of 124 people with a newly diagnosed autoimmune disease of the hematopoietic system - Pernicious Anemia. The group consisted of 85 women and 39 men aged ≥ 60 (61.3%), 50-59 (20.2%), 40-49 (12.1%), and 30-39 (6.5%) years old. Criteria for inclusion were age over 18 years; the diagnosis of PA based on a typical clinical picture, as well as fulfillment of at least 2 of 3 diagnostic criteria: a/ reduced serum B\textsubscript{12} level (below 200 pg/ml), b/ positive result for one of the antibodies: IFAb or/and APCA, c/ a positive therapeutic test, that is, precisely defined laboratory parameters, responses to a single parenteral administration of 1000 μg of vitamin B\textsubscript{12} in the form of an increase in the number of reticulocytes after 5-10 days (reticulocytic break), reduction of iron or LDH in the blood by 50% of baseline, remission of thrombocytopenia or/and neutropenia within 2 weeks, regression of anemia and hypersegmentation of granulocyte nuclei after 2-4
weeks. Exclusion criteria were neoplasm; chronic renal failure (eGFR < 30 ml/min/1.73m²); chronic hepatic failure (bilirubin above 34.2 µmol/l); symptomatic circulatory insufficiency; symptomatic respiratory failure; severe neurological diseases and mental disorders. In fact, within the examined group there were no persons with eGFR lower than 45 ml/min/1.73m².

The control group (C) consisted of 41 people, being 30 women and 11 men of comparable age to the study group. The C group has been created in order to compare prevalence of ANA and EmA between PA and C groups. Criteria for inclusion were age over 18 years; exclusion of autoimmune disease based on the interview and available test results; negative antibody result of IFAb and APCA; no clinical signs of organ dysfunction; no aberrations in blood count or blood smear; no aberrations in basic laboratory parameters (listed below), including vitamin B₁₂ serum concentration; exclusion of chronic pharmacotherapy; negative history of autoimmune diseases in the family. In both groups (PA, C) blood for testing up to 20 ml was collected in the morning, in fasting state, from the ulnar vein using a vacuum system.

Serum/plasma was stored at -75°C until antibody determination.

IFAb (IgG) was determined by Enzyme-Linked Immunosorbent Assay (ELISA) and quantified. The interpretation of test results accepted in the experiment was < 20 RU/ml - negative result; ≥ 20 RU/ml - positive result. Assays were made using the ELISA test kit from EUROIMMUN Medizinische Labordiagnostika AG, Germany. The upper cut-off limit recommended by the kit manufacturer is 20 Relative Units (RU)/ml. Since there is no international reference serum/plasma for IFAb assessment, the calibration is performed in relative units (RU/ml). The manufacturer evaluated the level of IFAb in 351 healthy blood donors using the EUROIMMUN ELISA test and at a cut-off point of 20 RU/ml, showed that all blood donors were negative (reference group). The linearity of the applied test was within the measurement range of 0.2-200 RU/ml. The lower detection limit of the test used is 1
RU/ml (analytical sensitivity). This limit was defined as three times the standard deviation of the blind test and was the lowest value of the determined IFAb titer. The test kit used does not show cross-reactions (analytical specificity). The kit also does not interfere with hemolytic, lipemic, and hyperbilirubinemia sera (up to 10 mg/ml hemoglobin, 20 mg/ml triglycerides, 0.4 mg/ml bilirubin). The coefficient variation (CV) for intra-assay-variation measurements and CV of inter-assay-variation measurements in various ranges, evaluated in order to control the repeatability, in the standard curve is 4-5.1% and 3.7-7.8%, respectively.

APCA (IgG) was determined by ELISA and assessed semi-quantitatively. The interpretation of test results accepted in the experiment was negative result; positive result. The assays were made using the ELISA test kit from EUROIMMUN Medizinische Labordiagnostika AG, Germany. The results were evaluated semi-quantitatively by calculating the so-called Ratio coefficient, i.e. the ratio of the extinction of the control or the patient to the extinction of the appropriate calibrator. The upper cut-off limit recommended by the kit manufacturer is 20 Relative Units (RU)/ml. Since there is no international reference serum/plasma for APCA assessment, the calibration is carried out in relative units (RU/ml). EUROIMMUN recommends the following interpretation of the test results: Ratio < 1.0 - negative, ≥ 1.0 - positive result; in the quantitative assessment < 20 RU/ml - negative result, ≥ 20 RU/ml - positive result. The manufacturer evaluated the level of APCA (IgG) in 200 healthy blood donors using the EUROIMMUN ELISA test and at a cut-off point of 20 RU/ml showed that 4.5% of blood donors were APCA-positive (reference group). The linearity of the applied test was within the measurement range of 2-200 RU/ml. The lower detection limit of the test used is 1 RU/ml. This limit of detection was defined as three times the standard deviation of the blind test and was the lowest value of the APCA titer determined. The test kit used does not show cross-reactions. The kit also does not interfere with hemolytic, lipemic and hyperbilirubinemia sera (up to 10 mg/ml hemoglobin, 20 mg/ml triglycerides, 0.4 mg/ml
bilirubin). Intra-assay-variation and inter-assay-variation CV in various ranges of the standard curve are 2.5-4.8% and 3.1-4.4%, respectively. The sensitivity and specificity of the ELISA used for the indirect immunofluorescence assay (IIFT) considered to be the reference method is 97.3% and 94%, respectively.

ANA was determined by indirect IIFT and qualitatively assessed. The interpretation of test results accepted in the experiment was negative result; positive result. The assays were made using a test kit from BioSystems, Spain, which is intended for in vitro testing of human anti-nuclear antibodies in serum. Human epithelial cells (HEp-2) are incubated with patient samples. In positive cases, ANA binds to the relevant antigens present in HEp-2 cells. The resulting antigen-antibody complexes are detected with goat anti-human IgG labeled with fluorescein isothiocyanate and visualized using a fluorescence microscope equipped with an excitation filter of 495 nm and an emission filter with a wavelength of 525 nm. The observation at the recommended dilution, specific fluorescence was regarded as a positive result. There are different patterns of fluorescent taints that can be found in the same serum. The pattern can be homogeneous, peripheral, speckle, nucleolar, centromeric. If one of these specific taints did not occur, the result was treated as negative for these autoantibodies (the result precludes their presence). Sensitivity and diagnostic specificity is 98.3% and 93%, respectively.

EmA (IgG) was determined by ELISA and scored semi-quantitatively. The interpretation of test results accepted in the experiment was negative result; positive result. Assays were performed using an Eagle Biosciences, USA ELISA test kit. The results were evaluated semi-quantitatively by calculating the so-called Ratio coefficient, i.e. the ratio of the extinction of the control or the patient to the extinction of the appropriate calibrator. The manufacturer recommends the following interpretation of the test results: Ratio < 1.0 - negative, ≥ 1.0 - positive result; in the quantitative assessment < 20 U/ml - negative, ≥ 20
U/ml - positive. The lower detection limit of the assay used is 3 U/ml. Intra-assay-variation and inter-assay-variation CV in different ranges of the standard curve are 2.4-6% and 6.1-7.9%, respectively. The sensitivity and specificity of the ELISA used for the IIFT test considered as the reference method is 97.3% and 94%, respectively.

Serum concentration of Vitamin B₁₂, iron, ferritin, folic acid; peripheral blood count with smear, platelets count determined by the use of manual method, INR, APTT, PT, fibrinogen, D-dimers; proteinogram, total protein concentration; basic biochemical tests (creatinine, glucose, bilirubin, ALT, electrolytes, lipid profile, uric acid, CRP), TSH, fT₄, fT₃ were determined using routine kits of biochemical analysers.

**Statistical methods and tools:**

SPSS software has been used for statistical analysis. In the statistical elaboration, variables were available in either the qualitative form (IFAb, APCA, ANA, EmA) and one parameter on the quantitative scale (IFAb). Relationship for which \( P < 0.05 \) were considered significant. To assess the relationship between the nominal variables, the Chi-square Pearson independence test was used. For small samples size and df=1 Fisher's exact test has been used. A hierarchy of risk of the appearance of antibodies in the study group was developed. In order to achieve this, the probability maximizing approach has been used, with the probability of the response being taken into consideration. The study uses a Multidimentional Correspondence Analysis (MCA) for the occurrence of certain groups of antibodies in the study group. Thanks to this analysis, groups of clusters of certain measured parameters, e.g. antibodies and diseases caused by them, can be selected.

**RESULTS**

Table 1
IFAb or APCA occur in over 60% of patients who fulfill the criteria for diagnosis of PA (n=76/124). APCA are found in these patients more often (n=57/124) than IFAb (n=38/124) and simultaneous occurrence of both antibodies occurs in 15% (19/124) of patients. The results were summarized in a probabilistic assessment of the risk [0-100%] of the appearance of antibodies involved in the pathogenesis of PA (Table 1). For the quantified IFAb [RU/ml], descriptive statistics were calculated: mean (SD) 40.71 (71.08); min. 0; max. 329.1; median 5.4 (data not included in the table). The statistical analysis shows that there is no significant difference in the occurrence of ANA and EmA between PA and C groups. However, it is worth noting that ANA were found in 16.1% of patients with PA and in 4.9% of persons from the control group, and the occurrence of EmA in both groups is at similar level of around 2-3%. Thus, a tendency for a difference in ANA titer between the PA and C groups can be seen (Table 2). The thesis whether in patients with PA there is an association between the occurrence of antibodies involved in the development of CTD and CD with antibodies involved in the pathogenesis of PA has been verified, and such statistically significant relationships were not found. It is also of interest that in 7.9% of the subjects diagnosed with IFAb, EmA were found at the verge of significance ($P = 0.050$) (Table 3). The analysis of the thread has been extended with the MCA of the occurrence of certain groups of antibodies, which allowed the creation of a statistically significant (Chi-square=4584.51; $P = 0.009$) model (distribution of factors and their clusters on the Burt matrix). Based on this, it has been shown that PA patients in which IFAb or APCA occur, are highly probable to be EmA positive (Figure 1).
DISCUSSION

Presence of either IFAb, or APCA, or, subsequently, both, constitutes one of the diagnostic criteria of PA. Thus, in clinical context, category "IFAb or APCA" has been introduced to encompass the whole population of examined PA patients. The aim of our paper is to define the group of patients in which risk of other autoimmune diseases would be the highest (i.e."autoimmunological alert" would be the most evident). Accordingly, in order to distinguish the group of patients in which both antibodies are present, “IFAb and APCA” category has been introduced. Introducing categories “IFAb”, “APCA” allowed us to determine occurrence of each antibody in the examined population of PA patients. In the examined group of PA patients (n=124), IFAb or APCA occur in 61.3%. APCA occur more often (46%) than IFAb (30.6%), and the simultaneous occurrence of both antibodies occurs in 15.3% (Table 1). This remains in line with the data from the previously mentioned literature as well as other publications. For instance, in patients with PA who underwent endoscopy of the upper gastrointestinal tract (n=34), IFAb were found in 52%, APCA in 97% [15], and the combination of both antibodies for PA yields 73% sensitivity (and 100% specificity) [18]. IFAb were positive in 38% and APCA in 56% of patients (n=50) [39]. In turn, in a study of the Korean population (n=83), IFAb or APCA were present in 85.5% of PA patients (which is close to our result). However, conversely to our results, in this population, IFAb occur more often (77.5%) than APCA (43.2 %), and the simultaneous presence of both antibodies occurs in 34.6% [7]. The highest prevalence of PA is seen in Northern Europeans, especially those living in the United Kingdom and Scandinavia [9]. Could the above described epidemiological diversity be related to a different constellation of the prevalence of IFAb and APCA?
The prevalence of the analyzed antibodies has also been described in other autoimmune diseases, such as autoimmune thyroid disease (AITD), including Hashimoto or Graves diseases, as well as type 1 diabetes (T1D), vitiligo and CD [12, 14, 18, 24, 26, 40, 41]. Genetic susceptibility for PA is suggested by a specific HLA-DR pattern, which is known to be associated with other autoimmune diseases [9, 12, 18, 21, 42, 43]. It has been proved that the genotypes of Human Leukocyte Antigen (HLA)-DRB1*03 and DRB1*04, which are known to be associated with other autoimmune disease such as T1D and AITD [21], are also associated with PA. This observation supports the role of autoimmunity in PA [18] (in patients suffering from CD majority is HLA-DQ2 and/or DQ8 positive [35, 37, 44]). Anti-Thyroid Peroxidase Antibodies (TPOAb) or Anti-Thyroglobulin Antibodies (TgAb), typical for AITD, are more frequently present (mainly TPOAb) in patients with PA who have IFAb or APCA. This correlation seems most evident in patients with simultaneous occurrence of IFAb and APCA [1]. Our conjecture that anti-adrenal and anti-pituitary antibodies, involved accordingly in some cases of Addison’s disease and hypopituitarism, could be more frequent in PA patients, have not been confirmed [1]. APCA are also more frequent in patients diagnosed with Graves’ disease, and vitiligo [5, 14, 22, 26, 31]. The coexistence of autoimmune diseases of the endocrine glands, with gastroenterological and rheumatic diseases, is referred to as Autoimmune Polyendocrine Syndrome (APS). Patients with APS had significantly higher frequencies of the HLA A24, A31, B8, B51, B62, DR3, and DR4 [45]. In order to continue our previous research [1], we have decided to test patients suffering from PA for biochemical screening indicators of CTD (ANA) and CD (EmA). At first we have also considered screening the study group for antibodies involved in Latent Autoimmune Diabetes of Adults. However, after familiarizing ourselves with literature regarding co-occurrence of PA and LADA, we have found the possibility unlikely [46].
The co-occurrence of autoimmune diseases constitutes such a well-known fact that it seems surprising how few studies exist which evaluate the usefulness of a so-called "autoimmune alert", i.e. measurement of the clinically accepted diagnostic exponents of these processes. This could prove especially useful during the prodromal stage of the subsequent autoimmune disease. With regard to CTD, in the last 20 years, very little other than case reports were described. Seven patients with an association of PA and Sjögren's syndrome have been described [47] as well as a group of 74 patients with PA, among whom 7 were affected with Sjogren syndrome, 5 diagnosed with antiphospholipid syndrome, and 1 was suffering from systemic lupus erythematosus (1.35%) [26]. APCA and PA were found in 1/194 patients with systemic lupus erythematosus [48]. In turn, in patients with CD, APCA occurred 3-10 times more frequently [14, 31]. APCA are also more common in first- and second-degree relatives of people with CD [14, 24]. CD is associated with increased risk of all malignancies, especially those affecting the gastrointestinal tract [49], while PA is associated with increased risk of stomach cancer [11, 50]. In this context, the assessment of the co-occurrence of CD and PA could be considered when oncological vigilance is discussed. Determination of the co-occurrence of only two non-haematological antibodies with IFAb or APCA can be seen as a limitation of our paper. Even though we were not able to determine a significant statistical difference in the occurrence of ANA and EmA between the examined group of PA patients and the control group (C), our analysis showed that individuals suffering from PA in which IFAb or APCA are present, have higher risk of EmA occurrence (Figure 1). Considering that 16.1% of PA patients in our study were ANA positive, CTD screening could also be considered.

Conclusion: Simultaneous determination of IFAb and APCA significantly increases the likelihood of confirming the diagnosis of PA. In patients with PA, screening for Connective Tissue Disease and Celiac Disease may be considered.
CONTRIBUTION STATEMENT

EM-S and DK conceived the concept of the study. EM-S, DK, WF, BM, JG-Sz, BK-K contributed to the design of the research. EM-S, DK, WF were involved in data collection. All authors analyzed the data, edited and approved the final version of the manuscript.

ACKNOWLEDGMENT

Funding: This work was supported by the Medical University of Silesia grants; No. KNW-1-135/N/3/0, KNW-1-056/N/4/0, KNW-1-158/K/5/0; to DK.

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Table 1. List of antibodies against Anti-Intrinsic Factor (IFAb) and Anti-Parietal Cell (APCA) acc. risk of their presence in Pernicious Anemia patients (PA).

| Anti-Intrinsic Factor (IFAb) and Anti-Parietal Cell (APCA) Antibodies in Pernicious Anemia patients |
|-----------------------------------------------------------------------------------------------|
| IFAb or APCA                                                                                   | 61.3% |
| APCA                                                                                           | 46.0% |
| IFAb                                                                                           | 30.6% |
| IFAb and APCA                                                                                  | 15.3% |
Table 2. Prevalence of antibodies involved in development of Connective Tissue Disease (CTD) and Coeliac Disease (CD) i.e. Anti-Nuclear (ANA) and Anti-Endomysium (EmA) Antibodies in Pernicious Anemia patients (PA) and in Control group (C).

| Antibodies | result | Pernicious Anemia (PA) | Control (C) | Chi-square\(^e\) test | \(P\) |
|------------|--------|------------------------|-------------|------------------------|-----|
| ANA        | negative | 104 | 83.90 | 39 | 95.10 | 0.07 |
|            | positive  | 20  | 16.10 | 2  | 4.90  |      |
| EmA        | negative | 120 | 96.80 | 40 | 97.60 | 0.80 |
|            | positive  | 4   | 3.20  | 1  | 2.40  |      |
| ANA and EmA| negative | 124 | 100   | 41 | 100   |      |
|            | positive  | 0   | 0     | 0  | 0     |      |

List of abbreviations: ANA - Anti-Nuclear Antibodies; C – Control; EmA - Anti-Endomysium Antibodies; PA – Pernicious Anemia; n – number of patients/controls; F - Fisher's exact test.
Table 3. Coexistence of antibodies involved in development of Connective Tissue Disease (CTD) and Coeliac Disease (CD) with antibodies involved in pathogenesis of pernicious anemia in Pernicious Anemia patients (PA).

| Antibodies / Chi-square<sup>f</sup> test | IFAb / negative | IFAb / positive | APCA / negative | APCA / positive | IFAb or APCA / negative | IFAb or APCA / positive | IFAb and APCA / negative | IFAb and APCA / positive | P |
|--------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------------|-----------------------|------------------------|------------------------|---|
| ANA                                  |                 |                 |                 |                 |                       |                       |                        |                        |---|
| negative                             | 73              | 84.90%          | 31              | 81.60%          | 0.65                  | 56                    | 83.60%                 | 48                    | 84.20%                | 0.92 | 42 | 87.50% | 62 | 81.60% | 0.38 | 87 | 82.90% | 17 | 89.50% | 0.47 |
| positive                             | 13              | 15.10%          | 7               | 18.40%          | 0.050                 | 11                    | 16.40%                 | 9                     | 15.80%                | 0.87 | 48 | 100.00% | 72 | 94.70% | 0.11 | 102 | 97.10% | 18 | 94.70% | 0.59 |
| EmA                                  |                 |                 |                 |                 |                       |                       |                        |                        |---|
| negative                             | 85              | 98.80%          | 35              | 92.10%          | 0.050                 | 65                    | 97.00%                 | 55                    | 96.50%                | 0.87 | 48 | 100.00% | 72 | 94.70% | 0.11 | 102 | 97.10% | 18 | 94.70% | 0.59 |
| positive                             | 1               | 1.20%           | 3               | 7.90%           |                       | 2                     | 3.00%                  | 2                     | 3.50%                 |                       | 0 | 0.00% | 4 | 5.30% | 0.00 | 3 | 2.90% | 1 | 5.30% | 0.00 |

List of abbreviations: ANA - Anti-Nuclear Antibodies; APCA - Autoantibodies against the Parietal Cells; EmA - Anti-Endomysium Antibodies; IFAb - Autoantibodies against the Intrinsic Factor IFAb; n – number of patients; F - Fisher's exact test.
Figure 1. Coexistence of antibodies involved in development of Coeliac Disease (CD) with antibodies involved in pathogenesis of pernicious anemia in Pernicious Anemia patients (PA).

Figure legends: 1 – study group; 0 – control group; APCA - Autoantibodies against the Parietal Cells; EmA - Anti-Endomysium Antibodies; IFAb - Autoantibodies against the Intrinsic Factor IFAb;