Artículo de investigación

Circulating markers of vascular damage as predictors of cardiovascular events in atherosclerosis and metabolic disorders

Циркулирующие маркеры повреждения сосудов как предикторы сердечно-сосудистых событий при атеросклерозе и метаболических нарушениях

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Abstract

The article presents the results of cluster analysis of the contribution of immune inflammation and endothelial dysfunction (ED) markers to the frequency and severity of cardiovascular events (CVE) in cohorts of patients with asymptomatic atherosclerosis (AAS), coronary artery disease (CAD), type 2 diabetes mellitus (T2DM) and metabolic syndrome (MS) during a 3-year prospective observation.

Results Circulating markers of ED and immune inflammation, such as ET-1, IL-1β, TNF-α, antibodies to collagen type I and III, and antibodies to chondroitine sulfate (CS) contribute to cardiovascular (CV) manifestation in AAS. In CAD patients ET-1, eNOs, antibodies to collagen, as well as IL-6 and vWf are the main contributors. In T2DM without clinical manifestation of CAD, the set of markers associated with the adverse events includes ET-1, eNOs, IL-6, anti-C, and anti-HA. In CAD combined with T2DM, the cluster of markers associated with the adverse events includes vWf, TNF-α, eNOs, IL-6, anti-C, anti-HA and CRP. In AAS without MS, the key contributors are ET-1

Анотація

В статье представлены результаты кластерного анализа вклада маркеров иммунного воспаления и эндотелиальной дисфункции (ЭД) в частоту и тяжесть сердечно-сосудистых событий в когортах пациентов с бессимптомным атеросклерозом (AAS), ишемической болезнью сердца, сахарным диабетом 2 типа (СД 2-го типа) и метаболическим синдромом (МС) в течение 3-летнего проспективного наблюдения. Циркулирующие маркеры ЭД и иммунного воспаления, такие как ET-1, IL-1β, TNF-α, антитела к коллагену I и III типов и антитела к хондроитинсульфату (CS) способствуют проявлению сердечно-сосудистых заболеваний (ССЗ) при ААС. У больных ИБС основными факторами являются ET-1, eNOs, антитела к коллагену, а также IL-6 и vWf. При СД 2-го типа без клинической манифестации ИБС набор маркеров, ассоциированных с побочными явлениями, включает ET-1, eNOs, IL-6, anti-C, и anti-HA. При ИБС в сочетании с СД2, кластер маркеров, ассоциированных с негативным событиям относятся vWf, TNF-α,

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and vWf, and the presence of anti-C and anti-ChS; in AAS/MS patients, the key markers are IL-1β, TNF-α, anti-C, anti-ChS, anti-HA, and CRP. In CAD without MS, the cluster of markers associated with adverse events includes ET-1, eNOs and anti-HA; in CAD/MS it includes anti-C, ET-1, and IL-6.

Conclusion. The obtained results confirm the role of systemic inflammation in the development of atherosclerosis-associated angiopathy in coronary pathology and disorders of carbohydrate metabolism, and also suggest a set of circulating markers as predictors of adverse CVE.

Keywords: Endothelial dysfunction, immune inflammation, cytokines, autoantibodies, atherosclerosis, ischemic heart disease, coronary artery disease, diabetes mellitus, metabolic syndrome.

eNOs, IL-6, anti-C, anti-HA и CRP. При ААС без МС ключевыми факторами являются ET-1 и vWf, а также наличие anti-C и anti-ChS; у пациентов с ААС/МС ключевыми маркерами являются IL-1β, TNF-α, anti-C, anti-ChS, anti-HA и CRP. При ИБС без МС кластер маркеров, ассоциированных с неблагоприятными явлениями, включает ET-1, eNOs и anti-HA; при ИБС/МС он включает anti-C, ET-1 и IL-6.

Полученные результаты подтверждают роль системного воспаления в развитии атеросклероз-ассоциированной ангиопатии при коронарной патологии и нарушениях углеводного обмена, а также предполагают набор циркулирующих маркеров в качестве предикторов неблагоприятных сердечно-сосудистых событий.

Ключевые слова: эндотелиальная дисфункция, иммунное воспаление, цитокины, аутоантитела, атеросклероз, ишемическая болезнь сердца, болезнь коронарной артерии, сахарный диабет, метаболический синдром.

Introduction

Atherosclerosis and CAD are some of the major global health problems today with highly unfavorable contribution to demographic indicators [Russian Statistical Yearbook, 2017]. The prevalence of atherosclerosis-associated diseases is increasing, although the death rate from CAD has significantly decreased in recent decades due to introduction of modern algorithms for early diagnosis and effective treatment [Russian Statistical Yearbook, 2017]. Another serious medical and social problem is diabetes mellitus (DM), which leads to vascular complications resulting in reduced quality of life and increased mortality. In particular, atherosclerotic angiopathies (first of all, CAD, myocardial infarction, chronic heart failure, and cerebral stroke) account for up to 80% of adverse outcomes in patients with T2DM [Algorithms…, 2017; de Rooij et al., 2009]. However, the early detection of AAS for assessment of cardiovascular risks (CVR) and effective prevention of cardiovascular events (CVE) presents serious difficulties [Boytsov et al., 2012; Kaptoge et al., 2014; Tarasov et al., 2017]. For example, the results of numerous studies indicate that the SCORE system, used to assess cardiovascular risks in a population, is not sufficiently informative; in particular, it does not reflect the presence of AAS which is typically associated with high or very high CVR [Kaptoge et al., 2014; Svistunov et al., 2018].

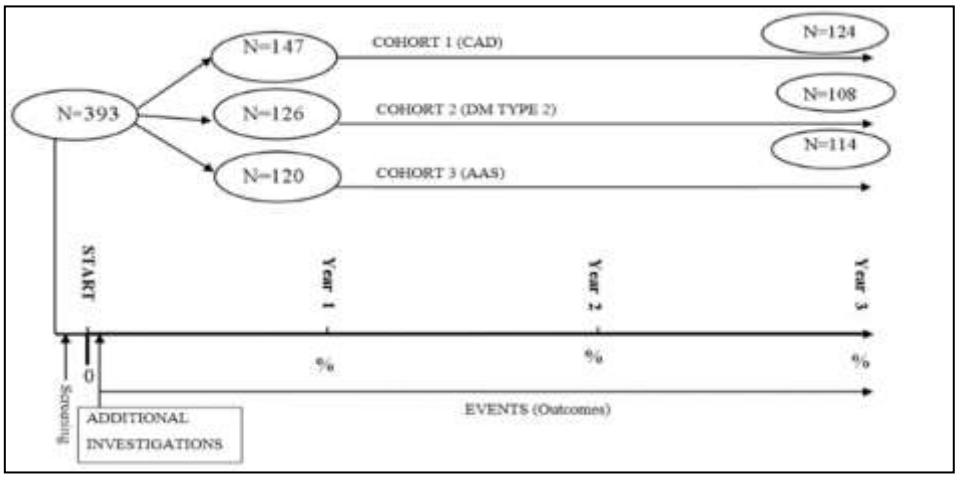
At present, the link between atherogenesis and endothelial dysfunction (ED) has been well established. In development of atherosclerosis, a low-intensity systemic inflammation plays an important role [Libby and Crea, 2010; Tarasov et al., 2016, 2017]. In this regard, such diagnostic and prognostic aspects of key mechanisms as endogenous risk factors for CAD and metabolic disorders are being actively studied. In our previous studies, it has been found that atherosclerosis-associated vascular lesions are characterized by overexpression of circulating markers of ED, pro-inflammatory cytokines and antibodies to the connective tissue components of the vascular wall [de Rooij et al., 2009; Svistunov et al., 2018]. The degree of increase in the level of the studied parameters depends on the severity of the clinical form of CAD, the presence of macrovascular complications in T2DM, as well as on the combination of these diseases. At the same time, AAS is also characterized by the presence of the above mentioned specific biomarkers, which suggests the possibility of using them for improved diagnostics of diseases associated with atherosclerosis and CV complications.

In light of these previous findings, the objective of this study is to comprehensively assess the role of cytokine imbalance and antibody production to the components of the connective tissue in the development of CVE in AAS, CAD and T2DM patients in the absence and in the presence of metabolic disorders.

Materials and methods

A total of 393 patients divided into three groups participated in the study (Fig. 1). The first group consisted of 147 persons with clinical and/or instrumental signs of CAD. The second group included 126 persons suffering from T2DM. The third group included 120 AAS patients without clinical and instrumental signs of CAD and T2DM; they were diagnosed with AAS based on the results of instrumental studies that were additionally carried out during the screening process. People with symptomatic atherosclerotic lesions of other vascular pools, except the coronary one, were not included in the group of patients with CAD and T2DM.

Figure 1. The study design.

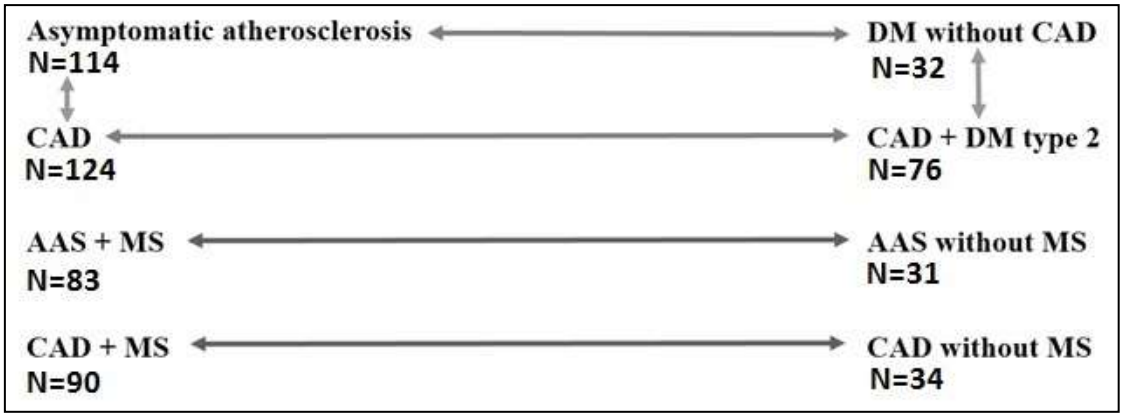


Throughout the entire observation period, patients with CAD and T2DM received a standard therapy according to the current national and international guidelines. Non-pharmacological and medical (statins) prophylaxis of CAD was performed for individuals with AAS. We initially determined the basal levels of soluble ED markers (von Willebrand factor (vWf), endothelin 1 (ET-1), endogenous NO synthase (eNOs)), auto-antibodies (antibodies to hyaluronic acid (a-HA), chondroitin sulfate (a-ChS), collagen (a-Coll)), and indicators of pro-inflammatory cytokine panel (tumor necrosis factor-α (TNF-α), interleukin 1 and 6 (IL -1, IL-6)), using an enzyme immunoassay. After 3 years, 124 people from the CAD group, 108 patients with T2DM and 114 people with AAS completed the study. To assess the pathogenetic differences regarding the effect of ED markers and immune inflammation molecules on the progression of CVD, we identified 8 cohorts for comparative analysis (Fig. 2).

To assess the role of the studied markers in the development and progression of atherosclerotic angiopathy, 2 cohorts were formed: cohort 1 included individuals with established AAS, and cohort 2 included patients with CAD. To assess the relationships between the studied markers and the atherosclerosis stage (preclinical or clinical), regardless of the presence of carbohydrate metabolism disorders, we examined cohort 3, which included patients with T2DM and AAS, and cohort 4 which consisted of patients with CAD and T2DM. To clarify the relationship of metabolic disorders and insulin resistance with the development of CVD and to analyze the role of the studied markers in this process, cohorts 5-8 were formed: cohort 5 included the patients from AAS group in combination with MS; cohort 6 – the group of patients with AAS but without MS; cohort 7 included patients with CAD in combination with MS; cohort 8 consisted of persons with CAD without MS. For a more objective assessment of pathogenic features of ED and immune inflammation in the presence of chronic hyperglycemia, it was important to conduct two

additional pairwise comparative analyses. Specifically, we compared cohort 3 (patients with T2DM without CAD) and cohort 1 (persons with AAS without T2DM); and, finally, we compared cohort 4 (patients with CAD in combination with T2DM) and cohort 2 (patients with CAD without T2DM).

Figure 2. Cohorts of patients formed to assess the role of the studied markers in the development and progression of CAD, T2DM and AAS with and without MS.



For cluster analysis, we used the following laboratory parameters as independent variables (predictors): pro-inflammatory cytokines, markers of ED, autoantibodies, high-sensitivity C-reactive protein (hs-CRP), and an indicator of endothelial function, determined by photoplethysmometric method (EFI-EF Index). We analyzed the clinical outcomes for patients over a three-year period of prospective observation as dependent variables, which were set as semi-continuous score values from 0 to 4depending on the nature of the complications from negligible to fatal: 0 - no adverse events for 3 years; 1 - manifestation of a chronic form of CAD; 2 -manifestation of acute coronary syndrome (ACS); 3 - death. With multiple endpoints in cluster analysis, we took into account the most adverse event. We used a K-means clustering algorithm with the number of iterations 50; the number of clusters (k) was determined empirically, based on the task to allocate a cluster of people with the highest frequency and severity of unfavorable cardiovascular events during the prospective observation period for the purpose of comparative assessment of the studied markers.

Results and discussion

As a result of the pairwise comparisons in the studied cohorts, we obtained from 2 to 4 clusters, each of which was characterized by a certain profile of the studied biomarkers. The centroids of the mean values of the studied parameters in clusters of patients from the comparison cohorts are presented in Table 1.

Among the patients with AAS we have distinguished 3 clusters. In terms of the frequency and severity of CVE, cluster 1 and cluster 2 did not have significant differences, while cluster 3 differed significantly from clusters 1 and 2 (more advanced disease). Cluster 3 (Table 1) was characterized by ED (high levels of ET-1, vWf and low levels of eNOS), significant deviations of the cytokine panel (elevated levels of IL-1β and TNF-α) and high levels of antibodies to C I and III types, a-ChS and a-HA. Cluster 3 was also characterized by higher values of hs-CRP and lower values of EFI, although the differences in these indicators between the clusters were insignificant. Among the patients with CAD in the absence of DM, we identified 4 clusters. The greatest frequency and severity of CVE was observed in cluster 1 (Table 1). It was characterized by pronounced ED (high ET-1 and vWf, and minimal concentration of NOs) in combination with moderate hypercytokinemia and increased levels of antibodies to connective tissue antigens.

Among the patients with T2DM without CAD 2 clusters were distinguished, whereas among T2DM/CAD persons, 3 clusters were identified. In T2DM without CAD, a high frequency of CV events was observed in cluster 1. In this cluster (Table 1) we did not find any signs of a true ED (high levels of ET-1 and vWf, and low levels of

eNOS and EFI), systemic inflammatory response with a significant increase in TNF-α and IL-6, as well as more active production of autoantibodies to C I, III types and a-HA. In the cohort of patients with CAD combined with T2DM, 3 clusters were identified. The highest frequency of adverse CVE was observed in cluster 3 (Table 1), which was characterized by higher levels of ET-1, vWf, TNF-α, as well as overexpression of autoantibodies to HA. In patients with AAS in the presence and absence of MS 3 clusters of patients were identified. Among the patients with AAS with MS, the adverse CVE more often developed in cluster 2, which was characterized by pronounced immune-mediated inflammatory changes with the maximal values of all pro-inflammatory cytokines (IL-1β, TNF-α and IL-6) and autoantibodies (anti-C, anti-CS and anti-HA), with moderate ED by type of activation.

Among the patients with AAS without MS, the adverse CVE more often developed in cluster 3, which was characterized by a higher level of antibodies to all components of the connective tissue, a significant increase in IL-6 and a moderately pronounced ED. Among the CAD/MS patients, 2 clusters were distinguished, while among the patients with CAD in the absence of MS, 3 clusters were identified. In the first case, the highest incidence and severity of adverse CVE occurred in patients in cluster 2 (Table 1), which was characterized by pronounced activation of endothelium with overexpression of ET-1 and vWf. In the same cluster, the marked immune-mediated inflammatory shifts were observed with the increase in all pro-inflammatory cytokines and production of autoantibodies to the components of the connective tissue, primarily to C types I and III.

Table 1. Centroids of average values of the studied parameters in cohorts of patients with AAS and CAD (1 pair), type 2 diabetes with CAD and without CAD (2 pairs), AAS with MS and without MS, and CAD with and without MS.

Centroids for k-means clustering (AAS (no DM))
Number of clusters: 3
Total number of training cases: 114

Cluster	ET-1	vWf	eNOs	IL-1	TNF	IL-6	a-Coll	a-ChS	a-HA	EFI	CRP	Events	Number of cases	Percentage(%)
1	1.63809 5	1.725714	280.3095	52.4523 8	14.6428 6	9.047 62	0.19476 2	1.373810	1.71738 1	18.261 90	3.333 333	0.21428 6	42	36.84211
2	0.73390 2	0.871707	355.0000	76.1707 3	21.7561 0	14.7073 2	0.21829 3	1.568293	1.73317 1	18.024 39	3.280 488	0.34146 3	41	35.96491
3	1.25806 5	1.649032	385.5806	92.9677 4	23.6774 2	11.8387 1	0.28838 7	2.219355	2.64935 5	17.709 68	3.435 484	1.83871 0	31	27.19298

Centroids for k-means clustering (CAD (no DM))
Number of clusters: 4
Total number of training cases: 124

Cluster	ET-1	vWf	eNOs	IL-1	TNF	IL-6	a-Coll	a-ChS	a-HA	EFI	CRP	Events	Number of cases	Percentage(%)
1	4.75500 0	1.800000 0	151.4500	105.050 0	22.9500 0	21.8500 0	0.24150 0	2.270000	3.03500 0	0.500 0	4.155 000	1.50000 0	20	16.12903
2	5.95625 0	1.681	230.3750	102.5	19.75	36.68	0.40625 0	2.562500	3.50000 0	-11.	5.168	0.81250 0	16	12.90323

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		250		625	000	750				8125	750			
3	5.623529	2.476471	259.5294	94.64 71	23.41176	27.000000	0.314118	3.005882	2.888235	-0.47066	4.247059	0.000000	17	13.70968
4	3.521127	1.598592	310.2676	85.4930	18.38028	23.492966	0.203803	1.998592	2.239437	9.5775	4.018310	0.239437	71	57.25806

Centroids for k-means clustering (DM+AAS)
Number of clusters: 2
Total number of training cases: 32

Cluster	ET-1	v Wf	eNOs	IL-1	TNF	IL-6	a-Coll	a-ChS	a-HA	EFI	CRP	Events	Number of cases	Percentage(%)
1	1.622500	4.887500	175.3125	181.1875	38.312500	35.312500	0.333125	1.768750	3.350625	-6.250000	4.556250	1.937500	16	50.00000
2	1.055625	4.725000	260.8750	174.4375	35.812500	25.187500	0.230000	1.856250	2.504375	2.68750	5.268750	0.250000	16	50.00000

Centroids for k-means clustering (DM+CAD)
Number of clusters: 3
Total number of training cases: 76

Cluster	ET-1	v Wf	eNOs	IL-1	TNF	IL-6	a-Coll	a-Chs	a-HA	EFI	CRP	Events	Number of cases	Percentage(%)
1	2.100000	5.333333	280.9608	179.8039	37.313733	28.058882	0.270980	2.186275	2.909020	-2.6863	4.603922	0.000000	51	67.10526
2	2.060000	6.050000	72.5000	209.5000	30.000000	39.500000	0.750000	2.750000	3.750000	-15.00000	7.100000	0.500000	2	2.63158
3	2.116957	6.113043	211.4348	180.6957	35.652173	34.130433	0.343913	2.286957	3.533478	-8.4783	5.330435	1.347826	23	30.26316

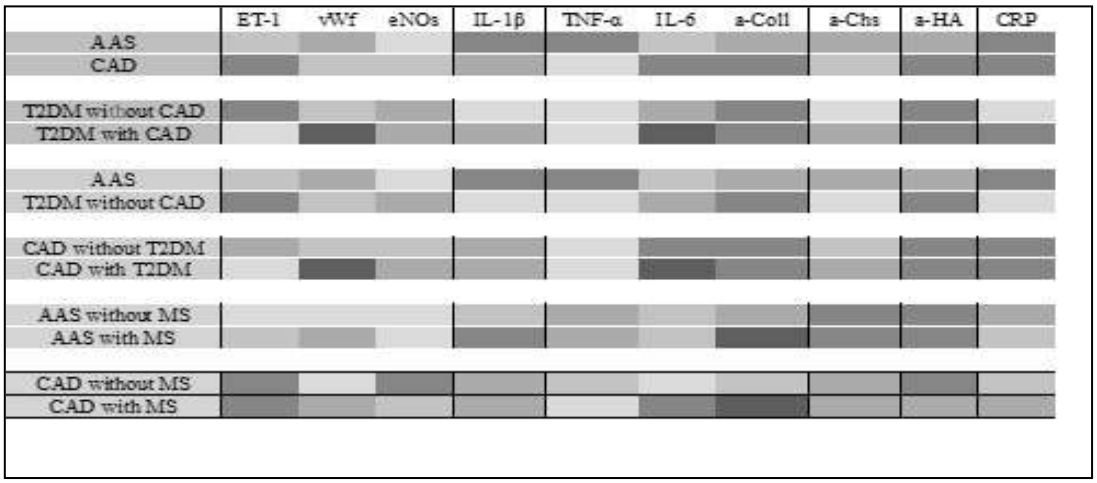
Centroids for k-means clustering (AAS+MS)
Number of clusters: 3
Total number of training cases: 83

Cluster	ET-1	v Wf	eNOs	IL-1	TNF	IL-6	a-Coll	a-ChS	a-HA	EFI	CRP	Events	Number of cases	Percentage(%)
1	0.852222	1.102222	331.5000	59.444444	18.305556	12.166667	0.239722	1.636111	1.886667	19.500000	3.194444	0.166667	36	43.37349
2	0.980000	1.473125	326.2500	96.500000	32.812500	13.562500	0.348750	2.562500	2.731250	17.06250	3.468750	2.000000	16	19.27711
3	1.911290	1.921	365.0968	79.67	14.29	11.51	0.193548	1.470968	1.946774	16.74	3.467	0.741935	31	37.34940

		935		742	032	513				194	742			
Centroids for k-means clustering (AAS+MS)														
Number of clusters: 3														
Total number of training cases: 83														
Cluster	ET-1	vWf	eNOs	IL-1	TNF	IL-6	a-Coll	a-ChS	a-HA	EFI	CRP	Events	Number of cases	Percentage(%)
1	0.852222	1.102222	331.5000	59.444444	18.305556	12.166667	0.239722	1.636111	1.886667	19.500000	3.194444	0.166667	36	43.37349
2	0.980000	1.473125	326.2500	96.500000	32.812500	13.562500	0.348750	2.562500	2.731250	17.062500	3.468750	2.000000	16	19.27711
3	1.911290	1.921935	365.0968	79.677742	14.290322	11.516133	0.193548	1.470968	1.946774	16.741944	3.467742	0.741935	31	37.34940
Centroids for k-means clustering (CAD+MS)														
Number of clusters: 2														
Total number of training cases: 90														
Cluster	ET-1	vWf	eNOs	IL-1	TNF	IL-6	a-Coll	a-ChS	a-HA	EFI	CRP	Events	Number of cases	Percentage(%)
1	3.633333	1.763636	299.1970	89.6061	21.166667	22.621211	0.225909	2.201515	2.690909	9.043939	4.043939	0.393939	66	73.33333
2	6.270833	1.829167	214.5000	102.958333	21.708333	33.958333	0.382083	2.662500	3.225000	-8.791667	4.775000	0.750000	24	26.66667
Centroids for k-means clustering (CAD (no MS))														
Number of clusters: 3														
Total number of training cases: 34														
Cluster	ET-1	vWf	eNOs	IL-1	TNF	IL-6	a-Coll	a-ChS	a-HA	EFI	CRP	Events	Number of cases	Percentage(%)
1	3.745833	1.741667	289.6250	82.2083	15.750000	25.583333	0.213750	2.004167	1.704167	5.458333	4.083333	0.125000	24	70.58824
2	5.440000	1.560000	147.6000	108.200000	11.200000	24.600000	0.176000	2.340000	3.620000	-9.800000	4.080000	1.400000	5	14.70588
3	5.720000	1.720000	114.4000	104.400000	25.200000	21.200000	0.210000	2.080000	2.160000	5.000000	4.680000	1.200000	5	14.70588

Among the patients with CAD in the absence of MS, a high incidence of CVE was observed in clusters 2 and 3. In these clusters (Table 1), the signs of pronounced ED and hyperproduction of pro-inflammatory cytokines and the studied autoantibodies were found. Cluster 2 with the most unfavorable disease progression was characterized by the highest rates of a-Chs and a-HA and the maximal level of IL-1β.

Figure 3. Centroids of average values of the investigated parameters in cohorts of patients with AAS and CAD (1 pair), type 2 diabetes with CAD and without CAD (2 pairs), AAS with MS and without MS, and CAD with and without MS.



As a result of the cluster analysis, we were able to assess the contribution of each of the studied parameters to the development of adverse CVE and to identify the profile of ED and immune inflammation circulating markers reflecting the frequency and severity of CV complications depending on the stage of atherosclerosis (AAS or clinically manifested coronary atherosclerosis), and also the presence of disorders of carbohydrate metabolism (metabolic syndrome or T2DM). Schematically, the data are summarized in Fig. 3.

As follows from these data, the greatest contribution to the development of CVE in patients with AAS without T2DM is made by circulating markers of ED and immune inflammation, such as ET-1, IL-1β, TNF-α, autoantibodies to C I and III, and CS. In patients with CAD without T2DM, ET-1, eNOs, antibodies to collagen, as well as IL-6 and vWf are the key contributors. In patients with T2DM without CAD, the set of markers associated with the development of adverse events includes ET-1, eNOs, IL-6, anti-C, and anti-HA, whereas in T2DM/CAD patients, this profile includes the reduced level of eNOs, the increased levels of vWf, TNF-α, IL-6, hs-CRP, and overexpression of antibodies to C and HA. In AAS/MS patients, along with hs-CRP, the key contributors are the increased levels of IL-1β, TNF-α, and antibodies to C, CS, HA. The development of CVE in the group with AAS but without MS is associated with the increased level of circulating markers of ED (ET-1, vWf), as well as antibodies to C and CS.

Obtained data are in consent with the results of numerous investigations which had demonstrated predictive value of the pro-inflammatory markers basal levels in CVE [Mueller, 2014; Ridker, 2014]. It was shown that increased levels of 'upstream' markers such as IL-6, IL-18 and TNF-α were all associated with future vascular events in an approximately log-linear manner, as were level of matrix metalloproteinase-9. However the magnitudes of these effects were smaller than that associated with the 'downstream' inflammatory biomarker, CRP. Since inflammation plays a key role in atherosclerotic plaque formation and further plaque destabilization biomarkers of plaque instability are logical candidates for early diagnosis of atherosclerotic CVD. Recent studies have shown that available assays for detection of these markers including myeloperoxidase, myeloid-related protein 8/14, pregnancy-associated plasma protein-A, and CRP have very

low diagnostic accuracy and therefore are not helpful in the early diagnosis of CVE. In this context searching for additional markers reflecting plague instability such as anti-connective tissue antibodies and/or ED agents seems to be appropriate for diagnostic and prognostic purposes.

Conclusion

The obtained results confirm the modern inflammatory concept of atherosclerosis and suggest that the mechanisms of immune inflammation play an important pathogenic role in the development of atherosclerosis and CAD, as well as in the development of vascular complications in clinically significant metabolic disorders such as MS and T2DM. It has been established that the contribution of immune inflammation and ED to the development of CV complications depends on the stage of the atherosclerotic process and the severity of carbohydrate metabolism disorders. The most typical profiles of the studied biomarkers, reflecting a high risk of adverse CVE in AAS, CAD, T2DM, and MS, as well as in a combination of atherosclerosis and carbohydrate metabolism disorders, were identified. The obtained data are of practical interest in connection with the possibility of their use for predicting CV complications in high-risk patients.

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