Thromboinflammation in high-risk human populations

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CHAPTER 1

General introduction and outline of the thesis

1. Endothelium: a key regulator in inflammation and Coagulation

The biological systems of coagulation and inflammation are intricately intertwined and delicately balanced, with a great deal of crosstalk between each other. Whereby inflammation not only leads to coagulation activation, but coagulation also considerably affects inflammatory activity. When any one component is out of balance, the entire balance is thrown off, resulting in a wide range of disorders with varying degrees of excess inflammation and thrombosis.

The endothelium is the fundamental regulator in both systems. Quiescent endothelial cells reveal an anti-coagulant surface phenotype by repressing tissue factor (TF) expression and releasing anti-thrombogenic factors (e.g, TF pathway inhibitor). Endothelial cells become activated and rapidly shift in response to inflammation in either acute illnesses such as sepsis and COVID-19 or chronic inflammatory states such as diabetes and obesity, disrupting the hemostatic balance which could lead to a procoagulant state [1]. TF plays a critical role in procoagulant phenotype switching and endothelial dysfunction. In experimental *in vitro* situations, a wide range of inflammatory cytokines, including tumor necrosis factor (TNF)-α, IL-1, and IFN-I beta, together with C-reactive protein (CRP) and lipopolysaccharides (LPS) have been shown to increase TF expression in endothelial cells [2-5]. Such an increase in TF initiates the extrinsic coagulation pathway which allows complex forming with circulating factor VIIa, enhancing the catalytic activity of the latter and triggering coagulation by activating coagulation factor X [6, 7] and participating in the prothrombinase complex (FVa: FXa). The prothrombinase complex (FVa: FXa) activates prothrombin (PT) to thrombin. Once the generated thrombin concentration exceeds a threshold beyond physiological conditions, it leads to a pro-inflammatory state and triggers a wide spectrum of endothelial responses [8]. This includes the induction of adhesion molecules that facilitate leukocyte binding and transmigration, such as E-selectin, P-selectin, intracellular cell adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1), as well as the disruption of the endothelial barrier function, the release of proinflammatory cytokines and complement activation [9-13].

Additionally, previous studies showed that the TF-FVIIa complex could stimulate proteinase-activated receptor (PAR) signaling, which induces the release of inflammatory cytokines and chemokines [14].

Endothelial cells of all blood vessels are covered with an endothelial glycocalyx, a tight matrix of glycosaminoglycans, such as heparan sulfate (HS) and chondroitin sulfate (CS) anchored in the cell membrane by a protein backbone, together as proteoglycans, and intertwined with hyaluronan (HA), a glycosaminoglycan not covalently linked [15, 16]. The endothelial glycocalyx together with associated proteins, such as lipase, growth factors, and chemokines, forming a bioactive surface layer [17], and plays a critical role in maintaining vascular integrity and homeostasis, regulating endothelial mechanotransduction, vascular permeability, coagulation, and inflammation [18, 19].

Under physiological conditions, the endothelial glycocalyx composition inhibits blood coagulation. For example, 3-O sulfate modification of HS inhibits Factor Xa activity, and further blocks thrombin generation [20]; besides, other components such as syndecan-1 and hyaluronic acid also interfere with clot formation (inhibits both intrinsic and extrinsic pathway) and affect fibrin polymerization [21]. Tissue factor pathway inhibitor (TFPI) in turn, binds to endothelial cells via HS and as such prevents initiation of coagulation by blocking the actions of the FVIIa-TF complex. HS binding and sequestering protein anti-thrombin III (ATIII) to the cell surface is capable of inhibiting thrombin activity produced by the coagulation cascade, whereas thrombomodulin (TM), also bound to HS binds thrombin, inhibiting fibrin generation. Subsequently, thrombin-TM complexes activate protein C, and activated protein C (APC) inactivates coagulation factors Va and VIIIa, thereby inhibiting further thrombin generation [22]. Intracellular Weibel-Palade bodies (WPB) in endothelial cells store von Willebrand factor (vWF), a protein enhancing the interaction with platelets. HS chains are reported to act as a relevant binding factor for vWF fibers at the endothelial cell surface [23]. In addition to its anti-coagulation function, the endothelial glycocalyx is involved in inflammation. As HS compositional changes can induce binding and signaling in inflammatory conditions, numerous studies have shown

that HS, via its protein-binding properties, regulates inflammatory responses [24-27]. For example, CCL2 is a well-known proinflammatory chemokine, playing a critical role in macrophage recruitment and polarization during inflammation [28]. HS, particularly 3-O-sulfated HS domains, can interact with C-C Motif Chemokine Ligand 2 (CCL2), which further regulates vascular integrity and homeostasis through the CCL2/CCR2 signaling pathway [29, 30]. Also, N- and 6-O-sulfated HS domains were remarkably increased on glomerular endothelium under inflammatory conditions, enhancing leukocyte adhesion *in vitro* [31]. Finally, loss of HA could lead to impaired microvascular perfusion and endothelial stability via disturbed angiopoietin1- TEK receptor tyrosine kinase (TIE2) signaling, stabilizing VE-cadherin cell-cell interactions [32].

Impairment of the endothelial glycocalyx during various pathological conditions, therefore, can result in the initiation of both the inflammation and coagulation system which results in thromboinflammation. Thromboinflammation, the activating interplay of thrombosis and inflammation not only drives cardiovascular disease but also induces acute severe illness, such as sepsis, acute respiratory distress syndrome (ARDS), and disseminated intravascular coagulation (DIC). In the current thesis, I specifically will discuss the role of thromboinflammation in the participants of the Netherlands Epidemiology of Obesity (NEO) study and high-risk patient populations during the COVID-19 pandemic and South-Asian Surinamese participants with type 2 diabetes mellitus (T2DM).

2. Thromboinflammation and gender

Accumulating evidence shows that sex-specific pathophysiological mechanisms play a growing role in the development of cardiovascular disease (CVD). Thromboinflammation, as one of the risk factors for CVD, also exhibits certain sex differences.

C-reaction protein (CRP), the most studied inflammatory marker, is primarily released from the liver following cytokine stimulation [33]. CRP has been shown to predict cardiovascular events in both men and women independently [34, 35]. However, various groups have reported remarkable sex differences in CRP, in which the CRP concentration in women is much higher than in men [36-42]. The well-documented sex differences in body fat distribution and sex hormone concentrations might be key determinants in sex dimorphism [43, 44]. For instance, CRP concentration was found to be higher in premenopausal women than in men, owing to the increasing effect of estrogens [36]. Meanwhile, the use of oral contraceptive drugs and postmenopausal hormone replacement therapy was also able to raise CRP levels [45, 46]. CRP is tightly correlated with both subcutaneous and visceral adipose tissue. Postmenopausal and perimenopausal women have more ectopic fat accumulation (i.e., visceral adipose tissue) than premenopausal women, and women have more subcutaneous fat than men, which contributes to higher CRP concentrations in women [47].

Women not only appear to have higher plasma levels of inflammatory factors, but they also have higher fibrinogen plasma levels than men of the same age and ethnic group [48], pushing the balance from fibrinolysis to clotting [49]. Plasma fibrinogen was reported to be associated with morbidity and mortality in coronary heart disease (CHD) patients [50-52] and to the extent of coronary atherosclerosis [53]. Besides, the levels of coagulation factors II, VII, X, IX, XI, and XII are higher in women than in men [54]. These elevated coagulation factors have been associated with an increased risk of CHD in multiple studies [55, 56].

Sex differences in presence of inflammatory- and coagulation factors therefore might lead to differences in microvascular health and contribute to a different clinical presentation and outcome of CVD. In previous decades, men were thought to be more susceptible to coronary heart disease (CHD) than women [57]. However, the risk of CHD in women is frequently underestimated due to the under-recognition of CHD and distinct clinical presentations, which eventually when discovered resulted in a poor prognosis [58].

Therefore, it is important to study the significance of microvascular dysfunction in the pathophysiology of CHD and how this may differ by sex.

3. Thromboinflammation and diabetes

T2DM is characterized by insulin resistance and insufficient compensatory insulin secretion, the mechanism of which varies by ethnicity.

Thromboinflammation is commonly observed in patients with diabetes [59]. A previous epidemiological study in the general population demonstrated that increased levels of fasting glucose, HbA1c, and postprandial glucose response were associated with higher activity of FVIII, FIX, and FXI, and to some extent also with increased concentration of fibrinogen, which provided some evidence between hyperglycemia and coagulation [60]. Another study revealed that platelets of patients with diabetes had an increased capacity of mediating microvascular thrombosis and inflammation during ischemia-reperfusion injury [61].

Several mechanisms might be involved in the diabetes-associated thromboinflammation process. One mechanism is associated with platelet aggregation/activation, such as platelet-derived chemokine C-X- C motif ligand 14 (CXCL14). CXCL14 is one of the potential players in the development of thromboinflammation in diabetes expressing proinflammatory properties through its involvement in thrombus formation, platelet migration, and monocyte migration [62, 63]. Besides, platelets can interact with inflammatory cells by regulating lipids in a paracrine manner [64]. A recent study showed that the platelet atypical chemokine receptor 3 (ACKR3)/ CXC-chemokine receptor 7 (CXCR7) interaction is capable of favoring antiplatelet lipids over an atherothrombotic lipidome and regulating thromboinflammation [65].

Another mechanism at play in diabetes involves the endothelial glycocalyx in platelet adhesion to the endothelium of damaged vessels [66]. Our previous study showed that loss of endothelial hyaluronan, a key component of the extracellular matrix, could lead to disturbed glomerular endothelial stabilization [18]. Besides, the endothelial glycocalyx is perturbed upon treatment with human diabetic serum [67]. The impaired endothelial glycocalyx could lead to increased ICAM1 and decreased eNOS expression [68], which might contribute to platelet activation [69, 70], thus forming a procoagulant cell surface and further promoting thromboinflammation.

Increasing studies investigate the biological functions of high-density lipoproteins (HDL) in pathophysiological conditions including cholesterol efflux mediation, capacity of anti-oxidation, anti-inflammation, and anti-thrombosis [71, 72]. Given the causal relationship between HDL function and diabetes [73-76], it may yet be another novel mechanism causing thromboinflammation in subjects with diabetes. Numerous epidemiological studies have shown that low levels of plasma HDL cholesterol (HDL-C) were associated with an elevated risk of T2DM and cardiovascular disease [77-79]. Also, the function of HDL was associated with incidence of cardiovascular disease and prognosis of heart failure [80-82]. Interestingly, accumulation of symmetric dimethylarginine, a marker associated with diabetes, could lead to HDL dysfunction, switching to an endothelial-damaging phenotype, and then mediating glycocalyx breakdown [83].

4. Thromboinflammation and COVID-19

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection caused the coronavirus disease 2019 (COVID-19), and its worldwide spreading led to a global pandemic since late 2019 [84]. Although the majority of COVID-19 patients are asymptomatic or only showed mild symptoms [85], it is worth noting that part of the patients suffered from respiratory illness in the early disease stage, and rapidly progressed

into respiratory failure [86, 87]. Additionally, there was an increased incidence of thromboembolism events during hospitalization in the intensive care unit (ICU) [88]. ARDS and coagulopathy are major contributors to the mortality of COVID-19 [89], while endothelial dysfunction is reported to participate in both ARDS and coagulopathy [90, 91]. Severe pulmonary endothelial damage was observed in autopsy of COVID-19 non-survivors [92], therefore, COVID-19 could be regarded as an endothelial disease. Endothelial dysfunction with subsequent COVID-19-related thromboinflammation might play a vital role in the pathogenesis of ARDS and coagulopathy.

Hyperinflammation is involved in the severe illness of COVID-19 resulting in a cytokine storm regarding to increased levels of pro-inflammatory cytokines [85, 93-97]. Although the observed cytokine storm in COVID-19 patients was lower than those with non-COVID-19 severe cases with ARDS, sepsis, and influenza virus infection [98, 99], COVID-19 patients exhibited decreased innate antiviral defenses accompanied by exploded inflammatory cytokine production [100]. NLR family pyrin domain containing 3 (NLRP3) inflammasome was activated by SARS-CoV-2 in the lungs of patients who died from COVID-19-associated ARDS. *In vitro* studies on primary human monocytes revealed that SARS-CoV-2 infection activated the NLRP3 inflammasome [101, 102]. Furthermore, IL-1 and IL-18, products of NLRP3 inflammasome, are elevated in the COVID-19 patients with critical illness and are associated with poor clinical outcomes [102].

In addition to the hyperinflammation, SARS-CoV-2 infection could also lead to a prothrombotic state. Patients with severe COVID-19 were associated with endogenous activation of coagulation and fibrinolysis; but different from sepsis-induced hypercoagulant status, patients with COVID-19 showed a loss of coagulation-initiating mechanisms [103]. Moreover, emerging evidence demonstrated that the complement system had a role in the maladaptive immune response that promotes hyperinflammation and thrombotic microangiopathy, increasing COVID-19 mortality [104]. SARS-CoV-2 was found to trigger complement-mediated endothelial damage, cause deregulations of the coagulation cascade, and further result in adverse clinical presentations [105-107]. Despite

the indirect effect of the complement activation, COVID-19-associated coagulopathy is also suggested to be caused by endothelial dysfunction. Endothelial activation markers such as von Willebrand factor (vWF) and Angiopoietin 2 (ANG2) were elevated in patients with SARS-CoV-2 infection and associated with disease severity [108, 109]. In COVID-19 patients, elevated circulating ANG2 levels have been linked to decreased respiratory function, hypercoagulable status, acute kidney injury, and increased mortality [109-112]. ANG2 also was shown to mediate the endothelial glycocalyx breakdown [113], and the MYSTIC study revealed that glycocalyx health, as measured by perfused boundary region (PBR), an inversed reflection of endothelial glycocalyx layer, was a prognostic predictor for COVID-19 and disease severity [109].

Early studies of lipid metabolism in patients revealed a role for high-density lipoproteins (HDL) protective factors in a variety of endothelial functions such as antioxidant, anti-inflammatory, anti-thrombotic, and even anti-infectious properties [114, 115]. Thromboinflammation in COVID-19 might be also induced by HDL dysregulation. Recently, it was observed that the metabolic lipid profile in COVID-19 patients in the ICU was different when compared to healthy controls or patients with cardiogenic shock in ICU [116]. It is worth noting that increasing evidence suggested that low serum HDL-cholesterol (HDL-C) levels at hospital admission are associated with disease severity and mortality in COVID-19 [117, 118]. However, other studies revealed that the HDL lipidome and proteome rather than quantitative HDL-C concentration play a more representative role in HDL function during disease [80, 119]. In addition to HDL-C concentrations in COVID-19, several studies showed significant inflammatory remodeling of the HDL proteome, associated with COVID-19 disease severity in both adult and pediatric COVID-19 patients [119-121].

Outline of this thesis

In this thesis, we address the role of thromboinflammation in different high-risk populations, such as women versus men in the general Dutch population, type 2 diabetes mellitus, and COVID-19 patients.

Recent research reveals that microvascular dysfunction more commonly in women is a growing determinant of sex difference in coronary heart disease. In **Chapter 2**, we examined the sex differences in the relationship between microvascular health and coagulation parameters in a middle-aged Dutch population and revealed a hitherto unreported sex-specific association between microcirculatory health and procoagulant status, which suggests considering microvascular health in the early development of coronary heart disease in women.

HDL particles exhibit large heterogeneity in size, density, and composition. The composition of HDL can partly affect various functions including mediating cholesterol efflux, anti-oxidation, anti-inflammation, and anti-thrombotic processes, which is becoming an important determinant in the development of microvascular complications in T2DM. In **Chapter 3**, we used ^{1}H nuclear magnetic resonance (NMR) spectroscopy and Bruker IVDr Lipoprotein Subclass Analysis (B.I.LISA^{TM}) software to determine the changes in plasma HDL (both particle size and lipid composition) in healthy individuals (Dutch white Caucasian [DwC], Dutch South Asian [DSA]) and individuals with T2DM. We also investigated the role of HDL in anti-thrombotic capacity, determined as the ability to suppress TNF-α induced thrombin generation in endothelial cells *in vitro*.

Lipids play an essential role in both the intrinsic and extrinsic pathways of prothrombin activation and dyslipidemia is one of the hallmarks of T2DM. Oxidized lipids could contribute to thrombus initiation and growth in oxidative stress-induced cardiovascular diseases. Following the previous study, in **Chapter 4**, we used the LC/MS-based Lipidyzer^{TM} platform to measure the same participants mentioned above to study the biological

mechanisms underlying the link between dyslipidemia and T2DM in different ethnic groups.

Evidence of pulmonary microvascular thrombosis and inflammation were found on autopsy of COVID-19 non-survivors, leading to the increasing concern on thromboinflammation in the disease pathogenesis. In **Chapter 5**, we explored how serum factors affect vascular integrity in patients with severe COVID-19 (glycocalyx function, barrier function, and anti-coagulation capacity). Serum from COVID-19 patients in the ICU could induce endothelial dysfunction, characterized by endothelial glycocalyx degradation, endothelial barrier failure, and hypercoagulable status, which could be targeted earlier in the disease by supplementation of heparin sulfate mimetics. As HDL has both anti-inflammatory and anti-thrombotic capacity regarding thromboinflammation, therefore, in **Chapter 6**, we measured blood HDL subclasses and lipid content concentrations in longitudinally collected serum samples from ICU and non-ICU, together with age-matched healthy controls using ^{1}H NMR spectroscopy and the validated B.I.LISA^{TM} software to identify HDL composition concerning disease progression, and endothelial function, and investigate whether specific HDL compositional changes could lead to different outcomes in the course of the disease.

In **Chapter 7**, we summarize and discuss the observations in this thesis, as well as future perspectives.

References

1 Szotowski B, Antoniak S, Poller W, Schultheiss HP, Rauch U. Procoagulant soluble tissue factor is released from endothelial cells in response to inflammatory cytokines. *Circ Res*. 2005; **96**: 1233-9. 10.1161/01.RES.0000171805.24799.fa.

2 Eisenreich A, Bogdanov VY, Zakrzewicz A, Pries A, Antoniak S, Poller W, Schultheiss HP, Rauch U. Cdc2-like kinases and DNA topoisomerase I regulate alternative splicing of tissue factor in human endothelial cells. *Circ Res*. 2009; **104**: 589-99. 10.1161/CIRCRESAHA.108.183905.

3 Napoleone E, Di Santo A, Lorenzet R. Monocytes upregulate endothelial cell expression of tissue factor: a role for cell-cell contact and cross-talk. *Blood*. 1997; **89**: 541-9.

4 Cirillo P, Golino P, Calabro P, Cali G, Ragni M, De Rosa S, Cimmino G, Pacileo M, De Palma R, Forte L, Gargiulo A, Corigliano FG, Angri V, Spagnuolo R, Nitsch L, Chiariello M. C-reactive protein induces tissue factor expression and promotes smooth muscle and endothelial cell proliferation. *Cardiovasc Res*. 2005; **68**: 47-55. 10.1016/j.cardiores.2005.05.010.

5 Chen Y, Wang J, Yao Y, Yuan W, Kong M, Lin Y, Geng D, Nie R. CRP regulates the expression and activity of tissue factor as well as tissue factor pathway inhibitor via NF-kappaB and ERK 1/2 MAPK pathway. *FEBS Lett*. 2009; **583**: 2811-8. 10.1016/j.febslet.2009.07.037.

6 Morrissey JH. Tissue factor interactions with factor VII: measurement and clinical significance of factor VIIa in plasma. *Blood Coagul Fibrinolysis*. 1995; **6 Suppl 1**: S14-9.

7 Yuan L, Cheng S, Sol W, van der Velden AIM, Vink H, Rabelink TJ, van den Berg BM. Heparan sulfate mimetic fucoidan restores the endothelial glycocalyx and protects against dysfunction induced by serum of COVID-19 patients in the intensive care unit. *ERJ Open Res*. 2022; **8**. 10.1183/23120541.00652-2021.

8 Popovic M, Smiljanic K, Dobutovic B, Syrovets T, Simmet T, Isenovic ER. Thrombin and vascular inflammation. *Mol Cell Biochem*. 2012; **359**: 301-13. 10.1007/s11010-011-1024-x.

9 Cheng S, Wu D, Yuan L, Liu H, Ei-Seedi HR, Du M. Crassostrea gigas-Based Bioactive Peptide Protected Thrombin-Treated Endothelial Cells against Thrombosis and Cell Barrier Dysfunction. *J Agric Food Chem*. 2022; **70**: 9664-73. 10.1021/acs.jafc.2c02435.

10 Papadaki S, Sidiropoulou S, Moschonas IC, Tselepis AD. Factor Xa and thrombin induce endothelial progenitor cell activation. The effect of direct oral anticoagulants. *Platelets*. 2021; **32**: 807-14. 10.1080/09537104.2020.1802413.

11 Giri H, Srivastava AK, Naik UP. Apoptosis signal-regulating kinase-1 regulates thrombin-induced endothelial permeability. *Vascul Pharmacol*. 2022; **145**: 107088. 10.1016/j.vph.2022.107088.

12 Durigutto P, Macor P, Pozzi N, Agostinis C, Bossi F, Meroni PL, Grossi C, Borghi MO, Planer W, Garred P, Tedesco F. Complement Activation and Thrombin Generation by MBL Bound to beta2-Glycoprotein I. *J Immunol*. 2020; **205**: 1385-92. 10.4049/jimmunol.2000570.

13 Puy C, Pang J, Reitsma SE, Lorentz CU, Tucker EI, Gailani D, Gruber A, Lupu F, McCarty OJT. Cross-Talk between the Complement Pathway and the Contact Activation System of Coagulation: Activated Factor XI Neutralizes Complement Factor H. *J Immunol*. 2021; **206**: 1784-92. 10.4049/jimmunol.2000398.

14 Kondreddy V, Wang J, Keshava S, Esmon CT, Rao LVM, Pendurthi UR. Factor VIIa induces anti-inflammatory signaling via EPCR and PAR1. *Blood*. 2018; **131**: 2379-92. 10.1182/blood-2017-10-813527.

15 Boels MG, Lee DH, van den Berg BM, Dane MJ, van der Vlag J, Rabelink TJ. The endothelial glycocalyx as a potential modifier of the hemolytic uremic syndrome. *Eur J Intern Med*. 2013; **24**: 503-9. 10.1016/j.ejim.2012.12.016.

16 Wang G, Tiemeier GL, van den Berg BM, Rabelink TJ. Endothelial Glycocalyx Hyaluronan: Regulation and Role in Prevention of Diabetic Complications. *Am J Pathol*. 2020; **190**: 781-90. 10.1016/j.ajpath.2019.07.022.

17 Reitsma S, Slaaf DW, Vink H, van Zandvoort MA, oude Egbrink MG. The endothelial glycocalyx: composition, functions, and visualization. *Pflugers Arch*. 2007; **454**: 345-59. 10.1007/s00424-007-0212-8.

18 van den Berg BM, Wang G, Boels MGS, Avramut MC, Jansen E, Sol W, Lebrin F, van Zonneveld AJ, de Koning EJP, Vink H, Grone HJ, Carmeliet P, van der Vlag J, Rabelink TJ. Glomerular Function and Structural Integrity Depend on Hyaluronan Synthesis by Glomerular Endothelium. *J Am Soc Nephrol*. 2019; **30**: 1886-97. 10.1681/ASN.2019020192.

19 Rabelink TJ, van den Berg BM, Garsen M, Wang G, Elkin M, van der Vlag J. Heparanase: roles in cell survival, extracellular matrix remodelling and the development of kidney disease. *Nat Rev Nephrol*. 2017; **13**: 201-12. 10.1038/nrneph.2017.6.

20 Chopra P, Joshi A, Wu J, Lu W, Yadavalli T, Wolfert MA, Shukla D, Zaia J, Boons GJ. The 3-O-sulfation of heparan sulfate modulates protein binding and lyase degradation. *Proc Natl Acad Sci U S A*. 2021; **118**. 10.1073/pnas.2012935118.

21 Buchheim JI, Enzinger MC, Chouker A, Bruegel M, Holdt L, Rehm M. The stressed vascular barrier and coagulation - The impact of key glycocalyx components on in vitro clot formation. *Thromb Res*. 2020; **186**: 93-102. 10.1016/j.thromres.2019.12.015.

22 Ito T, Maruyama I. Thrombomodulin: protectorate God of the vasculature in thrombosis and inflammation. *J Thromb Haemost*. 2011; **9 Suppl 1**: 168-73. 10.1111/j.1538-7836.2011.04319.x.

23 Kalagara T, Moutsis T, Yang Y, Pappelbaum KI, Farken A, Cladder-Micus L, Vidal YSS, John A, Bauer AT, Moerschbacher BM, Schneider SW, Gorzelanny C. The endothelial glycocalyx anchors von Willebrand factor fibers to the vascular endothelium. *Blood Adv*. 2018; **2**: 2347-57. 10.1182/bloodadvances.2017013995.

24 Pum A, Ennemoser M, Gerlza T, Kungl AJ. The Role of Heparan Sulfate in CCL26-Induced Eosinophil Chemotaxis. *Int J Mol Sci*. 2022; **23**. 10.3390/ijms23126519.

25 Jain P, Shanthamurthy CD, Leviatan Ben-Arye S, Woods RJ, Kikkeri R, Padler-Karavani V. Discovery of rare sulfated N-unsubstituted glucosamine based heparan sulfate analogs selectively activating chemokines. *Chem Sci*. 2021; **12**: 3674-81. 10.1039/d0sc05862a.

26 Sawant KV, Sepuru KM, Lowry E, Penaranda B, Frevert CW, Garofalo RP, Rajarathnam K. Neutrophil recruitment by chemokines Cxcl1/KC and Cxcl2/MIP2: Role of Cxcr2 activation and glycosaminoglycan interactions. *J Leukoc Biol*. 2021; **109**: 777-91. 10.1002/JLB.3A0820-207R.

27 Vallet SD, Clerc O, Ricard-Blum S. Glycosaminoglycan-Protein Interactions: The First Draft of the Glycosaminoglycan Interactome. *J Histochem Cytochem*. 2021; **69**: 93-104. 10.1369/0022155420946403.

28 Dzenko KA, Song L, Ge S, Kuziel WA, Pachter JS. CCR2 expression by brain microvascular endothelial cells is critical for macrophage transendothelial migration in response to CCL2. *Microvasc Res*. 2005; **70**: 53-64. 10.1016/j.mvr.2005.04.005.

29 Winkler S, Derler R, Gesslbauer B, Krieger E, Kungl AJ. Molecular dynamics simulations of the chemokine CCL2 in complex with pull down-derived heparan sulfate hexasaccharides. *Biochim Biophys Acta Gen Subj*. 2019; **1863**: 528-33. 10.1016/j.bbagen.2018.12.014.

30 van Gemst JJ, Kouwenberg M, Rops A, van Kuppevelt TH, Berden JH, Rabelink TJ, Loeven MA, van der Vlag J. Differential binding of chemokines CXCL1, CXCL2 and CCL2 to mouse glomerular endothelial cells reveals specificity for distinct heparan sulfate domains. *PLoS One*. 2018; **13**: e0201560. 10.1371/journal.pone.0201560.

31 Rops AL, van den Hoven MJ, Baselmans MM, Lensen JF, Wijnhoven TJ, van den Heuvel LP, van Kuppevelt TH, Berden JH, van der Vlag J. Heparan sulfate domains on cultured activated glomerular endothelial cells mediate leukocyte trafficking. *Kidney Int*. 2008; **73**: 52-62. 10.1038/sj.ki.5002573.

32 Wang G, de Vries MR, Sol W, van Oeveren-Rietdijk AM, de Boer HC, van Zonneveld AJ, Quax PHA, Rabelink TJ, van den Berg BM. Loss of Endothelial Glycocalyx Hyaluronan Impairs Endothelial Stability and Adaptive Vascular Remodeling After Arterial Ischemia. *Cells*. 2020; **9**. 10.3390/cells9040824.

33 Baumann H, Gauldie J. Regulation of hepatic acute phase plasma protein genes by hepatocyte stimulating factors and other mediators of inflammation. *Mol Biol Med*. 1990; **7**: 147-59.

34 Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med*. 2000; **342**: 836-43. 10.1056/NEJM200003233421202.

35 Ballantyne CM, Hoogeveen RC, Bang H, Coresh J, Folsom AR, Heiss G, Sharrett AR. Lipoprotein-associated phospholipase A2, high-sensitivity C-reactive protein, and risk for incident coronary heart disease in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. *Circulation*. 2004; **109**: 837-42. 10.1161/01.CIR.0000116763.91992.F1.

36 Wener MH, Daum PR, McQuillan GM. The influence of age, sex, and race on the upper reference limit of serum C-reactive protein concentration. *J Rheumatol*. 2000; **27**: 2351-9.

37 Wang TJ, Nam BH, Wilson PW, Wolf PA, Levy D, Polak JF, D'Agostino RB, O'Donnell CJ. Association of C-reactive protein with carotid atherosclerosis in men and women: the Framingham Heart Study. *Arterioscler Thromb Vasc Biol*. 2002; **22**: 1662-7. 10.1161/01.atv.0000034543.78801.69.

38 McConnell JP, Branum EL, Ballman KV, Lagerstedt SA, Katzmann JA, Jaffe AS. Gender differences in C-reactive protein concentrations-confirmation with two sensitive methods. *Clin Chem Lab Med*. 2002; **40**: 56-9. 10.1515/CCLM.2002.011.

39 Wong ND, Pio J, Valencia R, Thakal G. Distribution of C-reactive protein and its relation to risk factors and coronary heart disease risk estimation in the National Health and Nutrition Examination Survey (NHANES) III. *Prev Cardiol*. 2001; **4**: 109-14. 10.1111/j.1520-037x.2001.00570.x.

40 Ford ES, Giles WH, Mokdad AH, Myers GL. Distribution and correlates of C-reactive protein concentrations among adult US women. *Clin Chem*. 2004; **50**: 574-81. 10.1373/clinchem.2003.027359.

41 Khera A, McGuire DK, Murphy SA, Stanek HG, Das SR, Vongpatanasin W, Wians FH, Jr., Grundy SM, de Lemos JA. Race and gender differences in C-reactive protein levels. *J Am Coll Cardiol*. 2005; **46**: 464-9. 10.1016/j.jacc.2005.04.051.

42 Lakoski SG, Cushman M, Criqui M, Rundek T, Blumenthal RS, D'Agostino RB, Jr., Herrington DM. Gender and C-reactive protein: data from the Multiethnic Study of Atherosclerosis (MESA) cohort. *Am Heart J*. 2006; **152**: 593-8. 10.1016/j.ahj.2006.02.015.

43 Kvist H, Chowdhury B, Grangard U, Tylen U, Sjostrom L. Total and visceral adipose-tissue volumes derived from measurements with computed tomography in adult men and women: predictive equations. *Am J Clin Nutr*. 1988; **48**: 1351-61. 10.1093/ajcn/48.6.1351.

44 Sjostrom L, Kvist H. Regional body fat measurements with CT-scan and evaluation of anthropometric predictions. *Acta Med Scand Suppl*. 1988; **723**: 169-77. 10.1111/j.0954-6820.1987.tb05941.x.

45 Rexrode KM, Pradhan A, Manson JE, Buring JE, Ridker PM. Relationship of total and abdominal adiposity with CRP and IL-6 in women. *Ann Epidemiol*. 2003; **13**: 674-82. 10.1016/s1047-2797(03)00053-x.

46 Ridker PM, Hennekens CH, Rifai N, Buring JE, Manson JE. Hormone replacement therapy and increased plasma concentration of C-reactive protein. *Circulation*. 1999; **100**: 713-6. 10.1161/01.cir.100.7.713.

47 Cartier A, Cote M, Lemieux I, Perusse L, Tremblay A, Bouchard C, Despres JP. Sex differences in inflammatory markers: what is the contribution of visceral adiposity? *Am J Clin Nutr*. 2009; **89**: 1307-14. 10.3945/ajcn.2008.27030.

48 Vorster HH. Fibrinogen and women's health. *Thromb Res*. 1999; **95**: 137-54. 10.1016/s0049-3848(99)00033-x.

49 Kim PY, Stewart RJ, Lipson SM, Nesheim ME. The relative kinetics of clotting and lysis provide a biochemical rationale for the correlation between elevated fibrinogen and cardiovascular disease. *J Thromb Haemost*. 2007; **5**: 1250-6. 10.1111/j.1538-7836.2007.02426.x.

50 Cortellaro M, Boschetti C, Cofrancesco E, Zanussi C, Catalano M, de Gaetano G, Gabrielli L, Lombardi B, Specchia G, Tavazzi L, et al. The PLAT Study: hemostatic function in relation to atherothrombotic ischemic events in vascular disease patients. Principal results. PLAT Study Group. Progetto Lombardo Atero-Trombosi (PLAT) Study Group. *Arterioscler Thromb*. 1992; **12**: 1063-70. 10.1161/01.atv.12.9.1063.

51 Thompson SG, Fechtrup C, Squire E, Heyse U, Breithardt G, van de Loo JC, Kienast J. Antithrombin III and fibrinogen as predictors of cardiac events in patients with angina pectoris. *Arterioscler Thromb Vasc Biol*. 1996; **16**: 357-62. 10.1161/01.atv.16.3.357.

52 Benderly M, Graff E, Reicher-Reiss H, Behar S, Brunner D, Goldbourt U. Fibrinogen is a predictor of mortality in coronary heart disease patients. The Bezafibrate Infarction Prevention (BIP) Study Group. *Arterioscler Thromb Vasc Biol*. 1996; **16**: 351-6. 10.1161/01.atv.16.3.351.

53 ECAT angina pectoris study: baseline associations of haemostatic factors with extent of coronary arteriosclerosis and other coronary risk factors in 3000 patients with angina pectoris undergoing coronary angiography. *Eur Heart J*. 1993; **14**: 8-17. 10.1093/eurheartj/14.1.8.

54 Favaloro EJ, Soltani S, McDonald J, Grezchnik E, Easton L. Cross-laboratory audit of normal reference ranges and assessment of ABO blood group, gender and age on detected levels of plasma coagulation factors. *Blood Coagul Fibrinolysis*. 2005; **16**: 597-605. 10.1097/01.mbc.0000187250.32630.56.

55 Olson NC, Cushman M, Judd SE, Kissela BM, Safford MM, Howard G, Zakai NA. Associations of coagulation factors IX and XI levels with incident coronary heart disease and ischemic stroke: the REGARDS study. *J Thromb Haemost*. 2017; **15**: 1086-94. 10.1111/jth.13698.

56 Zakai NA, Judd SE, Kissela B, Howard G, Safford MM, Cushman M. Factor VIII, Protein C and Cardiovascular Disease Risk: The REasons for Geographic and Racial Differences in Stroke Study (REGARDS). *Thromb Haemost*. 2018; **118**: 1305-15. 10.1055/s-0038-1655766.

57 Bots SH, Peters SAE, Woodward M. Sex differences in coronary heart disease and stroke mortality: a global assessment of the effect of ageing between 1980 and 2010. *BMJ Glob Health*. 2017; **2**: e000298. 10.1136/bmjgh-2017-000298.

58 Khamis RY, Ammari T, Mikhail GW. Gender differences in coronary heart disease. *Heart*. 2016; **102**: 1142-9. 10.1136/heartjnl-2014-306463.

59 Einarson TR, Acs A, Ludwig C, Panton UH. Prevalence of cardiovascular disease in type 2 diabetes: a systematic literature review of scientific evidence from across the world in 2007-2017. *Cardiovasc Diabetol*. 2018; **17**: 83. 10.1186/s12933-018-0728-6.

60 van der Toorn FA, de Mutsert R, Lijfering WM, Rosendaal FR, van Hylckama Vlieg A. Glucose metabolism affects coagulation factors: The NEO study. *J Thromb Haemost*. 2019; **17**: 1886-97. 10.1111/jth.14573.

61 Maiocchi S, Alwis I, Wu MCL, Yuan Y, Jackson SP. Thromboinflammatory Functions of Platelets in Ischemia-Reperfusion Injury and Its Dysregulation in Diabetes. *Semin Thromb Hemost*. 2018; **44**: 102-13. 10.1055/s-0037-1613694.

62 Witte A, Rohlfing AK, Dannenmann B, Dicenta V, Nasri M, Kolb K, Sudmann J, Castor T, Rath D, Borst O, Skokowa J, Gawaz M. The chemokine CXCL14 mediates platelet function and migration via direct interaction with CXCR4. *Cardiovasc Res*. 2021; **117**: 903-17. 10.1093/cvr/cvaa080.

63 Witte A, Chatterjee M, Lang F, Gawaz M. Platelets as a Novel Source of Pro-Inflammatory Chemokine CXCL14. *Cell Physiol Biochem*. 2017; **41**: 1684-96. 10.1159/000471821.

64 Manke MC, Ahrends R, Borst O. Platelet lipid metabolism in vascular thrombo-inflammation. *Pharmacol Ther*. 2022; **237**: 108258. 10.1016/j.pharmthera.2022.108258.

65 Cebo M, Dittrich K, Fu X, Manke MC, Emschermann F, Rheinlaender J, von Eysmondt H, Ferreiros N, Sudman J, Witte A, Pelzl L, Borst O, Geisler T, Rath D, Bakchoul T, Gawaz M, Schaffer TE, Lammerhofer M, Chatterjee M. Platelet ACKR3/CXCR7 favors antiplatelet lipids over an atherothrombotic lipidome and regulates thromboinflammation. *Blood*. 2022; **139**: 1722-42. 10.1182/blood.2021013097.

66 Kohli S, Shahzad K, Jouppila A, Holthofer H, Isermann B, Lassila R. Thrombosis and Inflammation-A Dynamic Interplay and the Role of Glycosaminoglycans and Activated Protein C. *Front Cardiovasc Med*. 2022; **9**: 866751. 10.3389/fcvm.2022.866751.

67 Boels MG, Avramut MC, Koudijs A, Dane MJ, Lee DH, van der Vlag J, Koster AJ, van Zonneveld AJ, van Faassen E, Grone HJ, van den Berg BM, Rabelink TJ. Atrasentan Reduces Albuminuria by Restoring the Glomerular Endothelial Glycocalyx Barrier in Diabetic Nephropathy. *Diabetes*. 2016; **65**: 2429-39. 10.2337/db15-1413.

68 Wang G, Kostidis S, Tiemeier GL, Sol W, de Vries MR, Giera M, Carmeliet P, van den Berg BM, Rabelink TJ. Shear Stress Regulation of Endothelial Glycocalyx Structure Is

Determined by Glucobiosynthesis. *Arterioscler Thromb Vasc Biol*. 2020; **40**: 350-64. 10.1161/ATVBAHA.119.313399.

69 Loscalzo J. Nitric oxide insufficiency, platelet activation, and arterial thrombosis. *Circ Res*. 2001; **88**: 756-62. 10.1161/hh0801.089861.

70 Kong J, Yao C, Dong S, Wu S, Xu Y, Li K, Ji L, Shen Q, Zhang Q, Zhan R, Cui H, Zhou C, Niu H, Li G, Sun W, Zheng L. ICAM-1 Activates Platelets and Promotes Endothelial Permeability through VE-Cadherin after Insufficient Radiofrequency Ablation. *Adv Sci (Weinh)*. 2021; **8**: 2002228. 10.1002/advs.202002228.

71 Mineo C, Shaul PW. Novel biological functions of high-density lipoprotein cholesterol. *Circ Res*. 2012; **111**: 1079-90. 10.1161/CIRCRESAHA.111.258673.

72 Jialal I, Jialal G, Adams-Huet B. The platelet to high density lipoprotein -cholesterol ratio is a valid biomarker of nascent metabolic syndrome. *Diabetes Metab Res Rev*. 2021; **37**: e3403. 10.1002/dmrr.3403.

73 Constantinou C, Karavia EA, Xepapadaki E, Petropoulou PI, Papakosta E, Karavyraki M, Zvintzou E, Theodoropoulos V, Filou S, Hatziri A, Kalogeropoulou C, Panayiotakopoulos G, Kypreos KE. Advances in high-density lipoprotein physiology: surprises, overturns, and promises. *Am J Physiol Endocrinol Metab*. 2016; **310**: E1-E14. 10.1152/ajpendo.00429.2015.

74 Xepapadaki E, Nikdima I, Sagiadinou EC, Zvintzou E, Kypreos KE. HDL and type 2 diabetes: the chicken or the egg? *Diabetologia*. 2021; **64**: 1917-26. 10.1007/s00125-021-05509-0.

75 Ebtehaj S, Gruppen EG, Parvizi M, Tietge UJF, Dullaart RPF. The anti-inflammatory function of HDL is impaired in type 2 diabetes: role of hyperglycemia, paraoxonase-1 and low grade inflammation. *Cardiovasc Diabetol*. 2017; **16**: 132. 10.1186/s12933-017-0613-8.

76 Heier M, Borja MS, Brunborg C, Seljeflot I, Margeirsdottir HD, Hanssen KF, Dahl-Jorgensen K, Oda MN. Reduced HDL function in children and young adults with type 1 diabetes. *Cardiovasc Diabetol*. 2017; **16**: 85. 10.1186/s12933-017-0570-2.

77 Schmidt MI, Duncan BB, Bang H, Pankow JS, Ballantyne CM, Golden SH, Folsom AR, Chambless LE, Atherosclerosis Risk in Communities I. Identifying individuals at high risk for diabetes: The Atherosclerosis Risk in Communities study. *Diabetes Care*. 2005; **28**: 2013-8. 10.2337/diacare.28.8.2013.

78 Wilson PW, Meigs JB, Sullivan L, Fox CS, Nathan DM, D'Agostino RB, Sr. Prediction of incident diabetes mellitus in middle-aged adults: the Framingham Offspring Study. *Arch Intern Med*. 2007; **167**: 1068-74. 10.1001/archinte.167.10.1068.

79 Sacks FM, Hermans MP, Fioretto P, Valensi P, Davis T, Horton E, Wanner C, Al-Rubeaan K, Aronson R, Barzon I, Bishop L, Bonora E, Bunnag P, Chuang LM, Deerochanawong C, Goldenberg R, Harshfield B, Hernandez C, Herzlinger-Botein S, Itoh H,

Jia W, Jiang YD, Kadowaki T, Laranjo N, Leiter L, Miwa T, Odawara M, Ohashi K, Ohno A, Pan C, Pan J, Pedro-Botet J, Reiner Z, Rotella CM, Simo R, Tanaka M, Tedeschi-Reiner E, Twum-Barima D, Zoppini G, Carey VJ. Association between plasma triglycerides and high-density lipoprotein cholesterol and microvascular kidney disease and retinopathy in type 2 diabetes mellitus: a global case-control study in 13 countries. *Circulation*. 2014; **129**: 999-1008. 10.1161/CIRCULATIONAHA.113.002529.

80 Jia C, Anderson JLC, Gruppen EG, Lei Y, Bakker SJL, Dullaart RPF, Tietge UJF. High-Density Lipoprotein Anti-Inflammatory Capacity and Incident Cardiovascular Events. *Circulation*. 2021; **143**: 1935-45. 10.1161/CIRCULATIONAHA.120.050808.

81 Ajala ON, Demler OV, Liu Y, Farukhi Z, Adelman SJ, Collins HL, Ridker PM, Rader DJ, Glynn RJ, Mora S. Anti-Inflammatory HDL Function, Incident Cardiovascular Events, and Mortality: A Secondary Analysis of the JUPITER Randomized Clinical Trial. *J Am Heart Assoc*. 2020; **9**: e016507. 10.1161/JAHA.119.016507.

82 Emmens JE, Jia C, Ng LL, van Veldhuisen DJ, Dickstein K, Anker SD, Lang CC, Filippatos G, Cleland JGF, Metra M, Voors AA, de Boer RA, Tietge UJF. Impaired High-Density Lipoprotein Function in Patients With Heart Failure. *J Am Heart Assoc*. 2021; **10**: e019123. 10.1161/JAHA.120.019123.

83 Hesse B, Rovas A, Buscher K, Kusche-Vihrog K, Brand M, Di Marco GS, Kielstein JT, Pavenstadt H, Linke WA, Nofer JR, Kumpers P, Lukasz A. Symmetric dimethylarginine in dysfunctional high-density lipoprotein mediates endothelial glycocalyx breakdown in chronic kidney disease. *Kidney Int*. 2020; **97**: 502-15. 10.1016/j.kint.2019.10.017.

84 Cascella M, Rajnik M, Aleem A, Dulebohn SC, Di Napoli R. Features, Evaluation, and Treatment of Coronavirus (COVID-19). *StatPearls*. Treasure Island (FL), 2022.

85 Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020; **395**: 497-506. 10.1016/S0140-6736(20)30183-5.

86 Ferrando C, Suarez-Sipmann F, Mellado-Artigas R, Hernandez M, Gea A, Arruti E, Aldecoa C, Martinez-Palli G, Martinez-Gonzalez MA, Slutsky AS, Villar J, Network C-SI. Clinical features, ventilatory management, and outcome of ARDS caused by COVID-19 are similar to other causes of ARDS. *Intensive Care Med*. 2020; **46**: 2200-11. 10.1007/s00134-020-06192-2.

87 Grasselli G, Zangrillo A, Zanella A, Antonelli M, Cabrini L, Castelli A, Cereda D, Coluccello A, Foti G, Fumagalli R, Iotti G, Latronico N, Lorini L, Merler S, Natalini G, Piatti A, Ranieri MV, Scandroglio AM, Storti E, Cecconi M, Pesenti A, Network C-LI. Baseline Characteristics and Outcomes of 1591 Patients Infected With SARS-CoV-2 Admitted to ICUs of the Lombardy Region, Italy. *JAMA*. 2020; **323**: 1574-81. 10.1001/jama.2020.5394.

88 Cui S, Chen S, Li X, Liu S, Wang F. Prevalence of venous thromboembolism in patients with severe novel coronavirus pneumonia. *J Thromb Haemost*. 2020; **18**: 1421-4. 10.1111/jth.14830.

89 Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, Guan L, Wei Y, Li H, Wu X, Xu J, Tu S, Zhang Y, Chen H, Cao B. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. 2020; **395**: 1054-62. 10.1016/S0140-6736(20)30566-3.

90 Vassiliou AG, Kotanidou A, Dimopoulou I, Orfanos SE. Endothelial Damage in Acute Respiratory Distress Syndrome. *Int J Mol Sci*. 2020; **21**. 10.3390/ijms21228793.

91 Vallet B, Wiel E. Endothelial cell dysfunction and coagulation. *Crit Care Med*. 2001; **29**: S36-41. 10.1097/00003246-200107001-00015.

92 Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, Vanstapel A, Werlein C, Stark H, Tzankov A, Li WW, Li VW, Mentzer SJ, Jonigk D. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. *N Engl J Med*. 2020; **383**: 120-8. 10.1056/NEJMoa2015432.

93 Bonaventura A, Vecchie A, Wang TS, Lee E, Cremer PC, Carey B, Rajendram P, Hudock KM, Korbee L, Van Tassell BW, Dagna L, Abbate A. Targeting GM-CSF in COVID-19 Pneumonia: Rationale and Strategies. *Front Immunol*. 2020; **11**: 1625. 10.3389/fimmu.2020.01625.

94 Buckley LF, Wohlford GF, Ting C, Alahmed A, Van Tassell BW, Abbate A, Devlin JW, Libby P. Role for Anti-Cytokine Therapies in Severe Coronavirus Disease 2019. *Crit Care Explor*. 2020; **2**: e0178. 10.1097/CCE.0000000000000178.

95 Li J, Gong X, Wang Z, Chen R, Li T, Zeng D, Li M. Clinical features of familial clustering in patients infected with 2019 novel coronavirus in Wuhan, China. *Virus Res*. 2020; **286**: 198043. 10.1016/j.virusres.2020.198043.

96 Vecchie A, Bonaventura A, Toldo S, Dagna L, Dinarello CA, Abbate A. IL-18 and infections: Is there a role for targeted therapies? *J Cell Physiol*. 2021; **236**: 1638-57. 10.1002/jcp.30008.

97 Zanza C, Romenskaya T, Manetti AC, Franceschi F, La Russa R, Bertozzi G, Maiese A, Savioli G, Volonnino G, Longhitano Y. Cytokine Storm in COVID-19: Immunopathogenesis and Therapy. *Medicina (Kaunas)*. 2022; **58**. 10.3390/medicina58020144.

98 Kox M, Waalders NJB, Kooistra EJ, Gerretsen J, Pickkers P. Cytokine Levels in Critically Ill Patients With COVID-19 and Other Conditions. *JAMA*. 2020; **324**: 1565-7. 10.1001/jama.2020.17052.

99 Leisman DE, Ronner L, Pinotti R, Taylor MD, Sinha P, Calfee CS, Hirayama AV, Mastroiani F, Turtle CJ, Harhay MO, Legrand M, Deutschman CS. Cytokine elevation in severe and critical COVID-19: a rapid systematic review, meta-analysis, and comparison

with other inflammatory syndromes. *Lancet Respir Med*. 2020; **8**: 1233-44. 10.1016/S2213-2600(20)30404-5.

100 Blanco-Melo D, Nilsson-Payant BE, Liu WC, Uhl S, Hoagland D, Moller R, Jordan TX, Oishi K, Panis M, Sachs D, Wang TT, Schwartz RE, Lim JK, Albrecht RA, tenOever BR. Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. *Cell*. 2020; **181**: 1036-45 e9. 10.1016/j.cell.2020.04.026.

101 Toldo S, Bussani R, Nuzzi V, Bonaventura A, Mauro AG, Cannata A, Pillappa R, Sinagra G, Nana-Sinkam P, Sime P, Abbate A. Inflammasome formation in the lungs of patients with fatal COVID-19. *Inflamm Res*. 2021; **70**: 7-10. 10.1007/s00011-020-01413-2.

102 Rodrigues TS, de Sa KSG, Ishimoto AY, Becerra A, Oliveira S, Almeida L, Goncalves AV, Perucello DB, Andrade WA, Castro R, Veras FP, Toller-Kawahisa JE, Nascimento DC, de Lima MHF, Silva CMS, Caetite DB, Martins RB, Castro IA, Pontelli MC, de Barros FC, do Amaral NB, Giannini MC, Bonjorno LP, Lopes MIF, Santana RC, Vilar FC, Auxiliadora-Martins M, Luppino-Assad R, de Almeida SCL, de Oliveira FR, Batah SS, Siyuan L, Benatti MN, Cunha TM, Alves-Filho JC, Cunha FQ, Cunha LD, Frantz FG, Kohlsdorf T, Fabro AT, Arruda E, de Oliveira RDR, Louzada-Junior P, Zamboni DS. Inflammasomes are activated in response to SARS-CoV-2 infection and are associated with COVID-19 severity in patients. *J Exp Med*. 2021; **218**. 10.1084/jem.20201707.

103 Bouck EG, Denorme F, Holle LA, Middelton EA, Blair AM, de Laat B, Schiffman JD, Yost CC, Rondina MT, Wolberg AS, Campbell RA. COVID-19 and Sepsis Are Associated With Different Abnormalities in Plasma Procoagulant and Fibrinolytic Activity. *Arterioscler Thromb Vasc Biol*. 2021; **41**: 401-14. 10.1161/ATVBAHA.120.315338.

104 Skendros P, Mitsios A, Chrysanthopoulou A, Mastellos DC, Metallidis S, Rafailidis P, Ntinopoulou M, Sertaridou E, Tsironidou V, Tsigalou C, Tektonidou M, Konstantinidis T, Papagoras C, Mitroulis I, Germanidis G, Lambris JD, Ritis K. Complement and tissue factor-enriched neutrophil extracellular traps are key drivers in COVID-19 immunothrombosis. *J Clin Invest*. 2020; **130**: 6151-7. 10.1172/JCI141374.

105 Yu J, Yuan X, Chen H, Chaturvedi S, Braunstein EM, Brodsky RA. Direct activation of the alternative complement pathway by SARS-CoV-2 spike proteins is blocked by factor D inhibition. *Blood*. 2020; **136**: 2080-9. 10.1182/blood.2020008248.

106 Yu J, Gerber GF, Chen H, Yuan X, Chaturvedi S, Braunstein EM, Brodsky RA. Complement dysregulation is associated with severe COVID-19 illness. *Haematologica*. 2022; **107**: 1095-105. 10.3324/haematol.2021.279155.

107 Aiello S, Gastoldi S, Galbusera M, Ruggenenti P, Portalupi V, Rota S, Rubis N, Liguori L, Conti S, Tironi M, Gamba S, Santarsiero D, Benigni A, Remuzzi G, Noris M. C5a and C5aR1 are key drivers of microvascular platelet aggregation in clinical entities spanning from aHUS to COVID-19. *Blood Adv*. 2022; **6**: 866-81. 10.1182/bloodadvances.2021005246.

108 Schmaier AA, Pajares Hurtado GM, Manickas-Hill ZJ, Sack KD, Chen SM, Bhambhani V, Quadir J, Nath AK, Collier AY, Ngo D, Barouch DH, Shapiro NI, Gerszten RE, Yu XG, Collection MC-, Processing T, Peters KG, Flaumenhaft R, Parikh SM. Tie2 activation protects against prothrombotic endothelial dysfunction in COVID-19. *JCI Insight*. 2021; **6**. 10.1172/jci.insight.151527.

109 Rovas A, Osiaevi I, Buscher K, Sackarnd J, Tepasse PR, Fobker M, Kuhn J, Braune S, Gobel U, Tholking G, Groschel A, Pavenstadt H, Vink H, Kumpers P. Microvascular dysfunction in COVID-19: the MYSTIC study. *Angiogenesis*. 2021; **24**: 145-57. 10.1007/s10456-020-09753-7.

110 Vassiliou AG, Keskinidou C, Jahaj E, Gallos P, Dimopoulou I, Kotanidou A, Orfanos SE. ICU Admission Levels of Endothelial Biomarkers as Predictors of Mortality in Critically Ill COVID-19 Patients. *Cells*. 2021; **10**. 10.3390/cells10010186.

111 Henry BM, de Oliveira MHS, Cheruiyot I, Benoit JL, Cooper DS, Lippi G, Le Cras TD, Benoit SW. Circulating level of Angiopoietin-2 is associated with acute kidney injury in coronavirus disease 2019 (COVID-19). *Angiogenesis*. 2021; **24**: 403-6. 10.1007/s10456-021-09782-w.

112 Smadja DM, Guerin CL, Chocron R, Yatim N, Boussier J, Gendron N, Khider L, Hadjadj J, Goudot G, Debuc B, Juvin P, Hauw-Berlemont C, Augy JL, Peron N, Messas E, Planquette B, Sanchez O, Charbit B, Gaussem P, Duffy D, Terrier B, Mirault T, Diehl JL. Angiopoietin-2 as a marker of endothelial activation is a good predictor factor for intensive care unit admission of COVID-19 patients. *Angiogenesis*. 2020; **23**: 611-20. 10.1007/s10456-020-09730-0.

113 Lukasz A, Hillgruber C, Oberleithner H, Kusche-Vihrog K, Pavenstadt H, Rovas A, Hesse B, Goerge T, Kumpers P. Endothelial glycocalyx breakdown is mediated by angiopoietin-2. *Cardiovasc Res*. 2017; **113**: 671-80. 10.1093/cvr/cvx023.

114 Tanaka S, Couret D, Tran-Dinh A, Duranteau J, Montravers P, Schwendeman A, Meilhac O. High-density lipoproteins during sepsis: from bench to bedside. *Crit Care*. 2020; **24**: 134. 10.1186/s13054-020-02860-3.

115 Tran-Dinh A, Diallo D, Delbosc S, Varela-Perez LM, Dang QB, Lapergue B, Burillo E, Michel JB, Levoye A, Martin-Ventura JL, Meilhac O. HDL and endothelial protection. *Br J Pharmacol*. 2013; **169**: 493-511. 10.1111/bph.12174.

116 Schmelter F, Foh B, Mallagaray A, Rahmoller J, Ehlers M, Lehrian S, von Kopylow V, Kunsting I, Lixenfeld AS, Martin E, Ragab M, Meyer-Saraei R, Kreutzmann F, Eitel I, Taube S, Kading N, Jantzen E, Graf T, Sina C, Gunther UL. Metabolic and Lipidomic Markers Differentiate COVID-19 From Non-Hospitalized and Other Intensive Care Patients. *Front Mol Biosci*. 2021; **8**: 737039. 10.3389/fmolb.2021.737039.

117 Mahat RK, Rathore V, Singh N, Singh N, Singh SK, Shah RK, Garg C. Lipid profile as an indicator of COVID-19 severity: A systematic review and meta-analysis. *Clin Nutr ESPEN*. 2021; **45**: 91-101. 10.1016/j.clnesp.2021.07.023.

118 Chidambaram V, Shanmugavel Geetha H, Kumar A, Majella MG, Sivakumar RK, Voruganti D, Mehta JL, Karakousis PC. Association of Lipid Levels With COVID-19 Infection, Disease Severity and Mortality: A Systematic Review and Meta-Analysis. *Front Cardiovasc Med*. 2022; **9**: 862999. 10.3389/fcvm.2022.862999.

119 Souza Junior DR, Silva ARM, Rosa-Fernandes L, Reis LR, Alexandria G, Bhosale SD, Ghilardi FR, Dalcoquio TF, Bertolin AJ, Nicolau JC, Marinho CRF, Wrenger C, Larsen MR, Siciliano RF, Di Mascio P, Palmisano G, Ronsein GE. HDL proteome remodeling associates with COVID-19 severity. *J Clin Lipidol*. 2021; **15**: 796-804. 10.1016/j.jacl.2021.10.005.

120 Stadler JT, Mangge H, Rani A, Curcic P, Herrmann M, Pruller F, Marsche G. Low HDL Cholesterol Efflux Capacity Indicates a Fatal Course of COVID-19. *Antioxidants (Basel)*. 2022; **11**. 10.3390/antiox11101858.

121 Mietus-Snyder M, Suslovic W, Delaney M, Playford MP, Ballout RA, Barber JR, Otvos JD, DeBiasi RL, Mehta NN, Remaley AT. Changes in HDL cholesterol, particles, and function associate with pediatric COVID-19 severity. *Front Cardiovasc Med*. 2022; **9**: 1033660. 10.3389/fcvm.2022.1033660.