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ABSTRACT

BACKGROUND: The anti-DFS70 autoantibodies are one of the most commonly and widely described agent of unknown clinical significance, frequently detected in healthy individuals. It is not known whether the DFS70 autoantibodies are protective or pathogenic. One of the factors suspected of inducing the formation of anti-DFS70 antibodies is increased oxidative stress. We evaluated the coexistence of anti-DFS70 antibodies with selected markers of oxidative stress and investigated whether these antibodies could be considered as indirect markers of oxidative stress.

METHODS: The intensity of oxidative stress was measured in all samples via indices of free-radical damage to lipids and proteins such as total oxidant status (TOS), concentrations of lipid hydroperoxides (LPH), lipofuscin (LPS), and malondialdehyde (MDA). The parameters of the non-enzymatic antioxidant system, such as total antioxidant status (TAS) and uric acid concentration (UA), were also measured, as well as the activity of superoxide dismutase (SOD). Based on TOS and TAS values, the oxidative stress index (OSI) was calculated. All samples were also tested with indirect immunofluorescence assay (IFA) and 357 samples were selected for direct monospecific anti DFS70 enzyme-linked immunosorbent assay (ELISA) testing.

RESULTS: The anti-DFS70 antibodies were confirmed by ELISA test in 21.29% of samples. Compared with anti-DFS70 negative samples we observed 23% lower concentration of LPH ($P = .038$) and 11% lower concentration of UA ($P = .005$). TOS was 20% lower ($P = .014$). The activity of SOD was up to 5% higher ($P = .037$). The Pearson correlation showed weak negative correlation for LPH, UA, and TOS and a weak positive correlation for SOD activity.

CONCLUSION: In samples positive for the anti-DFS70 antibody a decreased level of oxidative stress was observed, especially in the case of samples with a high antibody titer. Anti-DFS70 antibodies can be considered as an indirect marker of reduced oxidative stress or a marker indicating the recent intensification of antioxidant processes.

KEYWORDS: Antibodies, oxidative stress, free radicals, biomarkers

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Introduction

Although many autoantibodies are detectable, not all are associated with specific connective tissue diseases, even though some of them are detected in very high titers.[1,2] In recent years, one of the most commonly and widely described autoantigen of unknown clinical significance is stress oncoprotein lens epithelium-derived growth factor p75 (LEDGF/p75), also known as dense fine speckled 70 kDa (DFS70) autoantigen. The clinical relevance of anti-DFS70 autoantibodies remains unknown and still requires investigation.[3,4] It is unclear whether anti-DFS70 autoantibodies play a pathogenic or protective role.[5] Furthermore, the underlying cause of anti-DFS70 formation is not known.

The dense fine nuclear speckled pattern was first described by Ochs et al[6] and the associated antigen was named DSF70 in 2000.[7] A year earlier, Toshimichi Shinohara's group, working independently, called it LEDGF/p75, not knowing that it was related to DFS70,[8] and a few years later the protein and the gene were named PSIP1 (PC4 and SFRS1 Interacting Protein 1).[9,10] According to the primary studies, DFS70/LEDGF/p75 was thought to be a crucial factor in lens epithelial cell proliferation, but further studies have shown that this protein is a common cell growth promoter or transcription factor that is activated in response to increased stress conditions in the cell microenvironment.[11-14] Examples of such situations include increased oxidative stress induced by the use of cytotoxic drugs that induce oxidative DNA damage or exposure to radiation.[15] The important role of DFS70/LEDGF/p75 as a regulator of gene transcription activated in response to inflammatory stress occurring in autoimmune diseases, cancer, and also in the pathophysiology of acquired immunodeficiency syndrome (AIDS) has also been demonstrated.[15-22] Expression of DFS70/LEDGF/p75 protein therefore increases the chance of cell survival under various stress conditions in both diseased and healthy individuals.

Reactive oxygen species (ROS) can induce posttranslational modifications in certain proteins that can be recognized by the immune system as neoepitopes that are source of autoantibody formation.[23-26] As suggested by Ortiz-Hernandez et al[27] the autoantibody response to DFS70/LEDGF/p75 could be considered as a possible marker of increased oxidative stress which, in a pro-inflammatory microenvironment, leads, on the one hand, to increased expression of this protein and on the other, may cause its post-translational modifications. Moreover, Wu et al observed that during enhanced oxidative stress, DFS70/LEDGF/p75 undergoes thioredoxin1 (Trx1) mediated post-translational modifications involving cysteine reduction to maintain its stress-modulating function. These findings clearly

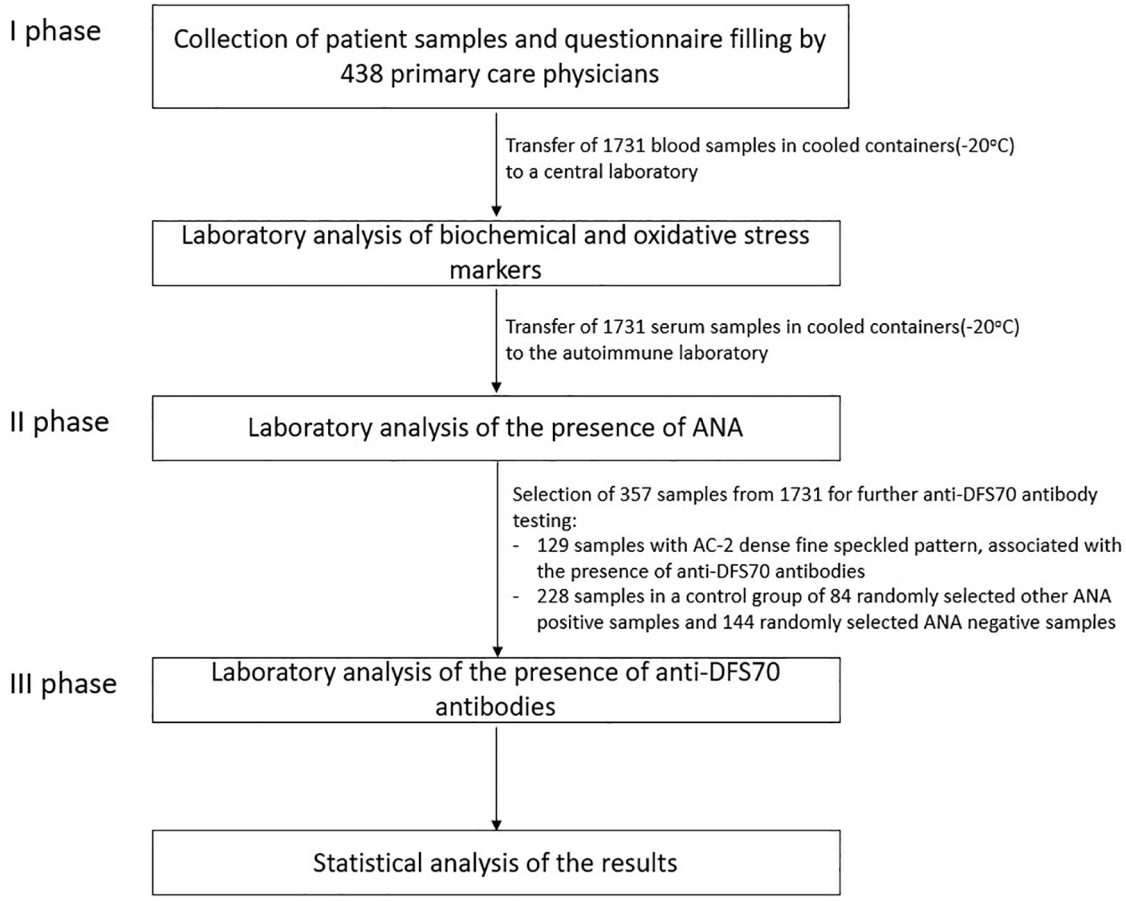

Figure 1. Research procedural stages.

show the potential for the protein to be modified.[28] Unfortunately, there is still no evidence that such stress-associated modifications increase the immunogenicity of this protein.

In this study, we evaluated the coexistence of anti-DFS70 antibodies with selected oxidative stress markers and their relationship with sociodemographic factors. We evaluated whether the anti-DFS70 could be considered to be an indirect marker of oxidative stress. In addition, we estimated the prevalence of anti-DFS70 antibodies in the Polish population.

Materials and Methods

A simplified scheme of the study is shown in Figure 1.

Design

A nationwide observational, cross-sectional study was carried out in Poland in the fourth quarter of 2015 and the first and second quarters of 2016.

Sampling

This study is part of a large research program, "Nationwide study of cardiovascular health in primary care in Poland—LIPIDOGRAM2015 and LIPIDOGEN2015," the design and rationale of which have been described in detail previously by Jóźwiak et al.[29] Briefly, the recruitment was carried out by 438 primary care physicians in 16 major administrative regions of Poland. Physicians/investigators were randomly selected from the Medical Data Management database. The expected number of patients recruited for LIPIDOGRAM2015 study (consecutive sample) was 13 000 to 14 000 with 13% to 15% (1700-2000) enrolled to the LIPIDOGEN2015 sub-study (random sample). The program included only adult patients over 18 years old. Each patient had to complete a questionnaire concerning medical and family history, concomitant diseases, and pharmacotherapy. The following criteria were used in the physical activity question: regular physical effort—increased activity of the musculoskeletal system, regularly for 2 to 2.5 hours/week, defined as exercising, walking, running, swimming, playing team games, dancing, and doing housework or household chores; or no regular physical activity—people who do not meet the criteria of regular physical activity; or others who did not provide detailed information on the level of their physical activity. In the question concerning the use of diet (hypolipemic, hypoglycemic, hypotensive) the following criteria were used: use of an appropriate diet—regular consumption of varied low cholesterol foods, moderate consumption of medium cholesterol foods, reduced consumption of saturated fats—in favor of monounsaturated and polyunsaturated fats, reduced consumption of carbohydrates and sweetened drinks, reduced consumption of table salt, increased consumption of

fish, increased consumption of fruit and vegetables and fiber-rich foods or no use of an appropriate diet—those not meeting the criteria for following an appropriate diet. Anthropometric measurements (height, body weight, waist circumference, and hip circumference) were performed at the doctor's office. In all enrolled patients, serum samples were obtained after ⩾12 hours of fasting. On the same day, measurements of blood pressure, heart rate, and fasting glucose were obtained in addition to lipid profiles. For the LIPIDOGEN2015 sub-study, saliva samples for DNA isolation and blood samples were collected to measure glycated hemoglobin, oxidative stress parameters, autoantibody levels, and inflammatory cytokine and apolipoprotein profiles.

For this study, we used 1731 serum samples from the above-mentioned LIPIDOGEN2015 sub-study. The tested group included 1043 women and 688 men. The blood samples were transferred in cooled containers (−20°C) to a central laboratory (Silesian Analytical Laboratories—SLA in Katowice, Poland) for biochemical analyses and then to the autoimmune laboratory (Euroimmun Poland Ltd. Customer Training Laboratory in Wroclaw, Poland) for Anti-nuclear Antibodies (ANA) determination.

Laboratory analyses

Measurements of total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) (direct immunological measurement) were performed and carried out using the same methodology and the same Siemens Advia 1800 analyser and Siemens reagents (Munich, Germany), within 12 hours of obtaining the blood sample. Fasting glucose levels were measured using Bionime glucometers (Taichung City, Taiwan) and Rightest strip tests (Taichung City, Taiwan).

For the evaluation of the intensity of oxidative stress, the indices of free-radical damage to lipids and proteins, enzymatic and non-enzymatic antioxidant system parameters in serum and erythrocytes were measured. In serum, indices of free-radical damage to lipids and proteins include: total oxidant status (TOS),[30] lipid hydroperoxides (LPH),[31] lipofuscin (LPS),[32] and malondialdehyde (MDA)[33] concentration. The activity of superoxide dismutase (SOD)[34] was determined in serum. In addition, parameters of non-enzymatic antioxidant systems, such as total antioxidant status (TAS)[35] and uric acid (UA) were also measured. The oxidative stress index (OSI) was calculated using the TOS/TAS formula. In erythrocytes, the indices of free-radical damage to lipids and proteins, such as LPS and MDA, were determined as was the activity of SOD.[34]

In the second phase, ANA were detected using an indirect immunofluorescence assay (IFA) in human laryngeal carcinoma cells (HEp-2) with commercially available Euroimmun Medizinische Labordiagnostika AG (Lübeck, Germany) test kits Mosaic Basic Profile 3 (catalog number FC 1800-2010-3). Sample incubation was carried out manually, according to the manufacturers' instructions, except that 998 samples were diluted with a threshold cut-off 1:160 as recommended by the current guidelines[36] and 733 patient samples were diluted with a threshold cut-off 1:100 as recommended by the test kit manufacturer's instruction. The samples were divided into 2 groups randomly. The results were evaluated on a EUROstar III fluorescence microscope (CarlZeiss Oberkochen, Germany). The test result included a qualitative assessment of the presence of ANA, estimation of antibody titer, and determination of the characteristic pattern according to the International Consensus on ANA Patterns (ICAP) nomenclature.[37] Results of IFA were collected and stored as digital images.

In the third phase, the concentration of anti-DFS70 antibodies was determined using ELISA. We used commercially available Euroimmun Medizinische Labordiagnostika AG (Lübeck, Germany) test kits (catalog number EA 159z-9601 G). Incubation was performed automatically with Euroimmun Analyzer I (Lübeck, Germany) according to the instructions included with the test kit. In this phase, we tested 357 preselected samples, of which 129 were samples with AC-2 dense fine speckled pattern (usually associated with the presence of anti-DFS70 antibodies) positive in the screening. The control group consisted of a random selection of 228 samples, out of which 84 were ANA positive in a screening test but with another type of pattern, and 144 were ANA negative samples. The ELISA results were expressed using the semi-quantitative Ratio index, which is the ratio of sample extinction to cut-off calibrator extinction. According to the ELISA test manual, Ratio index ⩾1.0 was considered positive.

Statistical analysis

Statistical analyses were carried out were performed using Statistica 13.3 (StatSoft, Tulsa, USA). Data are expressed as mean ± SD (for normal distribution) and median (nonparametric distribution) for continuous variables, and as a percentage for categorical variables. Univariate comparison of markers related to autoimmune diseases according to clinical variables was performed using the *U*-Mann-Whitney method for nonparametric variables or χ^2 test/Fisher exact test where appropriate. A 2-sided $P < .05$ was considered to indicate significance. Additionally, regression analysis was performed (R—multiple correlation coefficient, R^2 coefficient of determination, β*—regression standardized coefficient) and Pearson's correlation were calculated.

Results

The study included 357 patients attending primary health care practices (251 women and 106 men). Two hundred and twenty-six participants were diagnosed with a range of cardiovascular and renal disorders (eg, hypertension, coronary artery disease, dyslipidemia, diabetes, atrial fibrillation, kidney disease, or stroke), and 131 participants were apparently healthy individuals. The mean age of participants was 53 ± 12 years. The body

Table 1. Characteristics of the population.

	ALL (N = 357)		MALE (N = 106)		FEMALE (N = 251)	
	MEAN	SD	MEAN	SD	MEAN	SD
Age (years)	53.0	12.5	53.7	12.4	52.7	12.6
Height (cm)	166.8	8.53	176.0	6.45	162.9	5.94
Weight (kg)	78.6	15.9	90.2	13.8	73.7	14.1
BMI (kg/cm^2)	28.2	4.90	29.0	3.71	27.8	5.28
Waist circumference (cm)	93.2	13.4	100.9	9.46	90.0	13.5
Hips circumference (cm)	105.1	10.6	104.1	8.64	105.5	11.3
WHR	0.89	0.09	0.97	0.07	0.85	0.07

Abbreviations: BMI, body mass index; WHR, waist-hip ratio.

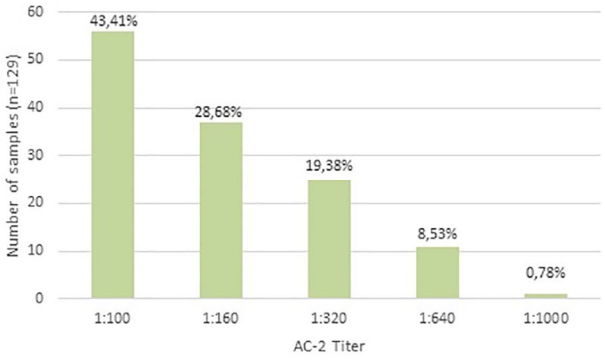

Figure 2. ANA AC-2 pattern distribution depending on the titer.

mass index (BMI) indicated that the participants were on average slightly overweight,[38] and the average waist-hip ratio (WHR) was above the normal range for both men and women.[39] No difference in age between men and woman ($P = .483$) was found (Table 1).

In the initial group of 1731 samples, from which we selected samples for the main study group, we found ANA in 15.02% (260/1731). The most frequent pattern was AC-2 dense fine speckled, detected in 50% (130/260) of all ANA positive samples, which accounted for 7.51% of all samples tested. One sample was AC-2 positive, but sufficient material was not available for further ELISA testing.

In our main study group, consisting of preselected 357 samples, 129 were determined to be AC-2 positive. This type of pattern is most often associated with anti-DFS70 antibodies.[3,36] The majority of them are samples in low titer (Figure 2). The presence of anti-DFS70 antibodies was confirmed by monospecific ELISA test in 21.29% (76/357) of samples. Extrapolation of these data allows us to estimate that the prevalence of anti-DFS70 in the Polish population is 4.39% (76/1731). Table 2 summarizes the presence of anti-DFS70 antibodies categorized by sample type. In a group of mixed patterns, there were 2 samples with additional pattern AC-21 and 1 sample each with patterns AC9/10, AC-27, and AC 25/26. In 6 of the samples classified as ANA negative, anti-DFS70 antibodies were found. In 4 samples, the Ratio index was low (1.08-1.56). In 1 sample, the Ratio index was raised (2.37) and was very high (6.47) in another. Table 3 presents the distribution of anti-DFS70 antibodies in ANA positive samples by staining pattern. The AC-2 pattern occurred most frequently. Anti-DFS70 antibodies are confirmed in samples characterized by AC-1, AC4/AC5, and AC-27 patterns.

Table 4 presents the distribution of anti-DFS70 antibodies according to the gender and age of participants. Anti-DFS70 are more prevalent in young people ($P = .008$), but when analyzed by sex, this was only the case for women ($P = .003$).

When comparing participants with and without cardiovascular and renal disorders, anti-DFS70 antibodies were present more commonly in those free of disease (27.5%, 36/131, $P = .03$). These antibodies were also observed less frequently in patients with arterial hypertension ($P = .023$). In our cohort, smokers seem to produce fewer antibodies, whether they still smoke ($P = .002$) or have done so in the past ($P = .006$). Comprehensive results of analysis of the prevalence of anti-DFS70 antibodies and various lifestyle factors, cardiovascular and renal diseases are shown in Table 5.

The relationship between the presence of anti-DFS70 antibodies and individual markers of oxidative stress is summarized in Table 6. We observed a 23% lower concentration of lipid hydroperoxides (LPH) ($P = .038$) and 11% lower concentration of uric acid (UA) ($P = .005$). Total oxidant status (TOS) was 20% lower ($P = .014$) than in patients without anti-DFS70 antibodies. Additionally, these results were accompanied by slightly higher (up to 5%) activity of superoxide dismutase (SOD) ($P = .037$) in red blood cells. Other oxidative stress markers did not differ significantly between the compared groups.

We also evaluated whether or not whether associations exist between higher concentrations of anti-DFS70 antibodies and individual oxidative stress markers. For this purpose, we arbitrarily divided the anti-DFS70 positive samples into 2 groups

Table 2. Anti-DFS70 antibodies distribution in tested group.

TESTED GROUP (N = 357)	DFS70 POSITIVE N = 76 N (%)	DFS70 NEGATIVE N = 281 N (%)
AC-2—dense fine speckled	65 (18.21)	59 (16.53)
Mixed pattern (AC-2 + other ANA[a])	1 (0.28)	4 (1.12)
Other ANA positive	4 (1.10)	80 (22.41)
ANA negative	6 (1.70)	138 (38.66)

Abbreviations: AC, anti-cell; ANA, anti-nuclear antibody; DFS70, dense fine speckled.
[a]Two samples additionally showed pattern AC-21 and 3 more samples additionally showed patterns AC9/10, AC-27, and AC 25/26, 1 additional type in each of the 3 samples.

Table 3. Anti-DFS70 antibody distribution depending on the ANA positive pattern.

TYPE OF PATTERN	ANTI-DFS70 (+)		ANTI-DFS70 (–)		*P*-VALUE
	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	
AC-1—Homogenous	2	74	8	273	.771
AC-2—Dense fine speckled	66	10	63	218	**<.001**
AC-3—Centromere	0	76	3	278	.844
AC4/AC5—Fine/large/coarse speckled	2	74	36	245	**.019**
AC6/AC7—Multiple/few nuclear dots	0	76	2	279	.879
AC-8—Homogenous nucleolar	0	76	1	280	.482
AC-9/AC-10—Clumpy/punctate nucleolar	0	76	28	253	**.004**
AC-15—Fibrillar linear	0	76	2	279	.879
AC-16—Fibrillar filamentous	0	76	1	280	.482
AC-21—Reticular/AMA	0	76	9	272	**.036**
AC-23—Rods and rings	0	76	1	280	.482
AC-25/AC-26—Spindle fibers/NuMA-like	0	76	3	278	.844
AC-27—Intercellular bridge	1	75	1	280	.879
AC-28—Mitotic chromosomal	0	76	1	280	.482

Abbreviations: AC, anti-cell; AMA, antimitochondrial antibodies; ANA, anti-nuclear antibody; NuMA, nuclear mitotic apparatus.

of low (1-4) or high (>4) ratio index. The results are presented in the Table 7. Statistically significant differences were observed for samples with high anti-DFS70 titer. Lower TOS ($P = .001$), LPH ($P = .002$), UA ($P = .003$) LPS in serum ($P = .001$), LDL ($P = .056$) tendency and higher SOD activity ($P = .045$) were reported in high ratio index of anti-DFS70 positive patients compared to the anti-DFS70 negative patients. Lower MDA ($P = .024$) in serum were reported in ratio index 1 to 4 of anti-DFS70 positive patients compared to the anti-DFS70 negative patients.

The Pearson correlation showed statistically significant but weak, negative correlations between anti-DFS70 prevalence and the levels of LDL ($P = .063$), LPH ($P = .003$), UA ($P = .004$), and TOS ($P = .006$). In addition, there was a weak positive correlation between anti-DFS70 prevalence and SOD activity ($P = .028$) (Table 8). Regression analysis showed that the parameters which influence the occurrence of anti-DFS70 antibodies included: concentration of lipid hydroperoxides (for LPH $\beta^* = -.16$), and concentration of uric acid (for UA $\beta^* = -.15$). The value of regression analysis was $R = .22$, $R^2 = .05$, $P < .001$.

We also made an extended analysis of the surprising results concerning the lack of association of cigarette smoking, with the presence of anti-DFS70 antibodies, as smoking is known

Table 4. Characteristics of the population depending on age in the occurrence of anti-DFS70 antibodies.

AGE	ALL (N = 357)			MALE (N = 106)			FEMALE (N = 251)			*P*-VALUE (MALE VS FEMALE)
	N	POSITIVE DFS70	POSITIVE DFS70 (%)	N	POSITIVE DFS70	POSITIVE DFS70 (%)	N	POSITIVE DFS70	POSITIVE DFS70 (%)	
<30	14	6	42.86	3	0	0.00	11	6	54.55	.989
⩽30-40	48	16	33.33	11	5	45.45	37	11	29.73	.339
⩽40-50	71	16	22.54	28	6	21.43	43	10	23.26	.090
⩽50-60	106	24	22.64	26	6	23.08	80	18	22.50	.713
⩽60-70	93	9	9.68	29	5	17.24	64	4	6.25	.073
⩾70	25	5	20.00	9	1	11.11	16	4	25.00	.346
P-value (vs age)[a]	**.008**			.363			**.003**			

Abbreviation: DFS70, dense fine speckled.
[a]Comparison male versus female dependently of age.

Table 5. The occurrence of anti-DFS70 antibodies depending on lifestyle and civilization diseases.

TESTED GROUP (N = 357)	ANTI-DFS70 NEGATIVE (N = 281) N (%)	ANTI-DFS70 POSITIVE (N = 76) N (%)	*P*-VALUE
Sex (% of men)	83 (29.5)	23 (30.3)	.903
Physical activity	116 (41.3)	35 (46.1)	.456
Dietary habits	172 (61.2)	52 (68.4)	.250
Tobacco smoking (current or in the past)	130 (46.3)	22 (28.9)	**.006**
Current tobacco smoking	45 (16.0)	2 (2.6)	**.002**
Alcohol consumption	194 (69.0)	45 (59.2)	.116
Chronic kidney disease	9 (3.2)	0 (0.0)	.115
Coronary artery disease	31 (11.0)	7 (9.2)	.649
Myocardial infarction	16 (5.7)	1 (1.3)	.112
Ischemic stroke	7 (2.5)	0 (0.0)	.166
Hemorrhagic stroke	1 (0.4)	0 (0.0)	.604
Atrial fibrillation	12 (4.3)	2 (2.6)	.515
Dyslipidemia	144 (51.2)	34 (44.7)	.315
Family hypercholesterolemia	10 (3.6)	1 (1.3)	.317
Diabetes mellitus	56 (19.9)	14 (18.4)	.770
Arterial hypertension	141 (50.2)	27 (35.5)	**.023**
Healthy individuals	95 (33.8)	36 (47.4)	**.030**

to increase the intensity of oxidative stress. The results are presented in Table 9. Anti-DFS70 positive individuals show a significant decrease in oxidative stress intensity, regardless of whether they are cigarette smokers or not. In anti-DFS70 positive non-smokers we observed a statistically significant 18% decrease in TOS ($P = .051$). Anti-DFS70 positive smokers had 28% lower LPH levels ($P = .032$), 17% lower UA levels ($P = .017$), and 12% higher SOD activity ($P = .002$). The gender

Table 6. The oxidative stress markers depending on occurrence of anti-DFS70 antibodies.

TESTED GROUP (N = 357)	ANTI-DFS70 NEGATIVE (N = 281)		ANTI-DFS70 POSITIVE (N = 76)		% CHANGE	*P*-VALUE
	MEAN	SD	MEAN	SD		
TC (mg/dl)	212.4	49.4	205.6	47.1	–3	.279
HDL-C (mg/dl)	54.2	15.4	55.7	18.6	3	.467
TG (mg/dl)	159.1	127.5	153.7	136.8	–3	.751
LDL-C (mg/dl)	137.6	44.4	130.2	42.4	–5	.192
Non-HDL (mg/dl)	158.2	48.4	149.9	46.7	–5	.179
TAS (mmol/l)	1.05	0.26	1.05	0.27	0	.939
TOS (μmol/l)	7.41	6.44	5.93	3.96	–20	**.014**
LPH (μmol/l)	4.15	3.82	3.19	2.46	–23	**.038**
UA (μmol/l)	376	108	336	94	–11	**.005**
MDA (μmol/l)	3.11	1.33	2.89	1.09	–7	.201
MDA nmol/gHb	297	108.2	290	95.68	–2	.655
LPS RF (serum)	362	174	338	140	–7	.266
LPS RF/gHb	609	236	583	245	–4	.404
SOD NU/ml (serum)	20.01	2.60	19.55	3.03	–2	.189
SOD NU/mgHb	183	28.63	191	37.26	5	**.037**

Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LPH, lipid hydroperoxides; LPS, lipofuscin; MDA, malondialdehyde; Non-HDL, non-high density lipoprotein cholesterol; SOD NU, superoxide dismutase nitrite units; TAS, total antioxidant status; TC, total cholesterol; TG, triglycerides; TOS, total oxidant status; UA, uric acid.

of smokers and non-smokers was not associated with the presence of anti-DFS70 antibodies.

Discussion

In this study, our principal finding was that reduced levels of oxidative stress were observed in anti-DFS70 positive samples, especially in samples with a high antibody titer. These findings suggest that anti-DFS70 antibodies can be considered as an indirect marker of antioxidant response. Secondly, the prevalence of anti-DFS70 in the Polish population is low and can be estimated at 4.39%.

The AC-2 pattern, defined by a dense and heterogeneous speckled staining in the nucleoplasm of interphase HEp-2 cells (sparing the nucleoli) and the metaphase chromosomal plate,[36] was the most commonly recognized pattern type in our study. The AC-2 pattern is related to anti-DFS70 antibodies, so we expected to confirm the presence of these antibodies, especially in these samples, but the percentage of confirmations was surprisingly low, at only 51.16% (66/129). Other investigators have also reported wide variations in concordance between suspected IFA and confirmation by DFS70 specific solid-phase assays.[17,40-45] For example, Carter et al[17] and Carbone et al[44] reported slightly lower percentages of confirmation in their studies—41% and 45.8%, respectively. There are several hypotheses that may explain these discrepancies. First, the experience of laboratory staff is of great importance because IFA is a highly subjective method. This is best seen in samples with a low autoantibody titer. More than half of the samples scored as AC-2 positive were very low titer samples, 43.41% (56/129) with a titer of 1:100 and 28.68% (37/129) with a titer of 1:160 (Figure 2). Furthermore, for such samples, the level of anti-DFS70 antibodies in the monospecific test may not be higher than the cut-off value (in our case Ratio index ⩾1). Unfortunately, there is no international standard for anti-DFS70 antibodies, so each test manufacturer must set an appropriate cut-off value for its own monospecific test. This may result in discrepancies in the interpretation of results of tests offered by different manufacturers. Additionally, we cannot exclude the possibility of some misidentification of the AC-2 pattern. It is possible that the antibodies that give an AC-2 pattern staining are in fact a rather heterogeneous group. It has been proposed recently to define a new separate pattern of staining called "non-DFS" or "pseudo-DFS,"[46] comprising nuclear speckled patterns with clear staining of the metaphase plate, but without the typical features of the AC-2 pattern.[47] The new pseudo-DFS pattern may be caused by autoantibodies other than anti-DFS70 or recognizing distinct DFS70/LEDGF/p75 protein epitopes[17] which may also explain the negative results of anti-DFS monospecific tests.[46,47] A further problem is that patterns similar to AC-2 or overlapping

Table 7. The oxidative stress markers depending on anti-DFS70 antibodies concentration.

TESTED GROUP (N = 357)	ANTI-DFS70 NEGATIVE		ANTI-DFS70 POSITIVE					
	RATIO INDEX 0-1 (N = 281)		RATIO INDEX 1-4 (N = 35)			RATIO INDEX >4 (N = 41)		
	MEAN	SD	MEAN	SD	*P*-VALUE	MEAN	SD	*P*-VALUE
TC (mg/dl)	212.4	49.4	208.4	42.33	.647	203.1	51.14	.264
HDL (mg/dl)	54.2	15.4	54.85	17.58	.816	56.44	19.55	.399
TG (mg/dl)	159.1	127.5	142.0	91.68	.443	163.8	166.6	.832
LDL (mg/dl)	137.6	44.4	138.0	41.13	.966	123.6	42.82	***.056***
Non-HDL (mg/dl)	158.2	48.4	153.6	39.83	.585	146.7	52.07	.159
TAS (mmol/l)	1.05	0.26	1.05	0.31	.888	1.04	0.23	.806
TOS (µmol/l)	7.41	6.44	6.73	4.61	.544	5.25	3.20	**.001**
LPH (µmol/l)	4.15	3.82	3.57	2.81	.387	2.86	2.10	**.002**
UA (µmol/l)	376	108	354.1	91.53	.261	321.9	94.65	**.003**
MDA (µmol/l)	3.11	1.33	2.58	1.02	**.024**	3.16	1.08	.797
MDA nmol/gHb	297	108.2	292.4	99.68	.829	288.8	93.34	.662
LPS RF (serum)	362	174	380.3	172.9	.550	301.0	91.93	**.001**
LPS RF/gHb	609	236	582.6	209.7	.535	583.1	274.5	.528
SOD NU/ml (serum)	20.01	2.60	19.96	2.97	.917	19.21	3.07	.287
SOD NU/mgHb	183	28.63	188.9	31.58	.251	193.2	41.80	**.045**

Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LPH, lipid hydroperoxides; LPS, lipofuscin; MDA, malondialdehyde; Non-HDL, non-high density lipoprotein cholesterol; SOD NU, superoxide dismutase nitrite units; TAS, total antioxidant status; TC, total cholesterol; TG, triglycerides; TOS, total oxidant status; UA, uric acid.

Table 8. Pearson's correlation between anti-DFS70 and selected parameters.

PARAMETERS	*R*	*P*-VALUE
LDL-C (mg/dl)	–.10	.063
TOS (µmol/l)	–.15	.006
LPH (µmol/l)	–.16	.003
UA (µmol/l)	–.16	.004
SOD NU/mgHb	.12	.028

Abbreviations: LDL-C, low-density lipoprotein cholesterol; LPH, lipid hydroperoxides; SOD NU, superoxide dismutase nitrite units; TOS, Total oxidant status; UA, uric acid.

staining that can obscure the presence of the AC-2. In our study, antibodies-DFS70 were detected in 2 samples evaluated as AC-1 (overlap) and in 2 samples evaluated as AC4/AC5 (can be similar), that fulfill these conditions. Moreover, in samples with the AC4/AC5 pattern, the likely occurrence of anti-DFS70 antibodies was statistically significantly higher ($P = .019$). We also observed 6 DFS70 positive samples in the ANA negative control group. Four of these were samples that were close to the cut-off, which confirms the possibility of discrepancies between IFA and monospecific assays for samples with low titers.

Severe systemic oxidative stress is considered to be one of the factors that may contribute to the formation of autoantibodies, including anti-DFS70.[23,48,49] Theoretically, the chain of events could be as follows: If conditions of oxidative stress prevail, antioxidative mechanisms are activated, including increased production of DFS70/LEDGF/p75, as demonstrated by Singh et al.[8] It is possible that under these conditions, the DFS70/LEDGF/p75 may undergo some posttranslational modifications induced by ROS, resulting in the formation of neoepitopes that may initiate an immune response and antibody production which can then cross-react and recognize native DFS70/LEDGF/p75 secondary to molecular mimicry. In our study, statistically significant changes in some oxidative stress markers were observed in patients with anti-DFS70 antibodies, but the observations were different from those previously assumed. In fact, lower concentrations of some markers were observed, accompanied by slightly higher SOD activity (Table 6).

These results indicate a generally decreased level of oxidative stress in subjects with anti-DFS70 antibodies. Moreover, the results presented in Table 7 show that this relationship is

Table 9. The oxidative stress markers and sex depending on occurrence of anti-DFS70 antibodies in relation to smoking (smokers—tobacco smoking current or in the past and non-smokers).

		ANTI-DFS70 NEGATIVE			ANTI-DFS70 POSITIVE			% CHANGE	*P*-VALUE
		N	MEAN	SD	N	MEAN	SD		
TOS (μmol/l)	All	281	7.41	6.44	76	5.93	3.96	–20%	**.014**
	Non-smokers	151	7.29	6.90	54	5.94	3.82	–18%	***.051***
	Smokers	130	7.55	5.88	22	5.90	4.37	–22%	.131
LPH (μmol/l)	All	281	4.15	3.82	76	3.19	2.46	–23%	**.038**
	Non-smokers	151	4.07	4.07	54	3.25	2.62	–20%	.094
	Smokers	130	4.25	3.51	22	3.05	2.09	–28%	**.032**
UA (μmol/l)	All	281	376	108	76	336	94	–11%	**.005**
	Non-smokers	151	372	102	54	345	88	–7%	.069
	Smokers	130	381	115	22	315	107	–17%	**.017**
SOD NU/mgHb	All	281	183	28.63	76	191	37.26	5%	**.037**
	Non-smokers	151	184	29.72	54	186	33.56	1%	.668
	Smokers	130	182	27.39	22	204	43.33	12%	**.002**
		N (%)			N (%)			*P*-VALUE	
Sex (% of men)	All	83 (29.5)			23 (30.3)			.903	
	Non-smokers	27 (17.9)			14 (25.9)			.206	
	Smokers	56 (43.1)			9 (40.9)			.850	

Abbreviations: LPH, lipid hydroperoxides; SOD NU, superoxide dismutase nitrite units; TOS, total oxidant status; UA, uric acid.

particularly relevant in patients with a high titer of anti-DFS70 antibodies and was not observed in patients with a lower titer of antibodies (except for a slightly lower MDA concentration). It is also appropriate to quote here the hypothesis presented by Infantino et al[5] which proposed that autoantibodies targeting the DFS70/LEDGF/p75 protein may be involved in the removal of protein cleavage fragments from debris generated during cell death and tissue damage. It is assumed that in such a case, the anti-DFS70 antibodies could be produced as a result of increased oxidative stress associated with the collapse of the anti-oxidative systems, leading to the death of cells either through apoptosis or necrosis.[50-52] Perhaps the overexpression of the PSIP1 gene, on the one hand, results in lowering the level of oxidative stress, increasing the likelihood that the cell will survive, and on the other hand, the excess protein needs to be removed—this can be supported by immune mechanisms associated with autoantibody production. As Singh et al[8] have shown in the abovementioned publication, under in vitro conditions of oxidative stress, the DFS70/LEDGF/p75 protein is released into the culture medium. If the same process also takes place in vivo, the DFS70/LEDGF/p75 protein could more easily become an autoantigen for the immune system that would not interfere with its primary protective functions. Posttranslational modifications, as a result of ROS cannot be excluded either.

The hypothesis put forward by Ortiz-Hernandez et al[27] is supported by our results. It presupposes that the autoantibody response to DFS70/LEDGF/p75 might be a "sensor" of increased oxidative stress.[27] Our results confirm that high titers of anti-DFS70 antibodies may be regarded as a "sensor" of the body's response to oxidative stress. Hence, the antibodies are associated with reduced levels of oxidative stress markers, and may indicate elevated oxidative stress in the recent past. Moreover, the surprising results presented in Table 5, demonstrating less frequent occurrence of anti-DFS70 antibodies in cigarette smokers, led to the conclusion that smoking in itself does not increase the risk of anti-DFS70 antibodies, but smokers in whom anti-DFS70 antibodies are present appear to cope much better with oxidative stress induced by smoking than anti-DFS70 negative individuals (Table 9). This observation supports the conclusion that the very presence of anti-DFS70 antibodies could be an indicator of efficient antioxidant systems within the body. These antibodies were consistently observed in healthy rather than diseased individuals.

The observation of low TOS level in patients with a high titer of anti-DFS70 may indicate that the presence of anti-DFS70 antibodies does not initiate the advanced immune response process usually associated with increased oxidative stress. Furthermore,

anti-DFS70 is unlikely to inhibit the function of DFS70/LEDGF/p75, otherwise, in both cases, we would expect to observe increased oxidative stress. However, according to Ochs et al[12] a neutral or even protective effect of these antibodies could be context-dependent, raising the possibility that they may also behave as pathogenic antibodies under certain conditions. This conclusion is supported by early studies, which showed that anti-DFS70 antibodies are cytotoxic to cultured lens and lens epithelial cells, possibly by blocking extracellularly released DFS70/LEDGF/p75 from reintroduction into the cells, thus preventing their protective action against stressors.[53,54] Alternatively, the expression of the PSIP1 gene or its receptor protein may decrease with age,[8] with the same adverse effects on oxidative stress levels and possibly with a decrease in anti-DFS70 antibodies. This would also explain why anti-DFS70 antibodies are rarely detected in elderly people who are often observed to have impaired antioxidant mechanisms.[8]

The possibility of a false correlation between the 2 phenomena cannot be excluded, owing to the fact that since DFS70 antibodies are detected more frequently in the population of young and healthy people (as our research has also shown), it should be a natural consequence that they are correlated with lower levels of oxidative stress markers.

Conclusions

In samples positive for the anti-DFS70 antibody, a decreased level of oxidative stress was observed, especially in the case of samples with a high antibody titer. Based on the observed associations, we still cannot be sure whether oxidative stress can directly contribute to the formation of anti-DFS70 autoantibodies, but the mere presence of anti-DFS70 antibodies is correlated with low levels of oxidative stress, so anti-DFS70 antibodies may be considered an indirect "sensor" or marker of the body's antioxidant response. Further temporal studies are needed to determine whether anti-DFS70 antibodies arise as a consequence of oxidative stress.

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Author Contributions

Paweł Krzemień: Conceptualization, Methodology, Investigation, Writing—Original Draft. Sławomir Kasperczyk: Conceptualization, Methodology, Formal analysis, Writing—Review and Editing. Maciej Banach: Conceptualization, Methodology, Project administration, Writing—Review & Editing. Aleksandra Kasperczyk, Michał Dobrakowski, Tomasz Tomasik, Adam Windak, Mirosław Mastej, Alberico Catapano, Kausik K Ray, Dimitri P Mikhailidis, Peter P Toth, George Howard, Greory YH Lip, Maciej Tomaszewski, Fadi J Charchar, Naveed Sattar, Bryan Williams, Thomas M MacDonald and Peter E Penson: Writing—Review and Editing. Jacek J Jóźwiak: Conceptualization, Methodology, Investigation, Supervision, Project administration, Writing—Review and Editing. All authors revised the article critically for important intellectual content. All authors gave final approval of the work have participated sufficiently in the work and take public responsibility for appropriate portions of the content.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Approval

Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and has been approved by the Bioethical Committee of the Chamber of Physicians (No.K.B.Cz.-0018/2015).

Informed Consent

Informed consent was obtained from all individuals included in this study.

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