Thromboinflammation in high-risk human populations

Yuan, L.

CHAPTER 7

Summary, general discussion,and future perspectives

Thromboinflammation, which is a main trigger for endothelial dysfunction, has emerged as a new term to describe the interconnection between the simultaneous activation of the coagulation pathway and immune response. In the present thesis, the role of thromboinflammation in different high-risk populations, such as women vs. men in the general Dutch population, patients with T2DM from different ethnicities, and COVID-19 patients are highlighted.

Thromboinflammation, gender differences, and cardiovascular disease

Thromboinflammation exhibits sex differences, contributing to the risk of cardiovascular disease. Endothelial glycocalyx, a key regulator of thromboinflammation, is also involved in various vascular complications.

In the present thesis, in **Chapter 2**, we observed an association between early (pre-clinical) microvascular health changes measured by SDF imaging and coagulation factor activation and discovered a striking sex difference in microvascular health, in which women showed a perturbed endothelial glycocalyx concomitant with a more procoagulable endothelial surface, and this association was not observed in men. Based on previous studies [1-3], specifically in women, an increase in endothelial glycocalyx is associated with an increased risk of CHD. These findings highlight the importance of sex differences in microcirculatory perturbation in CHD, suggesting the potential clinical utility of monitoring microcirculatory change specifically in women to prevent the development of CHD. In line with these findings are earlier studies revealing that women who were suspected of or had confirmed clinical ischaemic heart disease have less atherosclerosis than men and a lower prevalence of obstructive coronary artery disease (CAD), especially when they are young [4]. Additionally, more than 50% of women with angina tested negative for coronary angiography, further indicating sex differences in CHD development [5]. Therefore, based

on these previous findings, accumulating evidence shows that perturbation of the microcirculation is more evident to contribute to CHD in women [6].

Perturbation of the endothelial glycocalyx could lead to procoagulant status [7], and together with previous studies demonstrating that the underlying systemic presence of a hypercoagulable state was associated with the incidence of CHD [8-10], hints toward an association between endothelial glycocalyx health and CHD which is mediated by coagulation activation. From the Tromsø Study, a case-cohort design with 1495 participants included, revealed that Syndean-4, one of the core proteins of endothelial glycocalyx, was associated with myocardial infarction incidence. The observed association was also stronger in women than men, indicating the link between endothelial glycocalyx and CHD as well as gender differences [11]. Interestingly, such a sex-dependent association between CHD and impaired sublingual microvascular glycocalyx barrier function in women was also found in a study by Brands et al. [12].

Thromboinflammation, diabetes in different ethnicities, and HDL function and dyslipidemia

HDL exhibits functions including anti-inflammatory and anti-thrombotic capacities, which can regulate thromboinflammation in pathophysiological conditions [13]. In chronic situations such as CHD, diabetes, and chronic kidney disease, HDL could lose its protective function and even gain adverse functionality further exacerbating the disease progression [14-17].

The mechanism of T2DM disease progression varies in different ethnicities [18] and recent studies showed that changes in HDL function may be one of the underlying mechanisms resulting in disease development and progression. Given that reduction of low-density lipoproteins (LDL) by statin use is associated with insulin resistance and an increased risk

of hyperglycemic complications in T2DM, particularly HDL functionality, may therefore be a better candidate to monitor diabetes progression [19-21].

A study comparing HDL function in Dutch South Asians to Dutch white Europeans within three age groups (neonates, adolescents, and adults), reported that in the absence of T2DM, only the ability of HDL to prevent LDL oxidation was decreased in overweight Dutch South Asians when compared to obese Dutch white Caucasians [22]. Another single-center study in Rotterdam assessing the relation between the development of dyslipidemia and glucose intolerance found that small HDL fractions were associated with insulin resistance and beta-cell dysfunction in South Asian families at risk of T2DM [15].

In **Chapter 3**, we compared HDL composition in healthy individuals and patients with T2DM of Dutch South Asian and Dutch white Caucasian ethnicity. Here we observed that between both ethnic groups, HDL composition differed. The impaired HDL functionality found in Dutch South Asians with T2DM might be because of loss of the smallest HDL subfractions; while in Dutch white Caucasians with T2DM, increased triglyceride content in the smallest HDL fraction contributes to HDL dysfunction. In line with these findings, small and dense HDL particles have been shown to play a vital role in multiple functions, such as cellular cholesterol efflux mediation, anti-oxidative, anti-thrombotic, anti-inflammatory, and anti-apoptotic capacities [16]. In our current study, we found that concentrations of the smallest HDL subfractions except triglyceride content were negatively associated with disease duration in Dutch South Asians with T2DM rather than in Dutch white Caucasian, suggesting a more vulnerable HDL composition phenotype. Moreover, T2DM patients in both ethnic groups showed significantly reduced anti-thrombotic capacity due to impaired HDL function. Consistent with an earlier finding, impaired HDL function in T2DM patients in terms of the capacity to suppress TNF-induced vascular cell adhesion molecule-1 (VCAM-1) expression in endothelial cells *in vitro* was observed [23]. According to our findings, HDL dysfunction induced thromboinflammation deregulation might contribute to a higher risk of disease development and progression in South Asians with T2DM.

In oxidative stress-induced cardiovascular disease, oxidized lipids can activate platelets via CD36, demonstrating a link between dysregulated lipoprotein metabolism, oxidative stress, and thrombus formation [24]. Lipids are involved in both the intrinsic and extrinsic pathways of prothrombin activation and dyslipidemia is one of the hallmarks of T2DM. In **Chapter 4**, using a novel targeted quantitative LC/MS-based Shotgun Lipidomics Assistant (SLA) platform, we generated a comprehensive mapping of the circulating lipidome in Dutch South Asians and Dutch white Caucasians with or without T2DM. Based on lipidomics phenotyping, we observed detailed insight into the complexity of lipid metabolism and the interindividual variations within the various ethnic groups. It is worth noting that there were distinct differences in the lipidome mainly related to the metabolism of cholesteryl esters (CEs), diacylglycerides (DGs), phosphatidylethanolamines (PEs), sphingomyelins (SMs), and triglycerides (TGs) between T2DM and healthy controls in our study population, which were consistent with previous findings observed in the case-cohort study nested within the PREDIMED trial [25] and the longitudinal METSIM study [26]. However, with conflicting results as reported in these two studies based on Chinese populations that FFA, SM, and LPC lipid species were higher in Chinese with T2DM, whereas we found opposite results in Dutch with T2DM, the high variability in lipidomics profile between ethnicities is further indicated.

Thromboinflammation and diabetes-related complications

Lipoprotein and lipid metabolism in patients with T2DM could lead to the deregulation of thromboinflammation and further affect the development or exacerbation of diabetes-related complications. Several studies discovered that dyslipidemia was linked to an increased risk of diabetes-related microvascular complications such as neuropathy and retinopathy [27-29]. South Asians have a higher prevalence of diabetic retinopathy than white Caucasians and a shorter aggravation time, and the diabetic retinopathy lesions [30] tend to be distributed more centrally [31]. In **Chapter 3**, we specifically observed that

lower ApoA2 and HDL-4 subclass concentrations in Dutch South Asian individuals with T2DM were associated with higher odds of having diabetes-related pan-microvascular complications such as retinopathy and neuropathy.

In addition to diabetic neuropathy and retinopathy, South Asian patients with T2DM are more likely to develop microvascular complications such as diabetic nephropathy, with a higher incidence of micro- and macroalbuminuria in South Asians as compared to white European Caucasians with T2DM and a faster progression to end-stage renal disease [32, 33]. This could be because these patients have a higher burden of systemic and glomerular inflammation. Additionally, South Asians have a unique body composition, with a more abdominally obese phenotype and a high percentage of visceral fat, which is dominant in the production and secretion of certain inflammatory cytokines, that contribute to the chronic low-grade inflammatory state [34]. Meanwhile, in **Chapter 4**, we identified a unique lipid species, DG, which was associated with diabetic nephropathy and renal functions. The DG- protein kinase C (PKC)/ protein kinase D (PKD) signaling network could regulate redox balance and induce oxidative stress [35]. In T2DM, DG concentration increases and the accumulation of DG further activates the PKC/PKD, thus resulting in diabetic nephropathy [36]. It was notable that DG 18:1_18:2 was higher in diabetic nephropathy, particularly in South Asians with T2DM, and had the most correlations with various clinical parameters. Even in an external cohort of Chinese subjects with IgA nephropathy, the correlations with renal function persisted. Future studies concerning diabetic nephropathy in multiple ethnic groups with large sample sizes are needed to verify our findings.

Thromboinflammation and COVID-19

Hyperinflammation is a hallmark of COVID-19, and the inflammatory response to SARS-CoV-2 infection frequently causes remarkable activation of the coagulation cascade. The

subsequent process called thromboinflammation is characterized by systemic endothelial damage and loss of proper anti-coagulant properties [37, 38].

Hyperinflammation induced by COVID-19 could disrupt vascular integrity. The glycocalyx is capable of regulating endothelial cell integrity and homeostasis through vascular barrier permeability protective function, anti-inflammation, and anti-coagulation capacity. Buijsers et al. observed that the activity of endothelial glycocalyx-degrading enzyme heparanase, which was involved in vascular leakage and inflammation, was increased in COVID-19 patients and was associated with disease severity [39]. Additionally, Kümpers et al. revealed that non-anticoagulant heparin fragments could inhibit the activity of heparanase and prevent endothelial glycocalyx injury in response to COVID-19 serum [40]. Meanwhile, 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 [41]. In **Chapter 5**, we showed that loss of endothelial glycocalyx in response to serum from ICU COVID-19 patients induced endothelial dysfunction through increased IL-6, ICAM1, ANG2, and HPSE1 gene expression and activation of the NF-κB signaling pathway. In addition, damaged endothelial glycocalyx resulted in vascular leakage and disturbed cell-cell contact.

Inhibition of the protective ANG1/TIE2 signaling cascade by ANG2 is a central regulator in protecting the vasculature against thrombus formation and vascular stabilization [42, 43]. A number of studies indicated that circulating ANG2 levels were increased in COVID-19 and associated with a worse prognosis [44-46]. In line with these findings, we also found that ANG2 levels were increased in ICU COVID-19 patients (**Chapter 5**). The presence of endothelial glycocalyx is required for anti-coagulation property and maintenance of endothelial quiescence. We also showed that loss of endothelial glycocalyx in response to serum from ICU COVID-19 patients could form a pro-coagulant cell surface through increased tissue factor expression and increased secretion of ANG2 and vWF (**Chapter 5**). To further validate our hypothesis that glycocalyx damage contributed to thromboinflammation in COVID-19, we tested whether preservation of the endothelial

glycocalyx might be an effective intervention to improve vascular health. We found that the heparan sulfate mimetic fucoidan could restore endothelial functionality including restoration of the endothelial glycocalyx, ameliorating endothelial activation, and leading to protection of the endothelial barrier function and induced anti-thrombotic effects (**Chapter 5**).

Michalick et al. discovered that plasma mediators in patients with severe COVID-19 could lead to lung endothelial barrier failure [47]. Meanwhile, Stahl et al. showed that blood composition in critically ill COVID-19 patients could cause endothelial injury involving glycocalyx integrity loss and vascular destabilization [48]. A number of cytokines and chemokines were increased in COVID-19 patients; however, the observed cytokine storm in COVID-19 patients was lower than observed in non-COVID-19 severe cases with ARDS, sepsis, and influenza virus infection [49, 50]. The discrepancy suggests that other mediators such as oxidized lipids or dysfunctional lipoproteins might contribute to COVID-19 pathogenesis. Given that COVID-19, in the end, is an endothelial disease, in **Chapter 6**, we fulfill the missing puzzle of blood mediators leading to endothelial dysfunction in COVID-19. We found that increased levels of HDL triglyceride content and decreased levels of the smallest HDL subclass were associated with endothelial dysfunction and COVID-19 disease severity. Besides, dysfunctional HDL in COVID-19 patients had less anti-thrombotic capacity than healthy individuals, suggesting a higher risk of thromboinflammation. These findings were also in line with our previous observations that a disturbed endothelial glycocalyx in COVID-19 patients resulted in the formation of a pro-coagulant cell surface (**Chapter 5**).

Future perspectives and open questions

In this thesis, we emphasize thromboinflammation in high-risk populations such as females, South Asians, T2DM, and COVID-19 patients. We also show that perturbation of endothelial glycocalyx, dysfunction of HDL, and dysregulation of lipid metabolism can lead to thromboinflammation, which all can play a critical role in acute or chronic disease development.

In **Chapter 2**, we showed that glycocalyx function together with pro-coagulation factors could contribute to early CHD development in women. However, we still lack a detailed mechanism and follow-up information on non-obstructive CHD, which needs to be investigated. In **Chapter 3**, we showed the HDL compositional and functional changes in South Asians with T2DM. HDL function depended on both lipidome and proteome. Therefore, specific determination of the proteome in isolated HDL particles is the next step in unraveling the underlying mechanisms in HDL functional changes. In **Chapter 4**, we found a specific lipid that only appeared in renal diseases (diabetic nephropathy and IgA nephropathy). However, the concentration of this lipid is quite low, necessitating a more precise and in-depth measurement and analysis method on a renal disease cohort with a larger sample size. In **Chapter 5** and **Chapter 6**, we only compared healthy individuals with ICU COVID-19 patients; however, whether our findings were specifically characteristic of COVID-19 is still unknown, emphasizing the need for the inclusion of non-COVID-19 ICU patient samples.

We showed a reduction of HDL subfractions in diabetes and COVID-19. However, it is still unknown whether supplementation of HDL mimetic or HDL-raising agents could be a potential therapeutic strategy for COVID-19, diabetes, and diabetes-related complications, which needs to be explored further.

References

1 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.

2 Fibrinogen Studies C, Danesh J, Lewington S, Thompson SG, Lowe GD, Collins R, Kostis JB, Wilson AC, Folsom AR, Wu K, Benderly M, Goldbourt U, Willeit J, Kiechl S, Yarnell JW, Sweetnam PM, Elwood PC, Cushman M, Psaty BM, Tracy RP, Tybjaerg-Hansen A, Haverkate F, de Maat MP, Fowkes FG, Lee AJ, Smith FB, Salomaa V, Harald K, Rasi R, Vahtera E, Jousilahti P, Pekkanen J, D'Agostino R, Kannel WB, Wilson PW, Tofler G, Arocha-Pinango CL, Rodriguez-Larralde A, Nagy E, Mijares M, Espinosa R, Rodriquez-Roa E, Ryder E, Diez-Ewald MP, Campos G, Fernandez V, Torres E, Marchioli R, Valagussa F, Rosengren A, Wilhelmsen L, Lappas G, Eriksson H, Cremer P, Nagel D, Curb JD, Rodriguez B, Yano K, Salonen JT, Nyyssonen K, Tuomainen TP, Hedblad B, Lind P, Loewel H, Koenig W, Meade TW, Cooper JA, De Stavola B, Knottenbelt C, Miller GJ, Cooper JA, Bauer KA, Rosenberg RD, Sato S, Kitamura A, Naito Y, Palosuo T, Ducimetiere P, Amouyel P, Arveiler D, Evans AE, Ferrieres J, Juhan-Vague I, Bingham A, Schulte H, Assmann G, Cantin B, Lamarche B, Despres JP, Dagenais GR, Tunstall-Pedoe H, Woodward M, Ben-Shlomo Y, Davey Smith G, Palmieri V, Yeh JL, Rudnicka A, Ridker P, Rodeghiero F, Tosetto A, Shepherd J, Ford I, Robertson M, Brunner E, Shipley M, Feskens EJ, Kromhout D, Dickinson A, Ireland B, Juzwishin K, Kaptoge S, Lewington S, Memon A, Sarwar N, Walker M, Wheeler J, White I, Wood A. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis. *JAMA*. 2005; **294**: 1799-809. 10.1001/jama.294.14.1799.

3 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.

4 Reynolds HR, Bairey Merz CN, Berry C, Samuel R, Saw J, Smilowitz NR, de Souza A, Sykes R, Taqueti VR, Wei J. Coronary Arterial Function and Disease in Women With No Obstructive Coronary Arteries. *Circ Res*. 2022; **130**: 529-51. 10.1161/CIRCRESAHA.121.319892.

5 Lee BK, Lim HS, Fearon WF, Yong AS, Yamada R, Tanaka S, Lee DP, Yeung AC, Tremmel JA. Invasive evaluation of patients with angina in the absence of obstructive coronary artery disease. *Circulation*. 2015; **131**: 1054-60. 10.1161/CIRCULATIONAHA.114.012636.

6 Pries AR, Reglin B. Coronary microcirculatory pathophysiology: can we afford it to remain a black box? *Eur Heart J*. 2017; **38**: 478-88. 10.1093/eurheartj/ehv760.

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 Song CJ, Nakagomi A, Chandar S, Cai H, Lim IG, McNeil HP, Freedman SB, Geczy CL. C-reactive protein contributes to the hypercoagulable state in coronary artery disease. *J Thromb Haemost*. 2006; **4**: 98-106. 10.1111/j.1538-7836.2005.01705.x.

9 Tantry US, Bliden KP, Suarez TA, Kreutz RP, Dichiara J, Gurbel PA. Hypercoagulability, platelet function, inflammation and coronary artery disease acuity: results of the Thrombotic RIsk Progression (TRIP) study. *Platelets*. 2010; **21**: 360-7. 10.3109/09537100903548903.

10 Bratseth V, Pettersen AA, Opstad TB, Arnesen H, Seljeflot I. Markers of hypercoagulability in CAD patients. Effects of single aspirin and clopidogrel treatment. *Thromb J*. 2012; **10**: 12. 10.1186/1477-9560-10-12.

11 Solbu MD, Kolset SO, Jenssen TG, Wilsgaard T, Lochen ML, Mathiesen EB, Melsom T, Eriksen BO, Reine TM. Gender differences in the association of syndecan-4 with myocardial infarction: The population-based Tromso Study. *Atherosclerosis*. 2018; **278**: 166-73. 10.1016/j.atherosclerosis.2018.08.005.

12 Brands J, Hubel CA, Althouse A, Reis SE, Pacella JJ. Noninvasive sublingual microvascular imaging reveals sex-specific reduction in glycocalyx barrier properties in patients with coronary artery disease. *Physiol Rep*. 2020; **8**: e14351. 10.14814/phy2.14351.

13 von Eckardstein A, Nordestgaard BG, Remaley AT, Catapano AL. High-density lipoprotein revisited: biological functions and clinical relevance. *Eur Heart J*. 2022. 10.1093/eurheartj/ehac605.

14 Li Y, Zhao M, He D, Zhao X, Zhang W, Wei L, Huang E, Ji L, Zhang M, Willard B, Fu Z, Wang L, Pan B, Zheng L, Ji L. HDL in diabetic nephropathy has less effect in endothelial repairing than diabetes without complications. *Lipids Health Dis*. 2016; **15**: 76. 10.1186/s12944-016-0246-z.

15 Yahya R, Jainandunsing S, Rashid M, van der Zee L, Touw A, de Rooij FWM, Sijbrands EJG, Verhoeven AJM, Mulder MT. HDL associates with insulin resistance and beta-cell dysfunction in South Asian families at risk of type 2 diabetes. *J Diabetes Complications*. 2021; **35**: 107993. 10.1016/j.jdiacomp.2021.107993.

16 He Y, Ronsein GE, Tang C, Jarvik GP, Davidson WS, Kothari V, Song HD, Segrest JP, Bornfeldt KE, Heinecke JW. Diabetes Impairs Cellular Cholesterol Efflux From ABCA1 to Small HDL Particles. *Circ Res*. 2020; **127**: 1198-210. 10.1161/CIRCRESAHA.120.317178.

17 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.

18 Sattar N, Gill JM. Type 2 diabetes in migrant south Asians: mechanisms, mitigation, and management. *Lancet Diabetes Endocrinol*. 2015; **3**: 1004-16. 10.1016/S2213-8587(15)00326-5.

19 Mansi IA, Chansard M, Lingvay I, Zhang S, Halm EA, Alvarez CA. Association of Statin Therapy Initiation With Diabetes Progression: A Retrospective Matched-Cohort Study. *JAMA Intern Med*. 2021; **181**: 1562-74. 10.1001/jamainternmed.2021.5714.

20 Liew SM, Lee PY, Hanafi NS, Ng CJ, Wong SS, Chia YC, Lai PS, Zaidi NF, Khoo EM. Statins use is associated with poorer glycaemic control in a cohort of hypertensive patients with diabetes and without diabetes. *Diabetol Metab Syndr*. 2014; **6**: 53. 10.1186/1758-5996-6-53.

21 Sukhija R, Prayaga S, Marashdeh M, Bursac Z, Kakar P, Bansal D, Sachdeva R, Kesan SH, Mehta JL. Effect of statins on fasting plasma glucose in diabetic and nondiabetic patients. *J Investig Med*. 2009; **57**: 495-9. 10.2310/JIM.0b013e318197ec8b.

22 Bakker LE, Boon MR, Annema W, Dikkers A, van Eyk HJ, Verhoeven A, Mayboroda OA, Jukema JW, Havekes LM, Meinders AE, Willems van Dijk K, Jazet IM, Tietge UJ, Rensen PC. HDL functionality in South Asians as compared to white Caucasians. *Nutr Metab Cardiovasc Dis*. 2016; **26**: 697-705. 10.1016/j.numecd.2016.02.010.

23 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.

24 Jackson SP, Calkin AC. The clot thickens--oxidized lipids and thrombosis. *Nat Med*. 2007; **13**: 1015-6. 10.1038/nm0907-1015.

25 Razquin C, Toledo E, Clish CB, Ruiz-Canela M, Dennis C, Corella D, Papandreou C, Ros E, Estruch R, Guasch-Ferre M, Gomez-Gracia E, Fito M, Yu E, Lapetra J, Wang D, Romaguera D, Liang L, Alonso-Gomez A, Deik A, Bullo M, Serra-Majem L, Salas-Salvado J, Hu FB, Martinez-Gonzalez MA. Plasma Lipidomic Profiling and Risk of Type 2 Diabetes in the PREDIMED Trial. *Diabetes Care*. 2018; **41**: 2617-24. 10.2337/dc18-0840.

26 Suvitaival T, Bondia-Pons I, Yetukuri L, Poho P, Nolan JJ, Hyotylainen T, Kuusisto J, Oresic M. Lipidome as a predictive tool in progression to type 2 diabetes in Finnish men. *Metabolism*. 2018; **78**: 1-12. 10.1016/j.metabol.2017.08.014.

27 Rao H, Jalali JA, Johnston TP, Koulen P. Emerging Roles of Dyslipidemia and Hyperglycemia in Diabetic Retinopathy: Molecular Mechanisms and Clinical Perspectives. *Front Endocrinol (Lausanne)*. 2021; **12**: 620045. 10.3389/fendo.2021.620045.

28 Hukportie DN, Li FR, Zhou R, Zheng JZ, Wu XX, Zou MC, Wu XB. Lipid variability and risk of microvascular complications in Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial: A post hoc analysis. *J Diabetes*. 2022; **14**: 365-76. 10.1111/1753-0407.13273.

29 Narindrarangkura P, Bosl W, Rangsin R, Hatthachote P. Prevalence of dyslipidemia associated with complications in diabetic patients: a nationwide study in Thailand. *Lipids Health Dis*. 2019; **18**: 90. 10.1186/s12944-019-1034-3.

30 van Niel J, Geelhoed-Duijvestijn P, Numans ME, Kharagjitsing AV, Vos RC. Type 2 diabetes in South Asians compared to Europeans: Higher risk and earlier development of major cardiovascular events irrespective of the presence and degree of retinopathy. Results from The HinDu The Hague Diabetes Study. *Endocrinol Diabetes Metab*. 2021; **4**: e00242. 10.1002/edm2.242.

31 He Y, Verma A, Nittala MG, Velaga SB, Esmaeilkhanian H, Li X, Su L, Li X, Jayadev C, Tsui I, Prasad P, Sadda SR. Ethnic Variation in Diabetic Retinopathy Lesion Distribution on Ultra-widefield Imaging. *Am J Ophthalmol*. 2022; **247**: 61-9. 10.1016/j.ajo.2022.10.023.

32 Chandie Shaw PK, Vandenbroucke JP, Tjandra YI, Rosendaal FR, Rosman JB, Geerlings W, de Charro FT, van Es LA. Increased end-stage diabetic nephropathy in Indo-Asian immigrants living in the Netherlands. *Diabetologia*. 2002; **45**: 337-41. 10.1007/s00125-001-0758-5.

33 Chandie Shaw PK, Baboe F, van Es LA, van der Vijver JC, van de Ree MA, de Jonge N, Rabelink TJ. South-Asian type 2 diabetic patients have higher incidence and faster progression of renal disease compared with Dutch-European diabetic patients. *Diabetes Care*. 2006; **29**: 1383-5. 10.2337/dc06-0003.

34 Bakker LE, Sleddering MA, Schoones JW, Meinders AE, Jazet IM. Pathogenesis of type 2 diabetes in South Asians. *Eur J Endocrinol*. 2013; **169**: R99-R114. 10.1530/EJE-13-0307.

35 Wang QJ. PKD at the crossroads of DAG and PKC signaling. *Trends Pharmacol Sci*. 2006; **27**: 317-23. 10.1016/j.tips.2006.04.003.

36 Noh H, King GL. The role of protein kinase C activation in diabetic nephropathy. *Kidney Int Suppl*. 2007: S49-53. 10.1038/sj.ki.5002386.

37 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.

38 Connors JM, Levy JH. Thromboinflammation and the hypercoagulability of COVID-19. *J Thromb Haemost*. 2020; **18**: 1559-61. 10.1111/jth.14849.

39 Buijsers B, Yanginlar C, de Nooijer A, Grondman I, Maciej-Hulme ML, Jonkman I, Janssen NAF, Rother N, de Graaf M, Pickkers P, Kox M, Joosten LAB, Nijenhuis T, Netea MG, Hilbrands L, van de Veerdonk FL, Duivenvoorden R, de Mast Q, van der Vlag J. Increased Plasma Heparanase Activity in COVID-19 Patients. *Front Immunol*. 2020; **11**: 575047. 10.3389/fimmu.2020.575047.

40 Drost CC, Rovas A, Osiaevi I, Rauen M, van der Vlag J, Buijsers B, Salmenov R, Lukasz A, Pavenstadt H, Linke WA, Kumpers P. Heparanase Is a Putative Mediator of Endothelial Glycocalyx Damage in COVID-19 - A Proof-of-Concept Study. *Front Immunol*. 2022; **13**: 916512. 10.3389/fimmu.2022.916512.

41 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.

42 Lovric S, Lukasz A, Hafer C, Kielstein JT, Haubitz M, Haller H, Kumpers P. Removal of elevated circulating angiopoietin-2 by plasma exchange--a pilot study in critically ill patients with thrombotic microangiopathy and anti-glomerular basement membrane disease. *Thromb Haemost*. 2010; **104**: 1038-43. 10.1160/TH10-02-0138.

43 Higgins SJ, De Ceunynck K, Kellum JA, Chen X, Gu X, Chaudhry SA, Schulman S, Libermann TA, Lu S, Shapiro NI, Christiani DC, Flaumenhaft R, Parikh SM. Tie2 protects the vasculature against thrombus formation in systemic inflammation. *J Clin Invest*. 2018; **128**: 1471-84. 10.1172/JCI97488.

44 Price DR, Benedetti E, Hoffman KL, Gomez-Escobar L, Alvarez-Mulett S, Capili A, Sarwath H, Parkhurst CN, Lafond E, Weidman K, Ravishankar A, Cheong JG, Batra R, Buyukozkan M, Chetnik K, Easthausen I, Schenck EJ, Racanelli AC, Outtz Reed H, Laurence J, Josefowicz SZ, Lief L, Choi ME, Schmidt F, Borczuk AC, Choi AMK, Krumsiek J, Rafii S. Angiopoietin 2 Is Associated with Vascular Necroptosis Induction in Coronavirus Disease 2019 Acute Respiratory Distress Syndrome. *Am J Pathol*. 2022; **192**: 1001-15. 10.1016/j.ajpath.2022.04.002.

45 Maldonado F, Morales D, Diaz-Papapietro C, Valdes C, Fernandez C, Valls N, Lazo M, Espinoza C, Gonzalez R, Gutierrez R, Jara A, Romero C, Cerda O, Caceres M. Relationship Between Endothelial and Angiogenesis Biomarkers Envisage Mortality in a Prospective Cohort of COVID-19 Patients Requiring Respiratory Support. *Front Med (Lausanne)*. 2022; **9**: 826218. 10.3389/fmed.2022.826218.

46 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.

47 Michalick L, Weidenfeld S, Grimmer B, Fatykhova D, Solymosi PD, Behrens F, Dohmen M, Brack MC, Schulz S, Thomasch E, Simmons S, Muller-Redetzky H, Suttorp N, Kurth F, Neudecker J, Toennies M, Bauer TT, Eggeling S, Corman VM, Hocke AC, Witzenrath M, Hippenstiel S, Kuebler WM. Plasma mediators in patients with severe COVID-19 cause lung endothelial barrier failure. *Eur Respir J*. 2021; **57**. 10.1183/13993003.02384-2020.

48 Stahl K, Gronski PA, Kiyan Y, Seeliger B, Bertram A, Pape T, Welte T, Hoeper MM, Haller H, David S. Injury to the Endothelial Glycocalyx in Critically Ill Patients with COVID-19. *Am J Respir Crit Care Med*. 2020; **202**: 1178-81. 10.1164/rccm.202007-2676LE.

49 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.

50 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.