





# Cytokines in Abdominal Aortic Aneurysm: Master Regulators With Clinical Application

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Olesya A Puchenkova<sup>1</sup>, Vladislav O Soldatov<sup>1</sup>,  
Andrei E Belykh<sup>2,3</sup> , OlgaYu Bushueva<sup>4</sup>,  
Gennadii A Piavchenko<sup>5,6</sup> , Artem A Venediktov<sup>5</sup> ,  
Nikolay K Shakhpazyan<sup>7</sup> , Alexey V Deykin<sup>1</sup>, Mikhail V Korokin<sup>1</sup>  
and Mikhail V Pokrovskiy<sup>1</sup>

<sup>1</sup>Department of Pharmacology and Clinical Pharmacology, Belgorod State National Research University, Belgorod, Russia. <sup>2</sup>Department of Pathophysiology, Research Institute of General Pathology, Kursk State Medical University, Kursk, Russia. <sup>3</sup>Dioscuri Centre for Metabolic Diseases, Nencki Institute of Experimental Biology PAS, Warsaw, Poland. <sup>4</sup>Department of Biology, Medical Genetics and Ecology, Laboratory of Genomic Research at the Research Institute for Genetic and Molecular Epidemiology, Kursk State Medical University, Kursk, Russia. <sup>5</sup>Department of Histology, Cytology and Embryology, I.M. Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia. <sup>6</sup>Laboratory of Cell Pathology in Critical State, State Research Institute of General Reanimatology, Moscow, Russia. <sup>7</sup>Laboratory of Clinical Pathology, Institute of Human Morphology, Moscow, Russia.

**ABSTRACT:** Abdominal aortic aneurysm (AAA) is a potentially life-threatening disorder with a mostly asymptomatic course where the abdominal aorta is weakened and bulged. Cytokines play especially important roles (both positive and negative) among the molecular actors of AAA development. All the inflammatory cascades, extracellular matrix degradation and vascular smooth muscle cell apoptosis are driven by cytokines. Previous studies emphasize an altered expression and a changed epigenetic regulation of key cytokines in AAA tissue samples. Such cytokines as IL-6, IL-10, IL-12, IL-17, IL-33, IL-1 $\beta$ , TGF- $\beta$ , TNF- $\alpha$ , IFN- $\gamma$ , and CXCL10 seem to be crucial in AAA pathogenesis. Some data obtained in animal studies show a protective function of IL-10, IL-33, and canonical TGF- $\beta$  signaling, as well as a dual role of IL-4, IFN- $\gamma$  and CXCL10, while TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-12/IL-23, IL-17, CCR2, CXCR2, CXCR4 and the TGF- $\beta$  noncanonical pathway are believed to aggravate the disease. Altogether data highlight significance of cytokines as informative markers and predictors of AAA. Pathologic serum/plasma concentrations of IL-1 $\beta$ , IL-2, IL-6, TNF- $\alpha$ , IL-10, IL-8, IL-17, IFN- $\gamma$ , and PDGF have been already found in AAA patients. Some of the changes correlate with the size of aneurysms. Moreover, the risk of AAA is associated with polymorphic variants of genes encoding cytokines and their receptors: *CCR2* (rs1799864), *CCR5* (Delta-32), *IL6* (rs1800796 and rs1800795), *IL6R* (rs12133641), *IL10* (rs1800896), *TGFB1* (rs1800469), *TGFB1* (rs1626340), *TGFB2* (rs1036095, rs4522809, rs1078985), and *TNFA* (rs1800629). Finally, 5 single-nucleotide polymorphisms in gene coding latent TGF- $\beta$ -binding protein (*LTBP4*) and an allelic variant of *TGFB3* are related to a significantly slower AAA annual growth rate.

**KEYWORDS:** Aneurysm, AAA, cytokines, inflammation, TGF- $\beta$ , IL-6

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**CORRESPONDING AUTHOR:** Vladislav O Soldatov, Belgorod State National Research University, 85 Pobedy Str., Belgorod 308015, Russian Federation. Email: pharmsoldatov@gmail.com

## Introduction

Aortic aneurysm (AA) is a life-threatening disorder caused by an irreversible local enlargement of the aorta with high risk of rupture and consequent fatal bleeding. Unfortunately, the AA is usually an unpredictable finding due to its latent clinical course. In most cases, it is detected during a non-routine medical exam.<sup>1</sup> The AA are most commonly seen in the thoracic (thoracic aorta aneurysm, TAA) and abdominal (abdominal aorta aneurysm, AAA) aorta.<sup>2</sup> Despite their apparent similarity, AAA and TAA have different etiologies and underlying mechanisms. The more prevalent, AAA, is strongly associated with male sex, elderly age, dyslipidemia, increased blood pressure, and presence of atherosclerosis. In contrast, no evident correlations are found between the TAA progression and age,

sex, or other cardiovascular diseases but it involves a major genetic component and association with connective tissue disorders.<sup>3</sup>

The hallmarks of AAA are an aortic wall inflammation accompanied by a leukocytic infiltration and a degradation of the extracellular matrix (ECM),<sup>4</sup> meanwhile TAA is caused by a connective tissue component degradation. Also, the TAA features are a loss of vascular smooth muscle cells (VSMCs), a fragmentation and decrease in the number of elastic fibers, and an increased accumulation of proteoglycans.<sup>5-7</sup>

Thoracic and abdominal segments of the aorta have different embryological origins, and it is considered to be the most important cause for the pathogenetic differences between TAA and AAA.<sup>8</sup> The thoracic segment is composed of



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smooth muscle cells (SMCs) migrating from the neural crest, whereas the abdominal segment contains only primitive SMCs contributing to its formation.<sup>9</sup> This fact determines different responses of the segments to such destructive factors as high concentrations of homocysteine, angiotensin II (AngII), or transforming growth factor  $\beta$  (TGF- $\beta$ ) (more details in Ruddy et al).<sup>10</sup>

In this review, we focus on the importance of various cytokines in AAA pathogenesis describing their predictive value and probable therapeutic application.

### Cytokines are Key Regulators of AAA Development

Cytokines are crucial contributors of inflammatory alterations throughout the AAA course. Early stages of AAA are driven by an infiltration of the vascular wall by immune cells producing inflammatory factors. In subsequent stages, the increased production of cytokines leads to the activation of proteases and apoptosis of VSMCs resulting in ECM degradation and aortic wall thinning.<sup>11,12</sup> Finally, the rupture, which is a severe outcome of AA, also depends on cytokines upregulation.<sup>13</sup>

Dramatic shift of cytokine expression was revealed in the murine model of Angiotensin-II-(AngII)-induced AAA. An assay of cytokines in AAA tissue samples from AngII-treated *ApoE*<sup>-/-</sup> mice has been conducted with an antibody array of 200 cytokines, cytokine receptors, and related proteins; it has revealed that 21 of these 200 proteins are differentially expressed. These differentially expressed proteins are mostly associated with such functions as leukocytic migration and positive regulation of cell adhesion.<sup>14</sup>

Presumably, AAA is characterized by overexpression and activation of proinflammatory transcription factors leading to upregulation of certain cytokines and enhancement of downstream cellular responses.<sup>15-17</sup> Schönbeck et al has demonstrated that extracts of human AAA tissue predominantly contain T helper 2 (Th2)-associated cytokines and less abundant Th1-associated cytokines, whereas Th1-associated cytokines are found at higher concentrations in stenotic atherosclerotic vascular lesions.<sup>15,18</sup>

A number of studies on human samples highlight differential expression of cytokines in aneurysm tissue of AAA patients (Table 1).

### IL-6

IL-6 is a pleiotropic proinflammatory cytokine responsible for initiation of acute phase response.<sup>29</sup> Once IL-6 is secreted to the bloodstream from an altered region, systemic inflammatory response arises.<sup>30</sup> Numerous preclinical and clinical studies have uncovered a pathological role of high and dysregulated IL-6 expression in the development of inflammatory, autoimmune, and oncological diseases in humans.<sup>31</sup>

To initiate a signaling cascade, IL-6 binds to a heterodimeric complex consisting of membrane IL-6 receptor

(mIL-6R) and a subunit of gp130.<sup>32</sup> Only a few cells express mIL-6R, while almost all cells have gp130 on their surface. Cells synthesizing gp130 without mIL-6R do not interact with IL-6 but are able to respond to an IL-6 complex containing the soluble form of IL-6R (sIL-6R), which is generated by proteolytic cleavage of mIL-6R. This process has been termed *trans*-signaling. In contrast, to proinflammatory functions of IL-6 *trans*-signaling, IL-6 classic signaling mediates regenerative and anti-inflammatory activities.<sup>32,33</sup>

IL-6 takes part in the pathogenesis of various cardiovascular diseases. In particular, in patients with AAA, there is an increased level of circulating IL-6, which may correlate with the aorta diameter.<sup>34-36</sup> Moreover, Yuwen et al revealed that IL-6 is expressed within an AAA in patients but is undetectable in the corresponding tissue of a control group.<sup>20</sup> The concentration of IL-6 in an AA-affected aortic wall is severalfold higher than the concentration of IL-6 in progressive atherosclerotic stenotic lesions of the aorta, suggesting that IL-6 is a proinflammatory factor associated with dilatation rather than stenosis.<sup>37</sup> According to Nishihara et al, inhibition of sIL-6R by a monoclonal antibody reduces the aorta diameter in the murine model of CaCl<sub>2</sub>-induced AAA.<sup>38</sup> Paige et al showed that the prevention of AAA progression and of aorta rupture is achieved only via selective blockade of the *trans*-pathway of IL-6 signaling, whereas inhibition of both pathways (*trans*- and classic) does not correlate with improved survival.<sup>39</sup>

Jones et al reported that the 174C→G polymorphism in the *IL6* gene is associated with the worst survival among AAA patients and a higher level of IL-6 in blood plasma.<sup>40</sup> Another interesting finding of this study is that the aneurysm wall appears to be an important source of circulating IL-6. Smallwood et al presented an association between the IL-6-572G>C polymorphism in the promoter region of the *IL6R* gene encoding IL-6 receptor and the risk of AAA.<sup>41</sup> Moreover, while studying VSMCs isolated from patients with AAA and controls, Boddy et al found altered DNA methylation in *IL6R*.<sup>42</sup>

IgG4-associated diseases are recently described systemic disorders affecting various tissues, including the aorta (IgG4-AA: IgG4-associated aortic aneurysm). IgG4-AA is a consequence of tissue infiltration by plasma cells that produce IgG4.<sup>43</sup> Patients with this pathology have strikingly elevated levels of IL-6 in the tissue of the affected blood vessel and in peripheral blood.<sup>44</sup> In IgG4-AA, serum concentration of IL-6 correlates with higher IgG4 levels, which may perform an important function in the pathogenesis and progression of this disease. Numerous cell populations, including the endothelium, mesenchymal cells, and macrophages in the adventitia, promote IL-6 overexpression in IgG4-AA. IL-6 may be a useful biomarker of disease activity, and inhibition of IL-6 may serve as a new effective avenue for treatment of IgG4-AA.<sup>45</sup>

**Table 1.** Differential expression of cytokines and cytokine receptors in aortic samples collected from patients with AAA.

LEVEL	STUDY DESCRIPTION	UPREGULATED CYTOKINES	DOWNREGULATED CYTOKINES	REFERENCE
Protein	A 42-cytokine antibody-based protein array analysis of samples collected from 10 AAA patients and 9 non-AAA controls	IL-6, IL-1 $\alpha$ , IL-1 $\beta$ , TNF- $\alpha$ , TNF- $\beta$ , oncostatin M, IL-10, IL-8, ENA-78, GRO $\alpha$ , MCP-1, MCP-2, RANTES, G-CSF, M-CSF	-	Middleton et al <sup>19</sup>
Protein	A 274-cytokine antibody-based protein array analysis of homogenized human aortic tissues from 12 AAA patients and 6 non-AAA controls	MCP-1, RANTES, MDC, PARC, MIP-1b, PF4, GRO $\alpha$ , IL-8, IL-6, LIGHT, GDF15, EGF, TIMP-1, TNFSF5	MCP-2, PDGF-AA, BMP-4, bFGF, EGFR	Yuwen et al <sup>20</sup>
Protein	Fifty-two cytokines (from human AAA tissue samples from 4 patients) analyzed in a fluorescent-bead-based multiplex assay	IL-6, MIF, LIF, IFN- $\gamma$ , MCP-1, IL-8, MIG, IP-10, GRO $\alpha$ , HGF, VEGF	-	Ohno et al <sup>21</sup>
Protein	ELISAs of IL-8 and MCP-1 in cell cultures derived from AAA and non-AAA patients	MCP-1, IL-8	-	Koch et al <sup>22</sup>
Protein	ELISA of IL-1 $\beta$ in aortic biopsies from 15 AAA patients and 6 non-AAA patients	IL-1 $\beta$	-	Pearce et al <sup>23</sup>
Protein	ELISAs of TNF- $\alpha$ and IL-1 $\beta$ in aortic-tissue extracts from 7 AAA patients and 5 non-AAA patients	TNF- $\alpha$ , IL-1 $\beta$	-	Newman et al <sup>24</sup>
Protein	ELISAs of TNF- $\alpha$ , IL-6, and IL-8 in aortic tissue of 11 ruptured aneurysms and 32 asymptomatic aneurysms	TNF- $\alpha$ , IL-6, IL-8	-	Treska et al <sup>25</sup>
Protein	Measurement of the IL-10 level in the aortic tissue of 8 AAAs and 8 tissue samples affected by aortoiliac occlusive disease (AOD) by immunoenzyme assays	IL-10	-	Davis et al <sup>26</sup>
mRNA	Membrane-based complementary DNA expression array of total RNA (converted to cDNA) isolated from 4 AAAs and 4 normal abdominal aortic wall tissue samples	IL-8, CXCR4, PDGF-A, RANTES	-	Tung et al <sup>27</sup>
mRNA	Analysis of differential gene expression using a cDNA human cell interaction array of 265 genes	TNF- $\beta$ R	-	Armstrong et al <sup>28</sup>

Abbreviations: bFGF, basic fibroblast growth factor 2; BMP-4, bone morphogenetic protein 4; CXCR4 (fusin), C-X-C chemokine receptor type 4; EGF, epidermal growth factor; EGFR, EGF receptor; ENA-78 (CXCL5), epithelial neutrophil-activating peptide-78 (C-X-C motif chemokine 5); G-CSF (CSF3), granulocyte colony-stimulating factor (colony-stimulating factor 3); GDF15 (MIC-1), growth/differentiation factor 15 (macrophage inhibitory cytokine 1); GRO $\alpha$  (CXCL1), growth-regulated protein alpha (C-X-C motif chemokine 1); HGF, hepatocyte growth factor; IFN- $\gamma$ , interferon gamma; IL, interleukin; IP-10 (CXCL10), interferon  $\gamma$ -induced protein 10 (C-X-C motif chemokine 10); LIF, leukemia inhibitory factor; LIGHT (TNFSF14), tumor necrosis factor ligand superfamily member 14; M-CSF (CSF1), macrophage colony-stimulating factor (colony stimulating factor 1); MCP-1 (CCL2), monocyte chemoattractant protein 1 (C-C motif chemokine 2); MCP-2 (CCL8), monocyte chemoattractant protein 2 (C-C motif chemokine 8); MDC (CCL22), macrophage-derived chemokine (C-C motif chemokine 22); MIF, macrophage migration inhibitory factor; MIG (CXCL9), monokine induced by  $\gamma$ -interferon (C-X-C motif chemokine 9); MIP-1b (CCL4), macrophage inflammatory protein-1-beta (C-C motif chemokine 4); PARC (CCL18), pulmonary and activation-regulated chemokine (C-C motif chemokine 18); PDGF-A, platelet-derived growth factor A; PDGF-AA, platelet-derived growth factor alpha polypeptide; PF4 (CXCL4), platelet factor 4 (C-X-C motif chemokine 4); RANTES (CCL5), regulated upon activation, normal T-cell expressed and secreted (C-C motif chemokine 5); TIMP-1, tissue inhibitor of metalloproteinase 1 (metalloproteinase inhibitor 1); TNF, tumor necrosis factor; TNF- $\beta$ R, tumor necrosis factor- $\beta$  receptor; TNFSF5 (CD40-L), tumor necrosis factor ligand superfamily member 5 (CD40 ligand).

In addition, recent studies showed an association between elevated IL-6 levels and poor postoperative prognosis. A surgical intervention is necessary if an aneurysm is large or ruptured. This operation entails prolonged clamping of the vessel, thus contributing to ischemia. Splanchnic ischemia, a well-known complication of aortic surgery, triggers the synthesis of proinflammatory cytokines, the most important of which is IL-6. Elevated levels of this cytokine during this pathology and after the surgical operation cause serious systemic adverse reactions

in various organs/tissues, especially the lungs, kidneys, and myocardium, thereby resulting in multiple organ dysfunction syndrome.<sup>46</sup>

### IL-12

The IL-12 family is a group of heterodimeric cytokines that includes IL-12, IL-23, IL-27, and IL-35.<sup>47</sup> IL-12 is produced by activated antigen-presenting cells, especially macrophages,

and consists of subunits p35 and p40. IL-12 has an influence on hematopoiesis and is required for T-cell-dependent immune and inflammatory responses, regulation of cytokine synthesis by (and proliferation of) T and natural killer (NK) cells, and differentiation of T cells.<sup>48,49</sup> IL-12 and IL-23 control differentiation into (and proliferation of) Th1 and Th17 cells, respectively.<sup>50</sup> IL-23 is produced predominantly by macrophages and dendritic cells and activates memory T cells. IL-23 contains 2 subunits: p40 (just as IL-12 does) and p19 (which is absent in IL-12).<sup>48</sup> The impact of IL-12 on cells is mediated by a receptor complex consisting of IL-12R $\beta$ 1 and IL-12R $\beta$ 2. The receptor of IL-23 is composed of IL-12R $\beta$ 1 and IL-23R. Stimulation by IL-12 and IL-23 triggers receptor-associated Janus kinases (JAKs) and phosphorylation of STAT proteins in target cells. Janus tyrosine kinase Tyk binds to IL-12R $\beta$ 1, while JAK binds to IL-23R as well as IL-12R $\beta$ 2.<sup>51</sup>

IL-12 is a potent proinflammatory cytokine that is detectable at elevated concentrations in patients with AAA.<sup>52</sup> In a murine model of AngII-dependent AAA, IL12p40 deficiency increases TGF- $\beta$ 2 and IL-10 expression and reduces the inflammatory response. IL12p40-deficient mice have a predominantly anti-inflammatory profile, and such animals are protected from many inflammatory and immune-system-mediated conditions.<sup>53,54</sup> Yan et al demonstrated that in a murine model of elastase-induced AAA, administration of neutralizing monoclonal antibodies against IL-12 and IL-23 attenuates the development of AAA. Furthermore, IL12p40 inhibition significantly reduced macrophage expansion and suppressed the production of several macrophage-related inflammatory mediators, including IL-6, TNF- $\alpha$ , and matrix metalloproteinase 9 (MMP-9).<sup>55</sup>

### IL-17

IL-17A, the founding member cytokine of the IL-17 family, was cloned in 1993 and was originally named cytotoxic T lymphocyte-associated antigen 8.<sup>56</sup> The production of IL-17A is regulated by the transcription factor ROR $\gamma$ t.<sup>57-59</sup> Th17 cell and its secretory IL-17 have been repeatedly shown to be involved in the induction of tissue inflammation in autoimmune diseases such as rheumatoid arthritis, psoriasis, and multiple sclerosis.<sup>60,61</sup>

IL-17 has also been found as a crucial regulator of inflammation and apoptosis in AAA. In various experimental models of AAA, a decrease in the IL-17 level helps to weaken or prevent aneurysm development. For instance, anti-IL-17 activity was found as crucial for AAA attenuation mediated by cardiac glycosides digoxin and its derivatives. Normally cardiac glycosides are being prescribed to attenuate heart failure because of their ability to restore calcium imbalance in cardiomyocytes. According to Huh et al, digoxin and its derivatives can also inhibit Th17 differentiation and IL-17A production via selective antagonism of the ROR $\gamma$ t transcription factor. Wei et al also reported that digoxin arrests the progression of

experimental AAA by suppressing differentiation into Th17 cells and IL-17A expression.<sup>58,59</sup> In the model of elastase-induced AAA, treatment with mesenchymal stem cells causes inhibition of IL-17A attenuated aneurysm progression and reduced the levels of other inflammatory cytokines.<sup>62</sup> Furthermore, in a model of AngII-induced AAA and aortic dissection, 2 weeks of treatment with an anti-IL-17A antibody reduced aortic dilatation and prevented aneurysm dissection.<sup>63</sup> Clinical research with 476 AAA patients and 200 controls shows a positive correlation between plasma levels of IL-17A and cross-sectional AAA area.<sup>64</sup>

### TGF- $\beta$

The family of TGF- $\beta$ , including 3 isoforms (TGF- $\beta$ 1, TGF- $\beta$ 2, and TGF- $\beta$ 3), represents bifunctional regulators that either inhibit or stimulate cell proliferation. TGF- $\beta$  was originally isolated as a cytokine that, together with EGF, induces cell transformation and growth of selected fibroblast cell lines.<sup>65</sup> Later, it was proven that TGF- $\beta$  can either stimulate or inhibit cell proliferation, depending on the experimental conditions.<sup>66</sup> Research into the mechanisms underlying aneurysm and aortic dissection have shed light on the importance of TGF- $\beta$  activity for the maintenance of aortic-wall integrity.

There are 2 TGF- $\beta$  signaling pathways, and both play an important part in aneurysm formation. In the canonical pathway, TGF- $\beta$  binds to its type I or type II receptor on the cell surface. The resultant complex phosphorylates SMAD2 (mothers against decapentaplegic homolog 2) or SMAD3, thus leading to heterotrimerization with SMAD4, their nuclear translocation, and SMAD-dependent gene transcription.<sup>67</sup> In the noncanonical pathway, TGF- $\beta$  launches RhoA and mitogen-activated protein kinase cascades, the latter of which includes extracellular signal-regulated kinase (ERK), N-terminal Jun kinase (JNK), and p38. TGF- $\beta$  activates them, thereby causing their phosphorylation to p-ERK, p-JNK, and p-p38, respectively.<sup>68</sup> TGF- $\beta$  takes part in the pathogenesis of AAA and TAA. In AAA, TGF- $\beta$  controls key processes including ECM remodeling, survival and proliferation of VSMCs, and initiation of the immune/inflammatory response.<sup>69</sup>

Several connective tissue disorders highlight the role of TGF- $\beta$  in AAA. For instance, unbalanced TGF- $\beta$  signaling is recognized as one of the key factors in the pathogenesis of Marfan syndrome, a disease usually complicated by AA formation.<sup>70</sup> Normally, TGF- $\beta$  signaling is limited by fibrillin 1.<sup>71</sup> Because the gene encoding fibrillin 1 (*FBNI*) is mutated in Marfan syndrome, TGF- $\beta$  signaling is enhanced resulting in increased secretion of MMPs. Moreover, Loeys-Dietz syndrome, another Mendelian disease clinically overlapping with Marfan syndrome, is caused by a mutation in TGF- $\beta$ R1 (TGF- $\beta$  receptor 1), TGF- $\beta$ R2, TGF- $\beta$ 1, or TGF- $\beta$ 3 or in downstream effectors of the TGF- $\beta$  pathway: SMAD2 or SMAD3.<sup>72-75</sup>



According to Holm et al, SMAD-independent TGF- $\beta$  signaling is implicated in the pathogenesis of AA in mice with Marfan syndrome. In their study, selective inhibition of ERK1/2 in the noncanonical TGF- $\beta$  pathway slowed the progression of AA, whereas in SMAD4-deficient mice (canonical pathway), exacerbation of AA and earlier death of the mice were observed.<sup>76</sup> Zhang et al also demonstrated that depletion of SMAD4 in VSMCs leads to inflammation in the aortic wall, suggesting SMAD4-dependent TGF- $\beta$  signaling in VSMCs protects from AA formation and dissection.<sup>77</sup>

In a murine model of elastase-induced AAA, inhibition of TGF- $\beta$  activity promotes progression of aortic dilatation via reduction of SMAD2/3 phosphorylation.<sup>78</sup> In a model of AngII-induced AA, inhibition of TGF- $\beta$  increases dilatation and accelerates the rupture of AAA and TAA.<sup>2</sup> According to Angelov et al, systemic blockade of TGF- $\beta$  (neutralization by an antibody) aggravates the AAA course, whereas genetic inactivation of TGF- $\beta$  specifically in VSMCs significantly accelerates TAA development in combination with the spread of intramural hematomas, thinning of the media, and thickening of the adventitia.<sup>79</sup> Dai et al reported that overexpression of TGF- $\beta$ 1 in a pre-existing AAA by endovascular gene therapy helps to stabilize the vessel diameter, to preserve elastin, to decrease the infiltration by monocytes-macrophages and T lymphocytes, and to reduce the amounts of MMP-2 and MMP-9. The research showed, that in parallel with the suppression of the destructive process, the TGF- $\beta$ 1 overexpression triggered endoluminal reconstruction, replacing the thrombus with vascular smooth muscle and an intima rich in collagen and elastin.<sup>80</sup>

Thompson et al found that 5 SNPs in *LTBP4* and an allelic variant of *TGFB3* are associated with a significant decrease in AAA growth in a UK cohort. They demonstrated altered growth in carriers of 2 common haplotypes of *LTBP4* and a single haplotype of *TGFB3*.<sup>81</sup>

Thus, certain mechanisms underlying the impact of TGF- $\beta$  on the development of AAA are not clear enough. Possibly, the canonical TGF- $\beta$  signaling pathway prevents AA progression via SMAD4-dependent up-regulation of expression of VSMC-specific gene markers resulting in acceleration of proliferation and migration of VSMCs as well as increased production of collagen. In a similar way, during atherosclerotic vascular lesions, the canonical TGF- $\beta$  pathway promotes restenosis of the arteries, enhancing the proliferation of neointimal cells and the accumulation of collagen.<sup>82</sup>

For example, in atherosclerotic vascular lesions, this pathway promotes restenosis of the arteries, enhancing the proliferation of neointimal cells and the accumulation of collagen.

### *IL-10*

IL-10 is a major pleiotropic immunoregulatory cytokine that plays a key role in the alleviation of allergic and inflammatory

diseases.<sup>83</sup> IL-10 is produced by both innate- and adaptive-immunity cells, including dendritic cells, macrophages, mast cells, NK cells, eosinophils, neutrophils, B cells, regulatory T cells, and Th1, Th2, and Th17 lymphocytes.<sup>84</sup> IL-10 prevents the secretion of inflammatory cytokines and regulates the differentiation and proliferation of T cells, B cells, NK cells, mast cells, granulocytes, dendritic cells, keratinocytes, and endothelial cells.<sup>85</sup>

There is evidence that the immunoregulatory properties of IL-10 may be involved in AA development because the IL-10-1082 "A" polymorphism (associated with lower IL-10 production) is more common in patients with AAA, and patients with this polymorphism are at a higher risk of AAA. Plasma IL-10 concentrations are significantly lower in patients with AAA than in comparable patients with stenotic aortic atherosclerotic lesions. Besides, in a model of AngII-induced AAA, genetic or pharmacological depletion of IL-10 increases the susceptibility to aneurysm formation and rupture.<sup>12,86,87</sup>

IL-10 reduces inflammation in aneurysm tissue, thereby contributing to the proliferation and phenotypic transformation of VSMCs, inhibiting degradation of VSMCs and rupture of elastic fibers, and delaying aneurysm development in a rabbit model of elastase-induced AAA.<sup>88</sup>

Vucevic et al found a negative correlation between IL-6 and IL-10 expression levels in aortic samples derived from AAA patients. On the basis of this finding, they subdivided the AAAs into 2 cytokine-producing groups and showed that tissue samples in the IL-6<sup>high</sup>/IL-10<sup>low</sup> group contained higher percentages of granulocytes, HLA-DR<sup>+</sup> cells, and CD68<sup>+</sup> cells but lower percentages of lymphocytes and plasma cells as compared to the IL-6<sup>low</sup>/IL-10<sup>high</sup> group.<sup>89</sup>

### *IFN- $\gamma$*

IFN- $\gamma$  (originally called macrophage activating factor) is a key player in driving cellular immunity,<sup>90</sup> produced mainly by CD4<sup>+</sup> T cells.<sup>91</sup> Cellular responses to IFN- $\gamma$  are initiated by its interaction with a heterodimeric receptor consisting of interferon gamma receptor 1 and 2.<sup>92</sup> Downstream effects of IFN- $\gamma$  include macrophage activation, synthesis of MMPs and cysteine proteinases and inhibition of chemokines that attract cells to inflammatory sites.<sup>93,94</sup>

The inflammatory infiltrate of AAA is mainly composed of macrophages expressing MMP-12 and of T lymphocytes, which are the main producers of IFN- $\gamma$ . The participation of IFN- $\gamma$  in AAA pathogenesis has been proven by numerous research; for example, Schönbeck et al reported elevated levels of IFN- $\gamma$  in AAA tissues,<sup>18</sup> and serum of AAA patients.<sup>34</sup>

Nonetheless, the role of IFN- $\gamma$  in the development of AAA is dual: evidently, this cytokine promotes either proinflammatory or anti-inflammatory processes, depending on the disease stage, the model of AAA, and other unknown factors. According to King et al, IFN- $\gamma$  and its effector chemokine CXCL10 attenuate

AAA development, whereas deficiencies of both IFN- $\gamma$  and CXCL10 in mice induce AAA formation. The opposite effects of the IFN- $\gamma$  deficiency between AAA and aortic stenosis can be explained by the localization of these processes in different tissue layers of the vessel and by the accumulation of various types of inflammatory cells and cytokines in the affected tissue.<sup>95</sup> Shimizu et al also demonstrated that the IFN- $\gamma$  signaling blockade observed in IFN- $\gamma$  receptor-null mice enhances AAA formation after aortic allograft transplantation.<sup>96</sup>

In contrast, increased concentrations of IFN- $\gamma$  accelerate the AAA growth rate, and the serum concentration of IFN- $\gamma$  directly correlates with aneurysm progression according to Juvonen et al<sup>34</sup> In addition, Xiong et al showed the crucial role of IFN- $\gamma$  secreted by CD4<sup>+</sup> T-cells. In this study, CD4 deficient mice were resistant to AAA formation only before restoration of IFN- $\gamma$  levels.<sup>97</sup> Similar data were presented by Kuivaniemi et al: CD4<sup>-/-</sup> mice with decreased IFN- $\gamma$  expression were resistant to the induction of AAA.<sup>98</sup>

Presumably, IFN- $\gamma$  promotes AAA formation possibly via stimulating MMP synthesis, but later, in the already formed AA, IFN- $\gamma$  attenuates the progression of the pathology. Nevertheless, further research have to shed light on this cytokine in the focus of AAA.

#### *TNF- $\alpha$ and IL-1 $\beta$*

Both TNF- $\alpha$  and IL-1 are key proinflammatory cytokines of innate-immune-response initiation, mediating the recruitment, activation, and adhesion of circulating phagocytic cells and terminating the innate immune response.<sup>99</sup>

Kaneko et al reported increased expression of TNF- $\alpha$  converting enzyme (Tace) in human AAA samples as compared with normal aorta, suggesting up-regulation of TNF- $\alpha$  in aneurysm tissue. Moreover, temporal systemic deletion of Tace in mice with periaortic application of CaCl<sub>2</sub> resulted in decrease of AAA size and attenuation of extracellular matrix disruption and aortic wall inflammation.<sup>100</sup>

Xiong et al revealed that genetic or pharmacological blockade of TNF- $\alpha$  resulted in resistance to aneurysm formation in CaCl<sub>2</sub>-induced AAA murine model. TNF- $\alpha$  deficiency also attenuated matrix MMP 2 and MMP-9 expression as well as macrophage infiltration into the aortic tissue. In addition, Food and Drug Administration-approved TNF- $\alpha$  antagonist, infliximab, was shown to inhibit aneurysm growth.<sup>101</sup>

In a study on 7 AAA tissue samples and 5 control aortic-tissue samples, TNF- $\alpha$  proved to be upregulated significantly in the AAA extracts; IL-1 $\beta$  concentration was also significantly higher in the AAA tissue samples.<sup>24</sup>

Study on 34 subjects with AAA and 34 matched non-AAA controls revealed significantly increased IL-1 $\alpha$  and IL-1 $\beta$  plasma levels in AAA patients. Moreover, peripheral blood mononuclear cells derived from male patients with AAA were found to express significantly higher mRNA levels of *IL1B*

compared with control group.<sup>102</sup> In a model of AngII-induced AAA, inhibition of IL-1 $\beta$  by an antibody (01BSUR) inhibits the formation of AAA.<sup>103</sup> Johnston et al revealed significant protection against elastase-induced AAA formation in mice with genetic deletion of IL-1 $\beta$  or IL-1R.<sup>104</sup> Similarly, according to Meher et al, IL-1 $\beta$ -knockout mice demonstrated significantly lower infiltration of neutrophils to aorta and were protected from AAA. Moreover, this protection was abolished by adoptive transfer of wild-type neutrophils.<sup>105</sup>

In a model of  $\beta$ -aminopropionitrile-induced dissecting AA, Jiang et al found dramatic upregulation of IL-1 $\beta$  in aortic samples but not in the bloodstream. The study also revealed that IL-1 $\beta$  can locally participate in AAA formation via the activation of MMP-2 and MMP-9 and via degradation of elastin fibers because these alterations ultimately weaken biomechanical properties of the aortic wall.<sup>106</sup>

In contrast, Hingorani et al revealed that only TNF- $\alpha$ , but not IL-1 inhibition was able to completely block elastase-induced dilatation in a rat model of AAA.<sup>107</sup> A similar finding was reported by Batra et al, only TNF- $\alpha$  exerted an AAA-aggravating impact. Genetic deletion of IL-1 $\beta$  and IL-1R did not inhibit aneurysm formation. Moreover, IL-1R<sup>-/-</sup> mice developed larger aneurysms than did their wild-type controls.<sup>108</sup> Thus, both TNF- $\alpha$  and IL-1 $\beta$  are important factors for AAA progression but, apparently, depending on particular pathological conditions their role can be displayed differently.

Recently, B cell-activating factor (BAFF, BLyS, or TALL-1), a member of TNF family cytokines, was identified as a factor contributing to AAA formation. In a murine model of AAA, the level of lesion was decreased by the injection of BAFF antagonists both before and after the model induction. BAFF is a critical survival factor for mature B cells, supporting their differentiation as well as antibody class switching, and antibody secretion, that could explain its promoting role in the AAA development as a pro-inflammatory cytokine.<sup>109</sup>

#### *IL-4*

IL-4 is a signature cytokine of type 2 immune response and crucial regulator of atopic diseases.<sup>110</sup>

In a study by Shimizu et al, murine aortic allografts transplanted into IFN- $\gamma$ -deficient hosts manifested severe AAA formation, which was prevented by an anti-IL-4 antibody.<sup>96</sup> Liu et al revealed a protective function of eosinophil-secreted IL-4 in clinical observations and murine studies. Their results showed that eosinophil deficiency exacerbates the growth of AngII perfusion-induced AAA in *ApoE*<sup>-/-</sup> and eosinophil-deficient *ApoE*<sup>-/-</sup> $\Delta$ dblGATA mice. They concluded that eosinophils exert a protective action against AAA by releasing IL-4 and cationic proteins such as mEar1 to regulate macrophage and monocyte polarization and to block NF- $\kappa$ B (nuclear factor kappa-light-chain-enhancer of activated B cells) activation in aortic inflammatory and vascular cells.<sup>111</sup>

### IL-32

IL-32 is a cytokine inducing crucial inflammatory cytokines such as TNF $\alpha$  and IL-6 and its expression is elevated in various inflammatory and viral infectious diseases.<sup>112</sup> According to Bengts et al, the expression of proinflammatory cytokine IL-32 is significantly higher in AAA tissue samples than in control groups. Furthermore, IL-32 turned out to be predominantly expressed in VSMCs and T-cell-rich regions. The highest level of IL-32 mRNA was observed in the intima/media layer of AAA. However, there were no differences observed in the plasma levels of IL-32 between the control and experimental group.<sup>113</sup>

### IL-33

IL-33 is a tissue-derived nuclear cytokine from the IL-1 family abundantly expressed in endothelial cells, epithelial cells and fibroblast-like cells, both during homeostasis and inflammation.<sup>114</sup> Daily intraperitoneal administration of recombinant IL-33 or transgene IL-33 overexpression ameliorated periaortic CaPO<sub>4</sub> injury-induced and aortic elastase exposure-induced AAA in mice, as evidenced by reduced aortic reorganization, leukocytes infiltration and connective tissue degradation as well as lower apoptosis in the lesion.<sup>115</sup>

### Chemokine family

Chemokines are a family of small secreted peptide cytokines regulating various cell functions, first of all, immune-cell recruitment. They are known to play essential roles in a number of pathological conditions, including inflammation, atherosclerosis, altered hematopoiesis, and cancer. C-X-C motif chemokine ligand 12 (CXCL12, also referred to as SDF-1: stromal cell-derived factor 1) is a homeostatic CXC chemokine that possesses 7 isoforms and binds to CXCR4 and CXCR7 (C-X-C chemokine receptors type 4 and type 7).<sup>116</sup>

Using 32 aneurysm sac specimens from AAA patients and 12 matching nonaneurysmal aortic samples from transplant donors, Tanios et al revealed increased mRNA and protein expression of CXCR4 and CXCL12 in the AAA tissue samples. The mRNA expression of CXCR4 and CXCL12 was 9.6- and 4.6-fold higher in AAA than in nonaneurysmal aorta samples ( $P = .0004$  and  $P < .0001$ , respectively). Likewise, the protein level of CXCR4 was 3.2-fold higher in the AAA wall than in the nonaneurysmal aortic tissue ( $P < .0001$ ), although CXCL12 could not be detected.<sup>117</sup>

Michineau et al revealed increased levels of *CXCR4* mRNA in both the human AAA wall and the mouse aortic wall affected by CaCl<sub>2</sub>-induced AAA. Furthermore, in murine AAA model, CXCR4 blockade by AMD3100 decreased the infiltration by adventitial macrophages, inhibited aneurysm formation, and prevented aortic-wall destruction by reducing the mRNA levels of MMP-12, MMP-14, MCP-1, MIP-1 $\beta$ , MIP-2 $\alpha$ , RANTES, IL-1 $\beta$ , IL-6, TNF- $\alpha$ , and E-selectin.<sup>118</sup>

Finally, pharmacological inhibition of CXCR2<sup>119,120</sup> and genetic depletion of CCR2<sup>121</sup> markedly reduced AngII-induced AAA formation in *ApoE*<sup>-/-</sup> mice.

Current highlights of the role of listed cytokines in AAA progression are presented in Figure 1.

### Predictive Value of Cytokines

With all crucial involvement in the development of AAA cytokines might serve appropriate for diagnosis making and outcomes prediction. Herein we present a brief summary of research revealing predictive value of minimally invasive tests such as genotyping or biochemical analysis.

### Polymorphisms of genes

Unlike TAA, AAA has no major genetic component. Nevertheless, Table 2 presents 13 polymorphisms significantly associated with the risk of AAA.

### Serum and plasma levels of cytokines

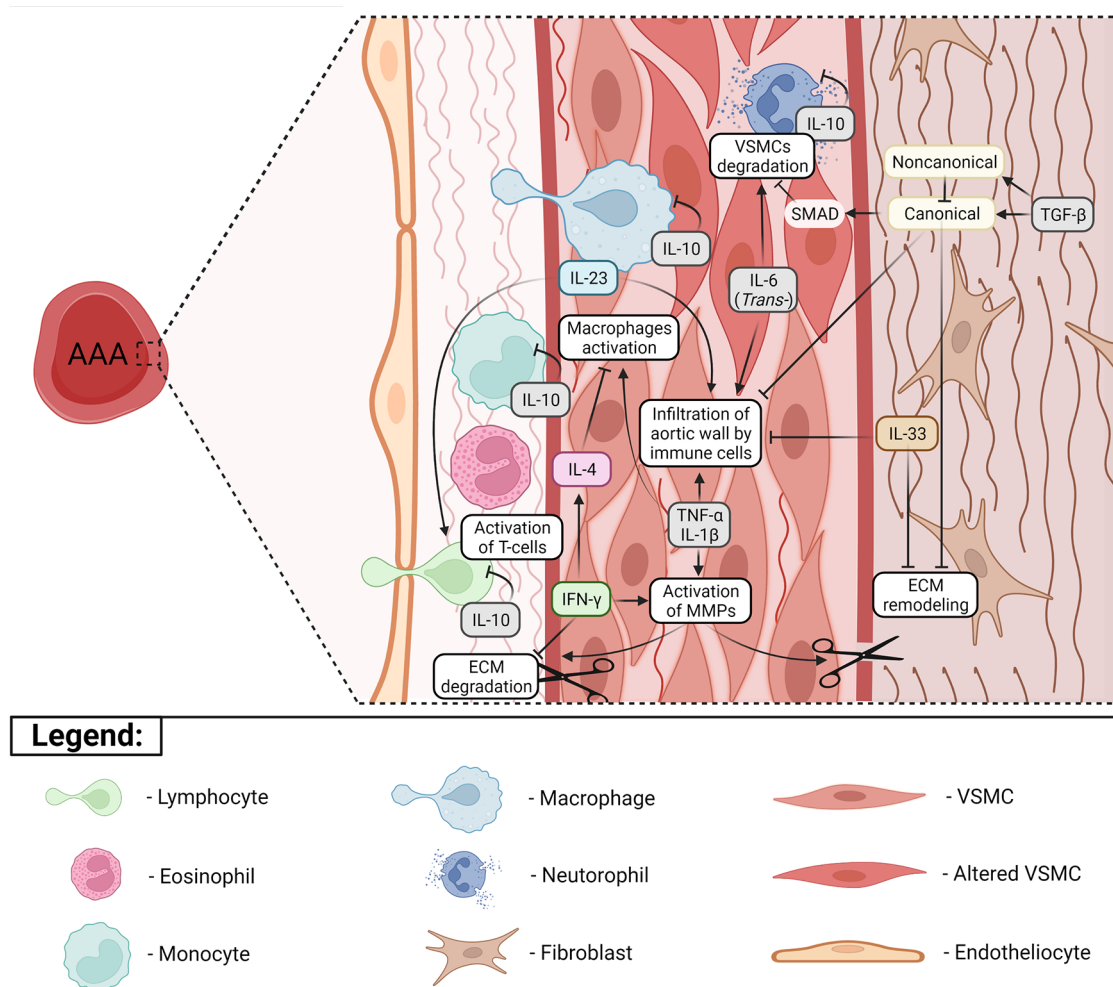
Measurement of blood serum/plasma cytokines' concentrations is one of the sensitive assays for the detection of a number of diseases. Besides inflammatory processes, blood levels of cytokines can be influenced by aging, physical exercise, psychiatric disorders, cancer, obesity, pain, and other factors.<sup>131,132</sup> The concentration levels of cytokines are usually measured in picograms per milliliter and can vary widely. For example, in healthy persons serum concentration of IL-10 varies from 0.01 to 19.8 pg/mL, while IFN- $\gamma$  concentration from 0.14 to 126.8 pg/mL.<sup>133</sup> The Table 3 summarizes studies revealing changed blood levels of cytokines in AAA patients (Table 3).

### Prospects of This Research Field

Nowadays, there is no effective pharmacotherapy of AAA that can mitigate the disease course and prevent ruptures. Therefore, a search for drug targets is obviously crucial in further studies. Cytokines are key participants in the regulation of AAA development (Figure 2), therefore managing their effects may become an appropriate step to alleviate the damage to the aortic wall. For instance, Bernal et al showed that therapy based on suppressor of cytokine signaling 1 (SOCS1) inhibits a common signaling pathway (JAK/STAT) of inflammatory cytokines, thus hindering AAA development in a murine model, as evidenced by limited aortic expansion and elastin degradation, preservation of VSMCs in the media, and reduced leukocytic infiltration.<sup>140</sup>

Noteworthy, detection of changed concentrations of cytokine do not clarify if it plays positive or negative role as levels of molecular participant can be imbalanced due to both adaptive or aggravating mechanisms. In fact, only effects of down- or upregulation of different cytokines pathways using pharmacological or genetic tools in experimental models of AAA can shed light on its role in the disease progression.





**Figure 1.** Contribution of several key cytokines in AAA formation. Aneurysmal damage of abdominal aorta is primarily driven by infiltration of the aortic wall by immune cells, to fit: lymphocytes, monocytes, macrophages and neutrophils. Subsequent inflammatory activation leads to increased production of matrix metalloproteases (MMPs), which cause remodeling of extracellular matrix (ECM), disrupting connective tissue fibers. Most crucial regulators orchestrating accumulation of immune cells are  $\text{TNF-}\alpha$ ,  $\text{IL-1}\beta$  (via trans-signaling) and  $\text{IL-6}$ . These cytokines are not only able to attract different types of immune cells but they also may be produced by a wide range of cells, both immune and mesenchymal. The same inflammatory effect can also be mediated by  $\text{IL-23}$  (mostly produced by macrophages), the cytokine which normally sends strong activating signals to T-lymphocytes. In contrast,  $\text{IL-10}$  is a cytokine which prevents inflammatory activation, arising beneficial effects in AAA. One of regulators with a controversial role in AAA formation is  $\text{IFN-}\gamma$ . On the one hand this cytokine activates degradation of ECM, but on the other hand  $\text{IFN-}\gamma$  may prevent macrophages activation via facilitating the release of  $\text{IL-4}$ . Another 2-faced cytokine is  $\text{TGF-}\beta$ , which can mediate both positive effects such as decrease of vascular smooth muscle cells (VSMCs) apoptosis via canonical SMAD-dependent pathway and negative effects via non-canonical pathway. Color indicates the type of cell which produces cytokine (blue—macrophages, pink—eosinophil, green—lymphocyte, beige—fibroblast, gray—various types of cells).

The pharmacological and genetic studies on AAA have confirmed by now the causative role of increased  $\text{TNF-}\alpha$ ,  $\text{IL-1}\beta$ ,  $\text{IL-6}$ ,  $\text{IL-12/IL-23}$ ,  $\text{IL-17}$ ,  $\text{CCR2}$ ,  $\text{CXCR2}$ ,  $\text{CXCR4}$ , and the  $\text{TGF-}\beta$  (non-canonical pathway) signaling as well as protective functions of  $\text{IL-10}$ ,  $\text{IL-33}$ , and canonical  $\text{TGF-}\beta$  signaling. The effects of  $\text{IFN-}\gamma$ ,  $\text{CXCL10}$  and  $\text{IL-4}$  on AAA are ambiguous and require further research (Figure 2B). In this way, we may consider that regarding these cytokines as drug targets is challenging for AAA treatment, thus the issue needs further investigation.

It is extremely relevant to look for new ways to predict the onset and course of AA, especially because of its high clinical significance and a long asymptomatic period in many patients. For

instance, Blumel et al offer a simple and elegant “thumb palm test” to reveal the ascending AA (a variant of TAA) with a high level of specificity.<sup>141</sup> Thanks to the literature review, we guess that a quantitative assessment of some cytokines may have certain diagnostic value in the AAA. Although, the cytokines use is seriously limited there, as their concentrations are able to change significantly even in the blood of generally healthy persons but also in a wide range of comorbidities. A possible solution is a search of appropriate integrative indicators and coefficients based on the ratio of various cytokines’ levels in AAA. This approach is already successfully applied in clinical practice when analyzing numerous biochemical parameters, namely the fibrinogen-to-albumin ratio as a new clinical marker of microinflammation<sup>142</sup>; the CRP/albumin ratio as



**Table 2.** Associations between polymorphisms in genes encoding cytokines and the risk or severity of AAA.

GENE	SNP/LOCI	REFERENCE	POPULATION/ COUNTRY	CASES/ CONTROLS	METHODS	RISK ALLELE	OR (P-VALUE)	MAIN CONCLUSIONS
<i>CCR2</i>	V64I (rs1799864)	Katrancioğlu et al <sup>122</sup>	Turkey	100 patients with AAA and 138 individuals with normal aortic diameter	Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP)	I	2.31 (0.01)	Significant relationship between <i>CCR2</i> V64I polymorphism (OR: 2.31, 95% CI: 1.19-4.46, P = .01) and presence of AAA in multivariate regression analysis
<i>IL6</i>	IL-6-572G>C (rs1800796)	Smallwood et al <sup>41</sup>	Australia	677 men with screen-detected AAAs and 656 age-matched controls	TaqMan-based PCR	C	5.78 (0.031)	Polymorphism is associated with AAA
	-174G/C (rs1800795)	Jabloniska et al <sup>123</sup>	Austria	104 patients with AAA and 112 healthy volunteers	PCR-RFLP	C	2.15 (0.015)	Polymorphism is associated with increased risk of AAA
<i>IL6R</i>	rs12133641	Jones et al <sup>40</sup>	104,830 European (U.S., Australia, Iceland, The Netherlands, U.K., New Zealand)	4972 European-ancestry cases, 99,858 European-ancestry controls. Replication: 2482 European-ancestry cases, 2750 cases, 2645 European-ancestry controls, 5263 controls	Genome-wide genotyping array (Affymetrix, Illumina)	A	1.14 (5 × 10 <sup>-19</sup> )	Polymorphism is associated with increased risk of AAA
<i>IL10</i>	IL-10-1082 (rs1800896)	Bown et al <sup>24</sup>	USA	389 patients with AAA and 404 healthy controls	Kompetitive allele-specific PCR (KASP)	A	1.50 (0.014)	"A" allele of IL-10 -1082G/A is significantly associated with AAA
		Bown et al <sup>25</sup>	UK	100 patients with AAA and 100 age-matched and sex-matched control subjects	Induced heteroduplex genotyping	A	1.8 (0.03)	"A" allele of IL-10 -1082G/A is significantly associated with AAA (OR = 1.8; 95% CI = 0.9-3.6; P = .03).
		Wang et al <sup>66</sup>	China	381 cases and 381 controls	PCR-RFLP	A	1.34 (0.04)	"A" allele of IL-10-1082G/A is significantly associated with AAA
<i>TGFB1</i>	rs1800469	Zuo et al <sup>26</sup>	China	155 cases and 310 controls	Typerr 4.0 software (Mass ARRAY Compact System; Sequenom, San Diego, CA, USA).	T	1.35 (0.03)	rs1800469-T (in <i>TGFB1</i> ) is associated with increased risk of AAA in Chinese population

(Continued)

Table 2. (Continued)

GENE	SNP/LOCI	REFERENCE	POPULATION/ COUNTRY	CASES/CONTROLS	METHODS	RISK ALLELE	OR (P-VALUE)	MAIN CONCLUSIONS
<i>TGFBFR1</i>	rs1626340	Baas et al <sup>127</sup>	Dutch	736 cases and 1024 controls	GoldenGate assay on Illumina BeadStation 500 GX (Illumina Inc., San Diego, CA, USA) with TaqMan Assays on Demand (Applied Biosystems, Foster City, CA, USA)	A	1.32 (0.001)	rs1626340 (in <i>TGFBFR1</i> ) correlates with AAA risk in Dutch population
<i>TGFBFR2</i>	rs1036095	Baas et al <sup>127</sup>	Dutch	736 cases and 1024 controls	GoldenGate assay on Illumina BeadStation 500 GX (Illumina) with Taqman Assays on Demand (Applied Biosystems)	C	1.32 (0.001)	rs1036095 and rs4522809 (in <i>TGFBFR2</i> ) are associated with increased AAA risk in Dutch population
	rs4522809					A	1.28 (0.0004)	
	g.42917C>T (rs1078985)	Golledge et al <sup>128</sup>	New Zealand	1043 subjects from New Zealand, of whom 654 had an AAA	Illumina Golden Gate assay on a BeadLab System (Illumina)	C	0.64 (0.020)	rs1078985 (in <i>TGFBFR2</i> ) is weakly associated with AAA among healthy male subjects according to a codominant model. In a recessive genetic model, the minor allele homozygote (CC) is associated with reduced prevalence of AAA (OR: 0.64 (95% CI: 0.45-0.93); P = .020)
<i>TNFA</i>	TNF- $\alpha$ 308G/A (rs1800629)	Jabloniska et al <sup>123</sup>	Austria	104 patients with AAA and 112 healthy volunteers	PCR-RFLP	A	1.97 (0.02)	TNF- $\alpha$ -238G/A gene polymorphism is associated with increased risk of AAA
<i>SMAD2</i> *	c.C833T, p.A278V (NM_001003652.3/ENS00000402690)	Zhang et al <sup>129</sup>	China	1 case	Exome sequencing and Sanger sequencing			Novel missense mutation (c.833CNT, p.A278V) in the <i>SMAD2</i> gene is the causal variant of familial AAA
<i>CCR5</i>	Delta-32 polymorphism	Ghilardi et al <sup>130</sup>	Italy	70 patients with AAA and 172 healthy controls	PCR	$\Delta \Delta$ 32	2.7 (0.002)	A genetic risk factor distinguishes AAA from arterial occlusive disease in humans: CCR5 Delta 32 deletion

\*Does not belong to the cytokine superfamily but is directly involved in TGF- $\beta$  signaling.

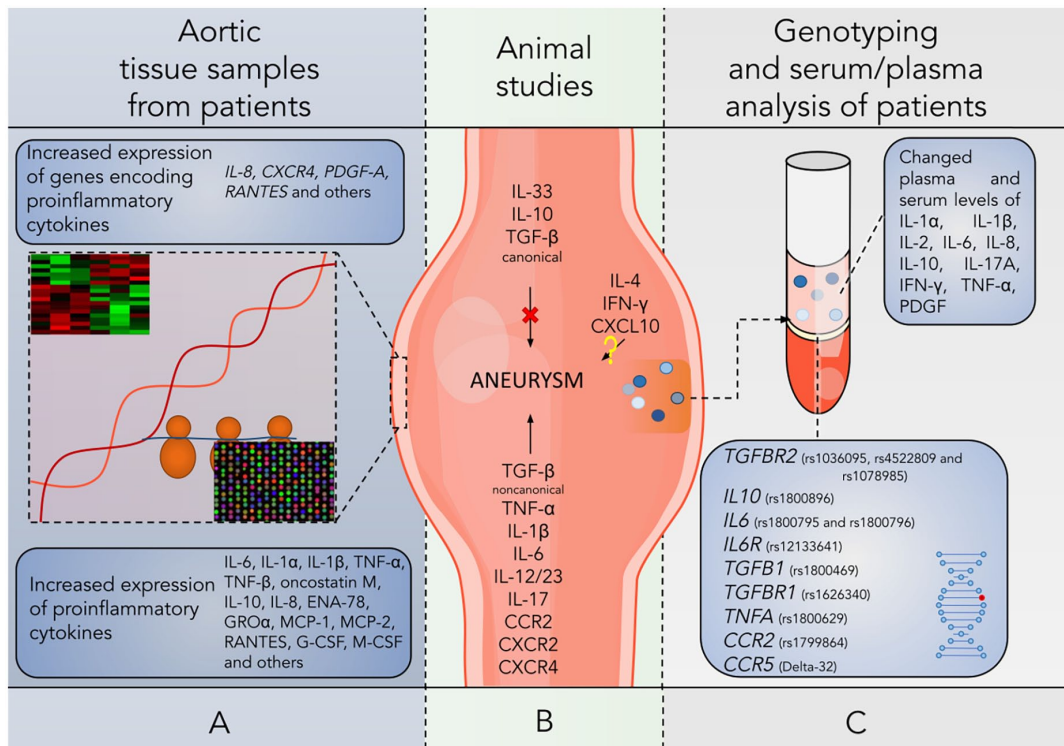
**Table 3.** Relation between AAA and plasma levels of various cytokines.

PROTEIN	COHORT	TYPE OF SAMPLE	PHENOTYPE/PREDICTIVE VALUE	REFERENCE
IL-1 $\alpha$	101 AAA patients	Serum	<ul style="list-style-type: none"> <li>IL-1<math>\alpha</math>, proinflammatory cytokine not normally detectable in the circulation was observed in the majority of patients with infrarenal AAA</li> <li>No correlations with maximum AAA diameter and growth rate</li> </ul>	Ahmad et al <sup>134</sup>
	34 pairs of AAA patients and age/sex-matched non-AAA patients	Plasma	Significantly increased IL-1 $\alpha$ plasma levels in AAA patients.	Wu et al <sup>102</sup>
IL-1 $\beta$	86 AAA patients/30 non-AAA subjects	Plasma	Increased plasma levels in AAA patients ( $P < .05$ )	Treska et al <sup>135</sup>
	345 patients with AAA and 30 healthy volunteers	Plasma	Increased plasmatic concentrations in AAA patients ( $P < .001$ )	Treska et al <sup>136</sup>
	9 samples from AAA patients and 15 samples from non-AAA patients	Plasma	IL-1 $\beta$ was not detectable in the plasma of AAA patients (n=9) but was detectable in most samples (7/10) in the control group (n=10)	Batra et al <sup>108</sup>
	34 pairs of AAA patients and age/sex-matched non-AAA patients	Plasma	Significantly increased IL-1 $\beta$ plasma levels in AAA patients.	Wu et al <sup>102</sup>
IL-2	86 AAA patients/30 non-AAA subjects	Plasma	Increased plasma levels in AAA patients ( $P < .05$ )	Treska et al <sup>135</sup>
	345 patients with AAA and 30 healthy volunteers	Plasma	Increased plasma concentrations in AAA patients ( $P < .001$ )	Treska et al <sup>136</sup>
IL-6	86 AAA patients/30 non-AAA subjects	Plasma	Increased plasma levels in AAA patients ( $P < .05$ )	Treska et al <sup>135</sup>
	120 non-AAA outpatients	Serum	Positive correlation of IL-6 serum levels with aortic diameter ( $P < .01$ )	Rohde et al <sup>36</sup>
	90 AAA patients	Plasma	IL-6 levels were increased with increasing AAA diameter and symptoms however not reaching significant correlation	Treska et al <sup>137</sup>
	27 AAA patients and 15 non-AAA subjects	Plasma	<ul style="list-style-type: none"> <li>Higher plasma levels in AAA patients</li> <li>Positive correlation between AAA surface area and mean plasma IL-6 concentration</li> </ul>	Dawson et al <sup>138</sup>
	360 AAA patients and 219 healthy subjects	Plasma	<ul style="list-style-type: none"> <li>Significantly (<math>P &lt; .0001</math>) increased plasma levels in AAA patients</li> <li>Significant difference in plasma levels between patients with different AAA sizes (<math>P = .0002</math>)</li> </ul>	Flondell-Sité et al <sup>139</sup>
	345 patients with AAA and 30 healthy volunteers	Plasma	Increased plasma concentrations in AAA patients ( $P < .001$ )	Treska et al <sup>136</sup>
	476 AAA patients and 200 non-AAA controls	Plasma	Reduced level in AAA patients	Liao et al <sup>64</sup>
IL-8	86 AAA patients/30 non-AAA subjects	Plasma	<ul style="list-style-type: none"> <li>Significant difference in plasma levels in relation to the size of AAA (<math>P &lt; .05</math>)</li> <li>Correlation of plasma levels with the size of the AAA in hypertonic patients (<math>p &lt; .05</math>)</li> </ul>	Treska et al <sup>135</sup>
	90 AAA patients	Plasma	IL-8 levels ( $P < .05$ ) showed a statistically significant correlation with the diameter of AAA	Treska et al <sup>137</sup>
	345 patients with AAA and 30 healthy volunteers	Plasma	Significant correlation with AAA diameter ( $P < .05$ )	Treska et al <sup>136</sup>
IL-10	476 AAA patients and 200 non-AAA controls	Plasma	<ul style="list-style-type: none"> <li>Reduced plasma levels in AAA patients, <math>P &lt; .001</math></li> <li>Significant protective factor against human AAA with OR 0.91 (<math>P = .011</math>)</li> <li>Positive correlation with maximal annual AAA growth rate (<math>P = .036</math>)</li> </ul>	Liao et al <sup>64</sup>

(Continued)

Table 3. (Continued)

PROTEIN	COHORT	TYPE OF SAMPLE	PHENOTYPE/PREDICTIVE VALUE	REFERENCE
IL-17A	476 AAA patients and 200 non-AAA controls	Plasma	<ul style="list-style-type: none"> <li>Reduced plasma levels in AAA patients, <math>P &lt; .001</math> (<math>P = .028</math>)</li> <li>Positive correlation with maximal cross-sectional AAA area (<math>P = .044</math>)</li> </ul>	Liao et al <sup>64</sup>
IFN- $\gamma$	476 AAA patients and 200 non-AAA controls	Plasma	<ul style="list-style-type: none"> <li>Significantly lower plasma IFN-<math>\gamma</math> levels in AAA patients (<math>P = .021</math>)</li> <li>Significant protective factor against human AAA with OR at 0.91 (<math>P = .044</math>)</li> <li>Positive correlation with maximal cross-sectional AAA area (<math>P = .009</math>)</li> </ul>	Liao et al <sup>64</sup>
TNF- $\alpha$	86 AAA patients/30 non-AAA subjects	Plasma	<ul style="list-style-type: none"> <li>Increased plasma levels in AAA patients (<math>P &lt; .05</math>)</li> <li>Correlation of plasma levels with AAA size in hypertensive patients (<math>P &lt; .01</math>)</li> </ul>	Treska et al <sup>135</sup>
	90 AAA patients	Plasma	<ul style="list-style-type: none"> <li>Increased plasma levels in AAA patients (<math>P &lt; .0001</math>)</li> <li>Negative correlation between plasma levels of TNF-<math>\alpha</math> and symptoms of AAA (<math>P &lt; .05</math>)</li> </ul>	Treska et al <sup>137</sup>
	360 AAA patients and 219 healthy subjects	Plasma	Significantly higher plasma levels in AAA patients ( $P < .0001$ )	Flondell-Sité et al <sup>139</sup>
	345 patients with AAA and 30 healthy volunteers	Plasma	Significantly lower concentrations in symptomatic patients with AAA rupture ( $P < .05$ ).	Treska et al <sup>136</sup>
PDGF	86 AAAs/30 non-AAA samples	Plasma	Increased plasma levels in AAA ( $P < .05$ )	Treska et al <sup>135</sup>



**Figure 2.** Summary representation of current data revealing implication of different cytokines in AAA. (A) Aneurysm samples collected from patients are characterized by expression of pro-inflammatory cytokines on the transcriptome and protein levels. (B) Study on mice show that pharmacological or genetic blockade of L-10, IL-33, and canonical TGF- $\beta$  signaling aggravate AAA course, whereas inhibition of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-12/IL-23, IL-17, CCR2, CXCR2, CXCR4 and the TGF- $\beta$  noncanonical pathway attenuate the disease. At the same time, animal studies show controversial role of IL-4, IFN- $\gamma$  and CXCL10. (C) Population genetics studies revealed that carriers of particular mutations of genes *CCR2* (rs1799864), *CCR5* (Delta-32), *IL6* (rs1800796 and rs1800795), *IL6R* (rs12133641), *IL10* (rs1800896), *TGFB1* (rs1800469), *TGFB1* (rs1626340), *TGFB2* (rs1036095, rs4522809, rs1078985), and *TNFA* (rs1800629) have increased risk of AAA development.



an independent prognostic marker in different types of cancer,<sup>143</sup> and as a scoring system for determining the stage and activity of an inflammatory disease<sup>142</sup>; the De Ritis (AST/ALT) ratio for the diagnosis and prognosis making in liver diseases, myocardial infarction, acute ischemic stroke, peripheral artery disease, and kidney injury<sup>144</sup>; the blood urea nitrogen-to-creatinine ratio as an independent prognostic indicator of poor outcomes in various diseases, such as heart failure, kidney injury, and stroke<sup>145</sup>; finally, the atherogenic index of plasma (a logarithm of the ratio between plasma triglyceride and high density lipoprotein cholesterol levels) as a sensitive marker of the risk to develop atherosclerosis and cardiovascular disease.<sup>146</sup> Identifying highly sensitive and specific relations between cytokines in the context of AAA seems to be an appropriate approach to create various test systems and diagnostic panels. This strategy undoubtedly requires additional research.

## Conclusion

Despite cytokines being key regulators of AAA progression, the certain mechanisms underlying their contribution have yet to be clarified. However, existing data opens up novel directions for the prevention and therapy of AAA and related disorders. One may also observe especially promising results revealing diagnostic and predictive value of cytokines for AAA patients. Obviously, further studies in this research field might bring some breakthroughs for prolongation of lifespan in persons struggling with AAA.

## Author Contributions

OAP—literature analysis, drafting the article, editing. VOS—main idea, conceptualization, drafting the article, critical revision of the content, editing. AEB—main idea, drafting the article, editing, graphical design. OYB—collection and analysis of data related to population genetics studies, editing. GAP, AAV, AVD, MVK, MVP, NKS—drafting the article, editing, references formalization. All authors read and approved the manuscript.

## Statement on Ethics

Ethical approval and informed consent were not required for the review.

## ORCID iDs

Andrei E Belykh  <https://orcid.org/0000-0001-9766-2104>

Gennadii A Pivachenko  <https://orcid.org/0000-0001-7782-3468>

Artem A Venediktov  <https://orcid.org/0000-0002-5604-0461>

Nikolay K Shakhpazyan  <https://orcid.org/0000-0003-3386-7746>

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