

# The Pathogenesis of Vascular Disease

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The prevalence of vascular disease has increased in both the United States and Europe since the end of World War II. The pathogenesis of vascular disease has been directly linked to changes in dietary habits and lifestyle practices and the discovery of penicillin by Sir Alexander Fleming in 1928, which led to a reduction in deaths secondary to bacterial infections. Multiple theories have evolved regarding the various factors associated with an increased risk of vascular disease. It is important to realize, however, that the study of the pathogenesis and subsequent treatment of vascular disease requires a "bigger picture" approach rather than consideration of just one or two factors. In this chapter, we review the contributions made by many investigators who have looked at one or more of these issues. We discuss the relationship (Fleming's Unified Theory of Vascular Disease<sup>1</sup>) between these factors (Figure 64.1) and their overall role in the pathogenesis of vascular disease, including coronary artery disease, carotid artery disease, and peripheral vascular disease. We also review the importance and benefit of looking at each of these contributing factors when evaluating and treating an individual with vascular disease.

## Sources of Endothelial Injury

The initiation of vascular disease begins with injury to the endothelial wall. This process can begin as soon as stretching of the endothelium occurs, which is while the child is within the mother's uterus and blood is pulsing through the arteries and veins. This pulsation of blood is necessary for survival, but it initiates the stretching of endothelial cells and the potentiation for injury. Clearly the human organism is designed to deal with this phenomenon or it would be incompatible with life itself. It is also clear from human history that longevity is not re-

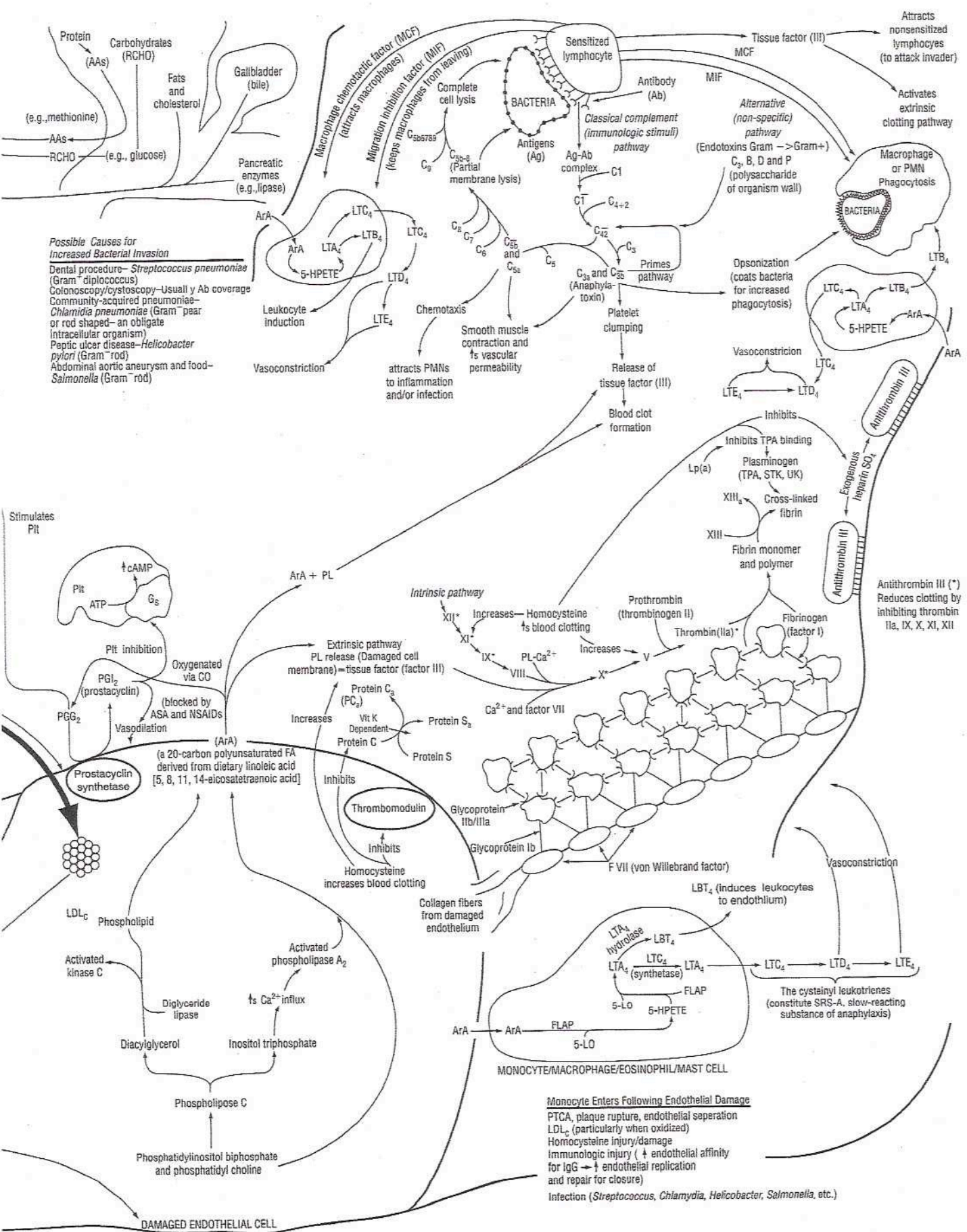
lated to medical science, in that Muhammad lived for 62 years, Gandhi for 79 years, Buddha for 80 years, and Methuselah for 969 years.

It is now clear that endothelial injury can occur from many causes. Such injuries can be caused by rupture of endothelial plaques after the formation of foam cells. This endothelial rupture may or may not expose collagen or other connective tissue, which can then precipitate the clotting cascade that is discussed later. Other causes include trauma to the blood vessel, free radical (e.g., oxygen) formation, bacterial invasion of the vessel, and formation of a thrombus, with subsequent activation of growth factors, inflammatory mediators, and vasoconstrictors and potential lumen occlusion.

Once the endothelial cells are damaged, the phospholipoproteins that are released include phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) and phosphatidylcholine (PC). Both undergo a series of changes and ultimately become a 20-carbon polyunsaturated fat known as arachidonic acid (AA), which is then released from the endothelial cell. Regardless of the cause of endothelial injury, the overall process is as shown in Figures 64.1 and 64.2.

The presence of fatty streaks, which has been documented in children<sup>2</sup> as young as 10 years, gives way to fibroproliferative infiltration by smooth muscle cells from the media. The mechanism for fibroproliferative infiltration is the release of such substances as platelet-derived growth factor (PDGF) and will be discussed later. Once the endothelium is injured or denuded, there is an increased receptiveness to immunoglobulin G and a vasospastic response to 5-hydroxytryptamine in the presence of thromboxane A<sub>2</sub> (discussed later). The vasodilative response to 5-hydroxytryptamine is inhibited in the presence of dysfunctional endothelium. The increased uptake of immunoglobulin G<sup>3</sup> is correlated with an increased replication of endothelium necessary to repair and replace damaged endothelium.





**Monocyte Enters Following Endothelial Damage**

- PTCA, plaque rupture, endothelial separation
- LDL<sub>c</sub> (particularly when oxidized)
- Homocysteine injury/damage
- Immunologic injury (↑ endothelial affinity for IgG → ↑ endothelial replication and repair for closure)
- Infection (*Streptococcus*, *Chlamydia*, *Helicobacter*, *Salmonella*, etc.)



## Dietary Factors Involved in Vascular Disease: The First Group of Factors

Numerous factors must be considered when looking at the dietary changes<sup>4-6</sup> required to reduce lipids. These changes include five major issues. The first is *caloric consumption*. It is now known that rats that consume twice the recommended number of calories necessary for survival demonstrate a decreased lifespan, with greater health problems, whereas rats that consume 70% of the recommended number of calories live twice as long as the average rat, with fewer health problems. There is no reason to believe this is different for humans.

Regardless of whether consumed calories begin as protein, carbohydrate, fat, or alcohol, excess caloric consumption is stored primarily as triglycerides or fats. This has been shown in the Tarahumara Indians, who have virtually no coronary artery disease despite relatively low levels of high-density lipoprotein cholesterol. When the Tarahumarans were placed on hypercaloric diets, they demonstrated a significant increase in plasma lipids<sup>7</sup> as well as weight. These fats eventually accumulate by way of production of very-low-density lipoprotein cholesterol and, subsequently, low-density lipoprotein cholesterol (LDLc), which is deposited in the subendothelial region of blood vessels. This is an excellent source of fatty acids, which are the primary energy source for organs such as the heart; however, excess accumulation results in monocyte and macrophage phagocytization and the initial development of fatty streaks.

Monocytes exit the lumina of blood vessels and become tissue macrophages that engulf the LDLc in an effort to remove the extracellular material. Eventually this phagocytized LDLc results in the macrophages' becoming foam cells. These macrophages release a number of chemical mediators, including PDGF, which causes vasoconstriction of the blood vessel. PDGF is in fact a more potent vasoconstrictor than angiotensin II. Also released is basic fibroblastic growth factor (bFGF), which enhances fibroblast formation in the medial layer and migration into the subendothelial region; this results in the more advanced stages of atherosclerotic disease (namely, fibroproliferation). The release of basic fibroblastic growth factor<sup>8</sup> from arteries after trauma or intervention (e.g., catheter-induced deendothelialization) increases intimal smooth muscle proliferation along with restenosis. Additionally, TGF $\beta$  is released, which also promotes fibroproliferation.

Probably more important than any other factor is the role of excessive dietary fat, particularly the *saturated fats*. It has been shown that dietary fat consumption is extremely important for two reasons: (1) fat has 9 calories per gram and (2) saturated fats, which pervade the diets

in most industrialized countries today, is atherogenic. The residents of these countries are plagued with vascular disease, cancer, and other related health problems. Regardless of the study considered, successful attempts at reducing cholesterol levels have required simultaneous reductions in dietary saturated fat intake. However, some quantity of dietary fat is necessary (essential fatty acids) for the healthy survival of the human species. The essential fatty acids are polyunsaturated fats (fatty acids) and include linoleic acid (9,12-octadecadienoic), linolenic acid (9,12,15-octadecatrienoic), and AA (5,8,11,14-eicosatetraenoic). AA can be synthesized in the body from linolenic acid. Studies in the 1970s and 1980s saw people lowering fat intake to less than 10% of their total caloric intake. When fat consumption is less than 8% of the daily total caloric intake, significant health issues arise, including immunologic problems.

In the 1940s and 1950s, the ability to preserve food increased as a result of our ability to hydrogenate food. This process of saturating a fat (*trans-fat*) was good for extending the shelf life of food, but not the "shelf life" of people. Saturated fats are particularly problematic, and even a single high-fat meal<sup>9</sup> has been shown to inhibit normal endothelial function by way of oxidative injury. The overall goal, therefore, is not to eliminate all fat from the diet but to reduce the amount of saturated fat and reduce the percentage of calories consumed as fat to approximately 15% of the total caloric intake, assuming the correct amount of calories<sup>4-6</sup> is being eaten daily.

The third dietary factor is *cholesterol intake*. Obviously, the ingestion of cholesterol is of concern because of the relationship between dietary cholesterol and very-low-density lipoprotein cholesterol production by the liver. There is also a relationship<sup>10</sup> between cholesterol levels, heart disease, and systolic blood pressure. However, the major contributing factor would appear to be not the amount of cholesterol in the diet but the amount of saturated fats found in foods that are also relatively high in cholesterol content. Of the cholesterol consumed daily, approximately 10% is absorbed across the gastrointestinal tract. Because the daily American diet includes an average of 250 to 500 mg of cholesterol, 25 to 50 mg are absorbed daily. However, the liver makes an average of 1000 mg of cholesterol daily. This explains why changes in dietary cholesterol intake *alone* may or may not be associated with changes in serum cholesterol levels and, subsequently, with the severity of atherosclerotic disease. This was first emphasized by Ancel Keys, who discussed the benefits of the Mediterranean diet. Dietary changes that do not take into account caloric and saturated fat intake, in addition to cholesterol, may demonstrate initial improvements but are unable to maintain control or reversal of vascular disease. This is the principal problem behind vegetarian diets, when *both* caloric and fat intake are not controlled.

A fourth dietary concern is protein and subsequently *homocysteine*, which is discussed in the next section as an independent risk factor. It is important to note here, however, that elevations in homocysteine result in increased phagocytosis of LDLc and LDLc-homocysteine-thiolactone complexes. This complex results in an increased oxidative load and further endothelial and subendothelial injury.

Finally, the role of *antioxidants* must be taken into consideration. Whether the oxygen free radicals (OFRs) are produced by way of damaged endothelium, excessive homocysteine, or responses to inflammatory or infectious agents, the effect of OFRs is the same. These extremely toxic compounds cause endothelial damage and dysfunction, resulting in vasoconstriction in addition to fibroblastic proliferation, increased phagocytosis of LDLc by macrophages, and further endothelial damage. Therefore, the ingestion of vitamins C and E, carotenoids such as beta carotene and lycopene, flavonoids (found in grape juice), selenium, flaxseed, or soy protein and the use of medications such as nitroglycerin have a positive (e.g., vasodilative) effect by reducing the levels of OFRs.<sup>9,11</sup> Other vitamins such as vitamin K must also be considered because of the effect on the extrinsic clotting pathway, particularly in individuals who take warfarin or related medications.

## The Role of Homocysteine: The Second Group of Factors

Homocysteine has been recognized as a risk factor<sup>12-16</sup> for vascular disease ever since researchers looked at the prevalence of elevated homocysteine levels in younger individuals who were thought to have premature coronary artery disease. The enzymatic pathway of homocysteine shows it to be a metabolic product of the essential dietary amino acid methionine. Some endothelial cells have been shown<sup>17</sup> to have less cystathionine  $\beta$ -synthase activity, which could potentially increase their risk of endothelial damage. Excessive accumulation of homocysteine results in several vascular responses. The first is an overwhelming of the endothelial tissue's ability to detoxify the OFRs generated from the homocysteine. The OFRs not only use up the nitrous oxide that would have a vasodilative effect but actually cause endothelial dysfunction and vasoconstriction.

The OFRs also stimulate fibroblast<sup>18,19</sup> and smooth muscle proliferation and migration to the subendothelial region, thereby enhancing the progression of vascular disease. Homocysteine is also metabolized to homocysteine thiolactone,<sup>20,21</sup> which increases the harmful acylate process and oxidative process of LDLc, thereby increasing phagocytosis of LDLc and LDLc-homocysteine-

thiolactone complexes by macrophages with the subsequent development of foam cells and fatty streaks.

Homocysteine increases the formation of thrombi and blood clots by interfering with heparin sulfate's activation of antithrombin III and decreasing antithrombin III levels. Homocysteine has also been shown to inhibit thrombomodulin expression, induce the expression of tissue factor, and reduce the binding of tissue plasminogen activator (TPA) to its endothelial receptor. Homocysteine also increases factors XI and V, decreases protein C activation, and increases the expression of thromboxane A<sub>2</sub> (TxA<sub>2</sub>). Homocysteine subsequently promotes a hypercoagulable state that has been shown to increase the risk for multiple vascular problems,<sup>22-26</sup> including myocardial infarction, cerebrovascular accidents, deep venous thrombosis, and peripheral vascular disease, particularly when coupled with stenotic lesions<sup>27,28</sup> where changes in entry (*a*) and exit (*w*) angles to and from a narrowed vessel will further promote thrombus formation.

## The Role of Hyperfibrinogenemia and Hypercoagulability: The Third Group of Factors

Damage to the endothelium of a blood vessel may occur from rupture of a plaque, cracking, fissuring, de-endothelialization (e.g., angioplasty), stretching of endothelial cells (advanced atherosclerosis), inflammatory or immunologic reactions (e.g., lupus anticoagulant), or surgical interventions. Once this has occurred with membrane phospholipid release (phosphatidylinositol 4,5-biphosphate and phosphatidyl choline), the connective tissue components are exposed. Paramount in this process is the release of AA and the exposure of collagen and von Willebrand's factor necessary for initiation of thrombus formation. However, as mentioned previously,<sup>27</sup> narrowed entry (*a*) and exit (*w*) angles also promote thrombus formation.

Once von Willebrand's factor and collagen are exposed, platelets are attracted to the area, where they bind to the damaged subendothelium (von Willebrand's factor-collagen complexes or polymers) and attach by means of platelet glycoproteins (glycoprotein Ib). When there is inadequate release of von Willebrand's factor (type I defect) or defective von Willebrand's factor (type II defect) is released, bleeding disorders (von Willebrand's disease) occur. When the platelets have no glycoprotein Ib (Bernard-Soulier syndrome), bleeding problems also occur. When there are no homeostatic abnormalities, the attachment of platelets to von Willebrand's factor-collagen complex occurs, with subsequent attraction of additional platelets that bind to each other by way of another glycoprotein known as glycopro-

tein IIb/IIIa. This glycoprotein is absent in individuals with Glanzmann's thrombasthenia, who also have bleeding disorders.

As shown in Figures 64.1 and 64.2, the release of AA leads to the production of antagonistic pathways (namely, prostacyclins and prostaglandins) that have opposing effects on platelet activation and vasomotor function. The AA also enters the monocyte/macrophage cells (mononuclear phagocytic/reticuloendothelial system), which is discussed later. The damaged endothelium (extrinsic pathway) also releases tissue thromboplastin (tissue factor), and the simultaneous disruption of blood flow (e.g., narrowed vessel, exposed subendothelium) initiates the intrinsic pathway for blood coagulation. Any factor that increases blood viscosity will also increase the tendency for blood clot formation. Such factors include cancer, inflammatory states, and hyperfibrinogenemia.

The extrinsic and intrinsic pathways converge where prothrombin/thrombinogen (factor II) is converted to thrombin (factor IIa), which is responsible for converting fibrinogen into fibrin monomers and subsequently fibrin polymers. Excessive fibrinogen augments this reaction and promotes thrombus formation. A pronounced effect of homocysteine is the promotion of a hypercoagulable state by increasing certain factors and inhibiting others. Likewise, lipoprotein(a), which has a molecular structure similar to those of LDLc and plasminogen,<sup>29</sup> impairs tissue plasminogen activation of plasminogen while inhibiting the binding of tissue plasminogen activator to the endothelial receptor for tissue plasminogen activator. This last effect is similar to one of the hypercoagulable properties of homocysteine.

One of the frequently unaddressed benefits of exercise is the beneficial impact on clotting factors. It has been demonstrated<sup>30</sup> that lower fibrinogen levels are seen in individuals who exercise regularly. Multiple factors that increase the potential for thrombus formation are reduced with exercise, including thromboxane A<sub>2</sub>.<sup>31</sup>

## The Role of Antioxidants: The Fourth Group of Factors

OFRs are extremely toxic for all living things. This includes not only the lytic effect on bacteria, which are phagocytized by polymorphonuclear leukocytes and monocytes/macrophages, but also the damage that can occur to the body itself. Multiple enzymatic pathways exist within the body to reduce these toxic products.

OFRs are reduced in endothelial cells (and elsewhere) by means of the enzyme glutathione peroxidase. If this enzyme is missing (as in hyperhomocysteinemia) or overwhelmed, the OFRs accumulate and cause endothelial damage. This oxidative stress has been shown to be associated with coronary artery disease<sup>32-35</sup> as well as cancer,<sup>36</sup> diabetes,<sup>37</sup> and even cardiac failure.<sup>38</sup> Whereas

high-density lipoprotein cholesterol serves as a scavenger mechanism for moving LDLc/cholesterol esters, antioxidants serve as free radical scavengers. Unlike high-density lipoprotein cholesterol, antioxidants do not merely move the offending agent around but catabolize the offending molecules.

Increased OFRs not only cause endothelial injury, but they also initiate a series of events including the oxidation of LDLc, which results in an increased propensity for phagocytosis of LDLc by macrophages. Increased levels of homocysteine also result (discussed previously) in the formation of LDLc-homocysteine-thiolactone complexes, which are also phagocytized by macrophages. These macrophages then release several substances (growth factors), to be discussed later. They include PDGF, basic fibroblastic growth factor, and tissue growth factor  $\beta$ . OFRs also cause endothelial dysfunction with subsequent vasoconstriction, which not only decreases blood flow but also enhances the potential for thrombosis.

In the presence of normally functioning endothelium, nitrous oxide, like nitroglycerin, results in inhibition of vascular smooth muscle (vasodilation) and decreases the proliferation and migration of fibroblasts and smooth muscle cells from the media (muscular) layer of the vessel into the subintimal layer. As the levels of OFRs increase, nitrous oxide (NO) is consumed, resulting in enhanced OFR effect without antagonism. Antioxidants have been shown to scavenge the OFRs and reduce the effects of vascular disease<sup>39,40</sup> independent of other risk potentiators or risk factors.

## Endothelial and Other Growth Factors: The Fifth Group of Factors

The basic role of the vascular system is to maintain the integrity of blood flow throughout the body. To do this, when damage occurs in one part of the vascular system, the affected area must take action to reduce the overall risk to the rest of the body. An underlying theme to living organisms is the use of a limited number of chemical reactions and mediators to produce various effects throughout the body. These chemical mediators are released from different cells of the body in an effort to carry out their individual tasks and communicate with other cells. Chemical mediators (factors) that have one effect in the vascular system have different effects in other parts of the body. For example, activation of smooth muscles in blood vessel walls results in vasoconstriction, whereas activation of smooth muscles in bronchial endothelium results in bronchospasm, even though the same chemical substances are being released.

When the endothelium is injured, collagen fibers are exposed and tissue factor is released. Both of these events stimulate the formation of blood clotting and fi-

brin development. The damaged endothelium releases AA, which stimulates prostaglandin, prostacyclin, and thromboxane A<sub>2</sub> formation. The AA also activates the leukotriene pathways, which are discussed in greater detail later. Platelets are activated by the formation of a fibrin clot (discussed previously), elevations in homocysteine levels, and prostaglandin synthesis. The platelets release PDGF, epinephrine, and additional thromboxane A<sub>2</sub>. The thromboxane A<sub>2</sub> attracts more platelets, and the epinephrine increases platelet aggregation and promotes vasoconstriction. The PDGF, which is also released from macrophages that phagocytize cholesterol, causes vasoconstriction.

Once released, PDGF stimulates fibroblast and smooth muscle proliferation and migration from the media to the intimal layer, where fatty streaks are converted to fibrous plaques.<sup>41,42</sup> As a result of PDGF, macrophages exhibit an increase in LDLc receptors (increased uptake of LDLc) and an increased influx of calcium into the macrophage. The increased phagocytosis of LDLc is associated with an increase in cholesterol synthesis.

Another factor released after endothelial injury is basic fibroblastic growth factor, which is angiogenic and has been found in greater than normal quantities in atherosclerotic vessels<sup>43</sup> and damaged<sup>44</sup> vessels. Although basic fibroblastic growth factor has been shown to be associated with collateralization of blood vessels, it has also been shown<sup>8</sup> to produce vasoconstriction and is released from macrophages. After angioplasty, vasoconstriction routinely occurs<sup>45</sup> in both distal and control regions<sup>46</sup> of the vessel. The three primary mitogenic effects of basic fibroblastic growth factor are smooth muscle and fibroblastic proliferation, as noted previously, and endothelial cell proliferation. Endothelial cell proliferation occurs by means of two mechanisms, the first being a direct effect and the second by upregulation<sup>47,48</sup> of vascular endothelial growth factor, thereby promoting angiogenesis. An additional function for heparin has been postulated<sup>49</sup> based on early research.

Tissue growth factor  $\beta$  is also released from macrophages and stimulates the proliferation and migration of fibroblasts and smooth muscle cells into the intimal layer, further promoting the advancement of fatty streaks to fibrous plaque formation. Other chemical mediators have been shown to be involved in other aspects of vascular disease. These are discussed independently in the following sections.

## The Role of Leukotrienes: The Sixth Group of Factors

The release of AA from damaged endothelial cells has been shown to lead to the production of prostaglandins and prostacyclins, which are antagonistic pathways that

have added to the understanding of the molecular mechanisms involved in thrombogenesis. Their actual role, although chemically complex, is rather limited in the overall unified theory of vascular disease. More important in the overall regulation and control are the chemical substances known as leukotrienes. These potent chemical mediators have been largely unrecognized until recently. The development of leukotriene inhibitors has resulted in a better understanding of decompression-induced pulmonary injury<sup>50</sup> and treatment options for bronchospastic disease,<sup>51,52</sup> which is the pulmonary equivalent of vasospastic problems.

Once AA is released, it can enter the leukotriene pathway in monocytes/macrophages, eosinophils, or mast cells. Leukotriene production occurs within these cells and gives rise to two pathways free of antagonists. The first is the production of leukotriene B<sub>4</sub> (LTB<sub>4</sub>), which attracts more leukocytes to the region, including the site of damaged endothelium and, as we shall see later, sites of bacterial invasion. The second pathway results in the production of three chemical mediators known as leukotriene C<sub>4</sub> (LTC<sub>4</sub>), leukotriene D<sub>4</sub> (LTD<sub>4</sub>) and leukotriene E<sub>4</sub> (LTE<sub>4</sub>). These three substances are known as cysteinyl leukotrienes and constitute what was formerly called slow-reacting substance of anaphylaxis. Both leukotriene D<sub>4</sub> and leukotriene E<sub>4</sub> cause vasoconstriction and are activated not only by endothelial injury but also by activated lymphocytes.

## The Role of the Complement Cascade (Classic and Alternative Pathways): The Seventh Group of Factors

Although frequently forgotten in the investigation of vascular disease, the complement cascade cannot be ignored. Like all reactions in the body, the role of complement is not limited to certain regions of the body and, as such, should be expected to have a potential role in either the cause of or response to vascular disease. There are two major pathways to the complement system that represent a humoral response to infectious processes. In conjunction with lymphocytes, antibodies, macrophages, and polymorphonuclear leukocytes, these two pathways defend the body and vascular system against foreign invasion. In the next section we discuss the issue of bacterial involvement in vascular disease.

The introduction of bacteria into the vascular space and into the intimal region of a blood vessel would result in activation of the complement pathways as it does elsewhere in the body. Sensitized lymphocytes produce antibody (Ab) to the bacterial antigen (Ag). The antigen-antibody complexes (immunologic stimulus) activate



the first component (C1) of the "classic" pathway, which leads to a series of reactions that produce several important components, including C3b, which results in opsonization (coating of the microbe to optimize phagocytosis), and C3a and C5a, which are anaphylatoxins that result in smooth (vascular) muscle contraction and an increase in vascular permeability. C5a also serves as a chemotactic or attractant for leukocytes. The classic pathway terminates as C5b-C6-C7-C8-C9, which results in bacteriolysis.

The alternative pathway is activated in the presence of endotoxins or shock resulting from the polysaccharide of the microbial wall. This is more prominent for gram-negative organisms but can occur with gram-positive microbes. The alternative pathway is also primed by the C3b produced by way of the classic pathway.

Once the lymphocyte recognizes the microbe and produces an antigen-antibody complex (interaction), the lymphocyte is sensitized. The sensitized lymphocyte releases three major chemical mediators: *transfer factor*, which attracts nonsensitized lymphocytes to the region where they become sensitized; *macrophage chemotactic factor* (MCF), which attracts macrophages to the region for phagocytosis and lysis of microbes (particularly after opsonization); and *migration inhibition factor* (MIF), which keeps the macrophages present and inhibits their leaving.

## The Role of Bacterial Involvement: The Eighth Group of Factors

The presence of bacterial infections is not a new problem, and recent work has shown that *Helicobacter pylori* is a major causative agent for gastric ulcers. Atherectomy specimens from coronary plaques in our laboratory and others have demonstrated bacterial agents in some of the lesions. Evidence to date demonstrates microbes not only in coronary plaques<sup>53-56</sup> but also in carotid artery stenosis,<sup>57</sup> which may lead to cerebrovascular accidents. These findings are not surprising, because it has long been recognized that disease in other vascular beds of the body has associated bacterial involvement (e.g., salmonella with abdominal aortic aneurysms).

The bacterial pathogens currently implicated are *Streptococcus pneumoniae*, *Chlamydia pneumoniae*, and *Helicobacter pylori*. The high prevalence of these bacterial pathogens probably accounts for their detection in atherosclerotic plaques and does not exclude other pathogens. Like rheumatic heart disease and valvular diseases that require prophylactic antibiotic coverage, individuals with vascular disease should be considered for prophylactic antibiotic coverage if there is any question about further vascular injury.

The presence of bacterial invasion in an atheromatous plaque may precipitate further problems by means of an inflammatory reaction, which may include either the complement or leukotriene pathway. The generation of OFRs may bring further problems as a result of the oxidative stress, as discussed previously.

## Putting It All Together: The Eight Groups of Factors

To date, research in several different areas of vascular disease has progressed independent of research in other areas. This limitation has allowed investigators to concentrate on specific areas of interest. However, it has also limited our understanding of the interrelatedness (Figure 64.3) of each of the different factors involved.

Significant research has demonstrated the presence of atheromatous plaques in both animal models and human subjects. Diet-induced hypercholesterolemia has demonstrated fatty plaques within 1 to 2 weeks in non-human primates. Monocytic involvement is seen early in the process, with the formation of fatty streaks. This has also been seen in children as young as 10 years. We have seen data with progression of disease in adults in as little as 104 days,<sup>58</sup> which suggests that once change has begun, it can progress quite rapidly. These changes are consistent with abrupt changes in coronary blood flow<sup>4,5</sup> that occur too suddenly to be accounted for by changes in LDLc levels alone. Such changes can be accounted for by changes in fibrinogen or viscosity<sup>27,28</sup> and require that these factors also be taken into account. Likewise, improvement in serum lipids has not always been associated with clinical improvement,<sup>59,60</sup> despite reductions in serum lipids. We do know, however, that total serum cholesterol levels should be reduced to below 150 mg/dL, triglycerides to below 150 mg/dL, and LDLc to less than 100 mg/dL to substantially reduce the risk and progression of vascular disease. Despite the frequent testing of cholesterol, this is too often ignored or left untreated.<sup>61</sup>

Several factors must be taken into account when evaluating a person for vascular disease, regardless of whether one is concerned about coronary artery disease, carotid disease, deep venous thrombosis, or other problems. First, you must determine the severity of LDLc and triglyceride levels to determine the load already present, which stimulates fatty streaks, calcium deposition, and fibroproliferation. We know that dietary changes, primarily reduction in calories and the amount of saturated fats, are the primary pivotal points around which reduction of this risk factor occurs. Cholesterol intake, too, must be addressed, but it is more related to foods with high saturated fat and caloric loads. In appropriate indi-

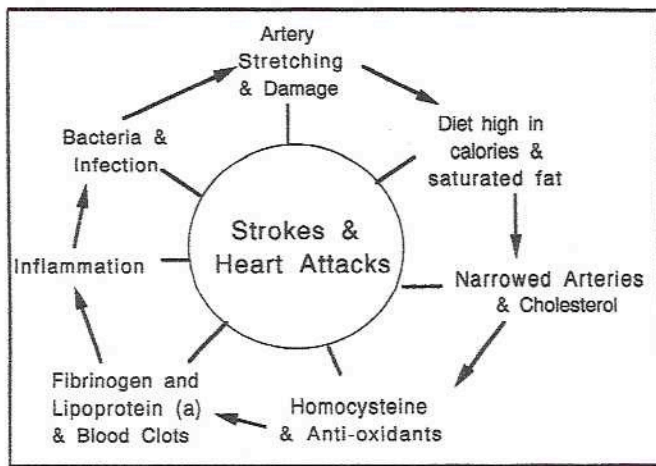


FIGURE 64.3. Damage to an artery from one group of factors may predispose the artery to further damage from other factors.

viduals, medications may in fact be necessary, but their benefit is significantly blunted without the necessary dietary changes. However, cholesterol is not the only detrimental factor affecting blood vessels. To complete the puzzle, the other pieces of the Fleming Unified Theory of Vascular Disease must be considered.

The presence of elevated homocysteine levels would suggest any of a number of other health issues (e.g., chronic renal failure, psoriasis, nitrous oxide and other medications) that need to be addressed directly, in addition to correcting nutritional factors. Treatment includes reducing contributing factors<sup>62</sup> and providing appropriate vitamin supplementation (vitamins B<sub>12</sub> and B<sub>6</sub> and folate) while monitoring plasma homocysteine levels in an attempt to reduce homocysteine to the normal range of 5 to 15 mmol/L. The oxidative stress that results from hyperhomocysteinemia not only produces endothelial injury but may also be a sign of an already taxed endothelial system. The resultant OFRs along with the homocysteine can promote vasoconstriction, macrophage phagocytosis of LDLc or LDLc-homocysteine-thiolactone complexes, and stimulation and migration of fibroblasts and smooth muscle cells into the subendothelial (intimal) region, where fibrous plaque formation progresses in the presence of fatty streaks. Homocysteine additionally increases the coagulability of blood, which can be particularly problematic in vessels that have stenotic lesions or in individuals who are otherwise predisposed to clotting tendencies. These problems can be addressed nutritionally and pharmacologically but only after they are considered.

Regardless of the etiology of endothelial injury, the release of AA leads to multiple pathways, each of which must be considered, as noted previously. Although research in the arena of prostaglandins and prostacyclins

has yielded much useful information, these are pathways that antagonize each other. Attempts to manipulate one has led to the blockage or the unrestrained expression of the other. However, the leukotriene pathway, when activated, has only one noted effect on blood vessels, that being the promotion of vasoconstriction and further inflammatory response. Although helpful in the bleeding scenario, this effect becomes nonproductive in vascular disease, where limitations in blood flow are the issue. Control of these cysteinyl leukotrienes has demonstrated promise in other disease states and suggests great potential for vascular disease. Recent work confirms another component of the Fleming Unified Theory hypothesis: that leukotrienes play a role in diseased coronary arteries. Like nitrous oxide, interleukins produce vasospasm in diseased arteries while leaving no effect on nonatherosclerotic coronary arteries.<sup>63</sup>

A review of most cardiology textbooks reveals the prevalence of cardiovascular disease problems in individuals with inflammatory and connective tissue diseases, whereas there is a paucity of such problems reported for individuals in immunodeficient states. Activation of these inflammatory pathways attracts leukocytes to the region where complement and bacterial involvement may have already been initiated. Several studies have implicated bacterial involvement in individuals who already exhibit vascular problems. Results suggest that the damaged vessel is predisposed to bacterial invasion, which must be considered and treated. Inflammation and damage to the endothelium would appear to be necessary for bacterial involvement to occur. However, once it has occurred, the presence of both complement and cellular responses to the invasion would be no different here than elsewhere in the body, and the importance of this inflammatory and infectious process has only recently been recognized.

The Fleming Unified Theory of Vascular Disease takes information not only from our laboratory but from throughout the world and recognizes the importance of various contributions made by many investigators. It is apparent that each group of factors should somehow be linked together if the studies reported to date are correct. The models shown in Figures 64.2 and 64.3 are simplified versions of how these eight groups of factors fit together. During a patient's initial screening for vascular disease, all eight groups of factors should be checked and treated when abnormal. Periodic reassessment of the patient's response to treatment should include rechecking the abnormal factors as well as diagnostic assessment of change, including nuclear imaging; ultrasound;  $\dot{V}O_2$ max evaluation; and, when indicated, angiography. The task before us now is to take this broader working model and apply it to the screening and treatment of vascular disease in each individual.

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# Autopsy Findings and Venous Thromboembolism in Patients With COVID-19

## A Prospective Cohort Study

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**Background:** The new coronavirus, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has caused more than 210 000 deaths worldwide. However, little is known about the causes of death and the virus's pathologic features.

**Objective:** To validate and compare clinical findings with data from medical autopsy, virtual autopsy, and virologic tests.

**Design:** Prospective cohort study.

**Setting:** Autopsies performed at a single academic medical center, as mandated by the German federal state of Hamburg for patients dying with a polymerase chain reaction-confirmed diagnosis of COVID-19.

**Patients:** The first 12 consecutive COVID-19-positive deaths.

**Measurements:** Complete autopsy, including postmortem computed tomography and histopathologic and virologic analysis, was performed. Clinical data and medical course were evaluated.

**Results:** Median patient age was 73 years (range, 52 to 87 years), 75% of patients were male, and death occurred in the hospital ( $n = 10$ ) or outpatient sector ( $n = 2$ ). Coronary heart disease and asthma or chronic obstructive pulmonary disease were the most common comorbid conditions (50% and 25%, respec-

tively). Autopsy revealed deep venous thrombosis in 7 of 12 patients (58%) in whom venous thromboembolism was not suspected before death; pulmonary embolism was the direct cause of death in 4 patients. Postmortem computed tomography revealed reticular infiltration of the lungs with severe bilateral, dense consolidation, whereas histomorphologically diffuse alveolar damage was seen in 8 patients. In all patients, SARS-CoV-2 RNA was detected in the lung at high concentrations; viremia in 6 of 10 and 5 of 12 patients demonstrated high viral RNA titers in the liver, kidney, or heart.

**Limitation:** Limited sample size.

**Conclusion:** The high incidence of thromboembolic events suggests an important role of COVID-19-induced coagulopathy. Further studies are needed to investigate the molecular mechanism and overall clinical incidence of COVID-19-related death, as well as possible therapeutic interventions to reduce it.

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Since it was first detected in December 2019, the novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) spread from the central Chinese province of Hubei to almost every country in the world (1, 2). Most persons with COVID-19 have a mild disease course, but about 20% develop a more severe course with a high mortality rate (3). As of 26 April 2020, more than 2.9 million persons have been diagnosed with COVID-19 and 210 000 of them have died (4). Why the new coronavirus seems to have a much higher mortality rate than the seasonal flu is not completely understood. Some authors have reported potential risk factors for a more severe disease course, including elevated D-dimer levels, a high Sequential Organ Failure Assessment score, and older age (5, 6). Because of the novelty of the pathogen, little is known about the causes of death in affected patients and its specific pathologic features. Despite modern diagnostic tests, autopsy is still of great importance and may be a key to understanding the biological characteristics of SARS-CoV-2 and the pathogenesis of the disease. Ideally, knowl-

edge gained in this way can influence therapeutic strategies and ultimately reduce mortality. To our knowledge, only 3 case reports have been published about COVID-19 patients who have undergone complete autopsy (7, 8). Therefore, in this study we investigated the value of autopsy for determining the cause of death and describe the pathologic characteristics in patients who died of COVID-19.

## METHODS

### Study Design

In response to the pandemic spread of SARS-CoV-2, the authorities of the German federal state of Hamburg ordered mandatory autopsies in all patients dying with a diagnosis of COVID-19 confirmed by polymerase chain reaction (PCR). The legal basis for this was section 25(4) of the German Infection Protection Act. Because of legal regulations, no COVID-19 death was exempted from this order, even if its clinical cause seemed obvious. The case series demonstrated herein consists of 12 consecutive autopsies, starting with the first known

**Table 1.** Patient Characteristics and Autopsy Findings

Case Number	Age, y	Sex	Preexisting Medical Conditions	Treatment	BMI, kg/m <sup>2</sup>	Clinical Cause of Death	PMI, d
1	52	Male	Obesity	CPR	38.8	Sudden cardiac death	1
2	70	Male	Parkinson disease, CHD, PAD, CKD	BSC	22.2	Respiratory failure, pneumonia	1
3	71	Male	AH, nicotine abuse, granulomatous pneumopathy	CA, MV	36.8	Respiratory failure, pneumonia	2
4	63	Male	T2DM, obesity, bronchial asthma	CA, MV, lysis of right ventricular thrombus, CPR	37.3	Cardiorespiratory failure, PE	1
5	66	Male	CHD	CPR	25.3	Sudden cardiac death	2
6	54	Female	Dementia, epilepsy, trisomy 21	BSC	29.6	Respiratory failure, aspiration pneumonia	1
7	75	Female	Atrial fibrillation, CHD, nicotine abuse	NIV	26.3	Respiratory failure, viral pneumonia	4
8	82	Male	Parkinson disease, T2DM, CHD	BSC	27.8	Respiratory failure, viral pneumonia	1
9	87	Female	Non-small cell lung cancer, COPD, CHD, CKD	BSC	15.4	Respiratory failure, viral pneumonia	4
10	84	Male	T2DM, AH, ulcerative colitis	BSC	20.7	Respiratory failure, viral pneumonia	5
11	85	Male	CHD, AH, bronchial asthma, atrial fibrillation	CA, MV, RRT	30.0	Cardiac arrest due to respiratory failure	2
12	76	Male	Obesity	CA, MV, CPR	34.4	PE	3

AB = acute bronchitis; ACVB = aortocoronary venous bypass; AH = arterial hypertension; aPC = activated pneumocytes; BMI = body mass index; BSC = best supportive care; CA = catecholamine therapy; CB = chronic bronchitis; CHD = coronary heart disease; CKD = chronic kidney disease; Co = congestion of small vessels; COPD = chronic obstructive pulmonary disease; CPR = cardiopulmonary resuscitation; DAD = diffuse alveolar damage; DVT = deep venous thrombosis; FB = fibroblasts; GC = giant cells; Gra = granulocytic infiltration; HI = hemorrhagic infarctions; HM = hyaline membranes; LAD = left anterior descending artery; LC = lymphocytes; MI = myocardial infarction; MV = mechanical ventilation; NET = neuroendocrine tumor; NIV = noninvasive ventilation; PAD = peripheral artery disease; PE = pulmonary embolism; PEG = percutaneous endoscopic gastrostomy; PIC = plasma cells; PMCT = postmortem computed tomography; PMI = postmortem interval; RCA = right coronary artery; RRT = renal replacement therapy; SM = squamous metaplasia; T2DM = type 2 diabetes mellitus; Thr = thrombi; VATS = video-assisted thoracoscopic surgery.

SARS-CoV-2-positive death occurring in Hamburg (the second largest city in Germany, with 1.8 million inhabitants). All autopsies were performed at the Department of Legal Medicine of University Medical Center

Hamburg-Eppendorf. The Ethics Committee of the Hamburg Chamber of Physicians was informed about the study (no. WF-051/20). The study was approved by the local clinical institutional review board and com-

Table 1—Continued

Cause of Death	Main Pathologic Findings	PMCT (Lungs)	Histology (Lungs)
PE, pneumonia	PE, DVT, pneumonia, obesity, cardiomegaly (660 g), splenomegaly (500 g), hepatomegaly (3880 g), shock organs (liver, kidneys), atherosclerosis	Diffuse bilateral pulmonary consolidations in each lobe	DAD: aPC, FB, GC, sparse HM, slight fibrosis Additional findings: Co, Thr
Pneumonia with bronchopneumonia	Pneumonia, CHD (stents in LAD and RCA, status post MI, cardiac aneurysm), contractures (with Parkinson syndrome), purulent bronchitis, cardiomegaly (515 g), shock liver	No PMCT	DAD: aPC, HM, sparse LC Additional findings: focal Gra, CB, AB
PE, pneumonia	PE, DVT, pneumonia, status post VATS (due to unspecified granuloma), CHD, anasarca, atherosclerosis	Emphysema; fine reticular pattern in each lobe; consolidations in the right lower and left lower lobes	DAD: SM, FB, aPC, HM Additional findings: Thr
PE, pneumonia	PE, DVT, pneumonia, obesity, cardiomegaly (605 g), ischemic colitis, shock liver	No PMCT	DAD: FB, aPC, HM, SM Additional findings: HI, Thr
Pneumonia	Pneumonia, DVT, CHD, status post MI	Consolidations in each lobe; reticular pattern in the right upper and lower lobes and in each left lobe	DAD: aPC, FB, HM, necrosis, LC Additional findings: surrounding small vessels, Thr
Pneumonia	Pneumonia, kidney infarctions, PEG tube	Consolidations in the right upper and middle lobes and in parts of the left upper and lower lobes; ground glass opacities in the right upper and lower lobes and in the left upper lobe; reticular pattern in the right middle and lower lobes and in each left lobe	Gra, AB, Co (no DAD)
Pneumonia	Pneumonia, lung emphysema, CHD, left cardiac dilatation, calcification of the mitral ring, cardiac pacemaker, atherosclerosis	Reticular pattern in each lobe; small areas of consolidation in the right lower, left upper, and left lower lobes	DAD: HM, aPC, SM Additional findings: emphysema, Co
Bronchopneumonia	Pneumonia, emphysema, DVT, CHD, status post ACVB, status post MI with left cardiac aneurysm, atherosclerosis	Emphysema; diffuse consolidations in each lobe; reticular pattern in the right upper and lower lobes and in the left lower lobe; bilateral pleural effusion	Gra, emphysema (no DAD)
Purulent bronchitis	Pneumonia, purulent bronchitis, CHD, status post MI, cachexia, bullous emphysema, NET in the lung, atherosclerosis	Emphysema; round tumor in the right lower lobe; small areas of consolidation in the right upper and lower lobes and in the left upper lobe; reticular pattern in the right upper and lower lobes and in each left lobe	Gra, AB, emphysema (no DAD) Additional findings: NET composed of small cells
Pneumonia, septic encephalopathy	Pneumonia, emphysema, septicemia, status post MI, atrophic kidneys	Reticular pattern in the right upper and lower lobes and in each left lobe; consolidations in the right middle and lower lobes and in each left lobe; ground glass opacities in the right upper and middle lobes and in parts of the left upper lobe; bilateral pleural effusion	Emphysema, Co, Gra, CB, fibrosis (no DAD)
Pneumonia	Pneumonia, DVT, minor PE, emphysema, CHD, cardiomegaly (650 g), atherosclerosis	Diffuse consolidations in each lobe; reticular pattern in the right middle and lower lobes and in each left lobe; ground glass opacities in the right upper and middle lobes and in the left upper lobe; bilateral pleural effusion	DAD: HM (sparse), GC, aPC Additional findings: emphysema, Co, Gra
PE	PE with lung infarctions, DVT, pneumonia, purulent tracheobronchitis, pneumonia, cardiomegaly (745 g), emphysema, obesity	No residual ventilation in either lung except for small areas in the right upper and middle lobes and in the left upper and lower lobes; bilateral pleural effusion	DAD: HM, aPC, fibrosis Additional findings: LC, PIC, HI, Thr, Co

plied with the Declaration of Helsinki. In all deceased patients, postmortem computed tomography (PMCT) and a complete autopsy, including histopathologic and virologic evaluation, were performed. Clinical records were checked for preexisting medical conditions and medications, current medical course, and antemortem diagnostic findings.

#### PMCT, Autopsy, and Histologic Examination

Computed tomographic examination was done at the Department of Legal Medicine with a Philips Brilliance

16-slice multidetector scanner in accordance with an established protocol (9). In brief, full-body computed tomography was performed from top to thigh (slice thickness, 1 mm; pitch, 1.5; 120 kV; 230 to 250 mAs), complemented by dedicated scans of the thorax with higher resolution (slice thickness, 0.8 mm; pitch, 1.0; 120 kV; 230 to 250 mAs). We performed external examinations and full-body autopsies on all deceased persons with SARS-CoV-2 positivity (PCR confirmed) as soon as possible after taking proper safety precautions



**Table 2.** Overview of Laboratory Results Taken at the Time of Hospitalization\*

Case Number	Hemoglobin, g/dL	MCV, fL	Platelets, × 10 <sup>9</sup> /L	Leukocytes, × 10 <sup>9</sup> /L	INR	aPTT, s	D-dimer, µg/L	LDH, µkat/L	Creatinine, µmol/L
Normal range	14.0–17.5	80.0–94.0	150–400	3.8–11.0	–	23–30	<500	2.00–4.10	53.4–99.1
1	NA	NA	NA	NA	NA	NA	NA	NA	NA
2	12.1	92	144	7.4	1.3	NA	NA	5.92	228.8
3	14.9	100	190	9.2	2.1	42	NA	6.32	102.9
4	13.3	88	478	7.1	1.1	21	23,100	11.07	65.6
5	14.4	NA	NA	NA	NA	NA	NA	NA	NA
6	14.0	95	135	3.4	1.0	30	NA	6.12	59.5
7	12.1	98	125	6.9	1.5	57	28,800	7.97	76.3
8	14.8	79	186	7.1	1.2	29	2100	9.84	99.1
9	10.7	98	210	5.3	1.0	23	NA	2.70	99.1
10	16.5	88	219	15.5	1.1	29	>200,000	10.50	129.6
11	9.9	78	304	11.6	1.1	45	5700	11.40	83.9
12	16.8	90	141	3.8	0.95	32	NA	6.32	67.1

aPTT = activated partial thromboplastin time; AST = aspartate aminotransferase; CRP = C-reactive protein; INR = international normalized ratio; LDH = lactate dehydrogenase; MCV = mean corpuscular volume; NA = not available; PCT = procalcitonin.

\* Patients in cases 1 and 5 died out of the hospital after a sudden cardiac arrest. Values are either nonexistent (case 1) or taken from a blood gas analysis (case 5).

(using personal protection equipment with proper donning and doffing), following guidelines from the German Association of Pathologists, which are closely aligned with relevant international guidelines. The recently published recommendations for the performance of autopsies in cases of suspected COVID-19 were taken into account (10). The interval from death to postmortem imaging and autopsy (postmortem interval) ranged from 1 to 5 days. During autopsy, tissue samples for histology were taken from the following organs: heart, lungs, liver, kidneys, spleen, pancreas, brain, prostate and testes (in males), ovaries (in females), small bowel, saphenous vein, common carotid artery, pharynx, and muscle.

For virologic testing, we took small samples of heart, lungs, liver, kidney, saphenous vein, and pharynx and sampled the venous blood.

Tissue samples for histopathologic examination were fixed in buffered 4% formaldehyde and processed via standard procedure to slides stained with hematoxylin-eosin. For the lung samples, we also used the keratin marker AE1/AE3 (Dako) for immunohistochemistry.

#### Quantitative SARS-CoV-2 RNA Reverse Transcription PCR From Tissue

Tissue samples were ground by using ceramic beads (Precellys lysing kit) and extracted by using automated nucleic acid extraction (MagNA Pure 96 [Roche]) according to manufacturer recommendations. For virus quantification in tissues, a previously published assay was adopted with modifications (11). One-step real-time PCR was run on the LightCycler 480 system (Roche) by using a 1-step RNA control kit (Roche) as master mix. The C<sub>t</sub> (cycle threshold) value for the target SARS-CoV-2 RNA (fluorescein) and whole-process RNA control (Cy5) was determined by using the second derivative maximum method. For quantification, standard in vitro-transcribed RNA of the E gene of SARS-CoV-2 was used (12). These samples were also

analyzed in a study focusing on renal tropism of SARS-CoV-2 (Puelles V, et al. Multi-organ and renal tropism of SARS-CoV-2. In preparation).

#### Statistical Analysis

Data that were normally distributed are presented as means (SDs); data outside the normal distribution are presented as medians (ranges). Categorical variables were summarized as counts and percentages. All data were analyzed with Statistica, version 13 (StatSoft).

#### Role of the Funding Source

The sponsor was not involved in the design or conduct of the study, nor in the analysis of the data or the decision to submit the manuscript.

## RESULTS

### Clinical Data

The median age of the 12 patients included in this study was 73 years (interquartile range, 18.5); 25% were women. For all patients, preexisting chronic medical conditions, such as obesity, coronary heart disease, asthma or chronic obstructive pulmonary disease, peripheral artery disease, diabetes mellitus type 2, and neurodegenerative diseases, could be identified (Table 1). Two patients died out of the hospital after unsuccessful cardiopulmonary resuscitation, 5 died after treatment in the intensive care unit, and the remaining 5 had an advanced directive for best supportive care and died in the non-intensive care ward. Laboratory results for clinical chemistry, hematology, and coagulation were not available for the patients who died out of the hospital. In the remaining patients, the most striking features of the initial laboratory test were elevated levels of lactate dehydrogenase (median, 7.83 µkat/L [range, 2.71 to 11.42 µkat/L]), D-dimer (available for 5 patients; median, 495.24 nmol/L [range, 20.38 to >1904.76 nmol/L]), and C-reactive protein (median, 189 mg/L [range, 18 to 348 mg/L]), as well as mild

Table 2—Continued

AST, U/L	Sodium, mmol/L	Potassium, mmol/L	CRP, mg/L	PCT, mg/L	Antimicrobial and Coagulation Therapy
<50	135-145	3.6-4.6	<5	<0.5	None
NA	NA	NA	NA	NA	Rivaroxaban: 20 mg once daily
54	154	4.6	303	NA	Piperacillin/tazobactam: 4.5 g three times daily
					Meropenem: 1 g three times daily
52	139	4.45	304	NA	Levofloxacin: 400 mg twice daily
					Enoxaparin: 4000 IU once daily
54	139	3.5	133	0.1	Cefpodoxime: 200 mg twice daily
NA	141	8.7	NA	NA	None
NA	132	4.4	18	0.2	None
97	141	3.8	213	0.3	Edoxaban: 30 mg once daily
NA	141	4.6	92	NA	None
NA	135	4.6	39	NA	None
NA	140	3.5	348	16.2	None
77	141	4.5	268	0.3	None
77	136	4.3	167	0.5	Certoparin: 3000 IU once daily

thrombocytopenia in 4 of 10 patients. A procalcitonin test had been performed in 6 patients, and the results were negative in all but 1 patient with pneumonia (case 10). Table 2 provides an overview of the initial laboratory results.

### PMCT

In 2 cases (2 and 4), PMCT was not possible for logistic reasons. In the remaining cases, PMCT demonstrated mixed patterns of reticular infiltrations and severe, dense, consolidating infiltrates in both lungs in the absence of known preexisting pathology (such as emphysema or tumor). A juxtaposition of antemortem and postmortem findings is demonstrated in Figure 1. A complete summary of PMCT findings is presented in Table 1.

### Autopsy

In 4 cases (1, 3, 4, and 12), massive pulmonary embolism was the cause of death, with the thrombi deriving from the deep veins of the lower extremities. In another 3 cases (5, 8, and 11), fresh deep venous thrombosis was present in the absence of pulmonary embolism. In all cases with deep venous thrombosis, both legs were involved (Figure 2). In 6 of the 9 men (two thirds) included in the study, fresh thrombosis was also present in the prostatic venous plexus (Appendix Figure 1, available at [Annals.org](#)).

In all 12 cases, the cause of death was found within the lungs or the pulmonary vascular system. However, macroscopically differentiating viral pneumonia with subsequent diffuse alveolar damage (a histologic diagnosis) from bacterial pneumonia was not always possible. Typically, the lungs were congested and heavy, with a maximum combined lung weight of 3420 g in case 11. The mean combined lung weight was 1988 g (median, 2088 g). Standard lung weights for men and women are 840 g and 639 g, respectively (13, 14). Only cases 6 and 9 presented with a relatively low lung weight: 550 g and 890 g, respectively (Appendix Table 1, available at [Annals.org](#)). The lung surface often displayed mild pleurisy and a distinct patchy pattern, with pale areas alternating with slightly protruding and firm,

deep reddish blue hypercapillarized areas. On the cutting surfaces, this pattern was also visible (Figure 2). The consistency of the lung tissue was firm yet friable. In 8 cases, all parts of the lungs were affected by these changes. Cases 6, 7, and 9—occurring in the 3 women of the case series—presented with changes compatible with focal purulent bronchopneumonia. Macroscopically, no changes were observed outside the lungs and respiratory tract, except for splenomegaly in 3 cases, which suggested a viral infection.

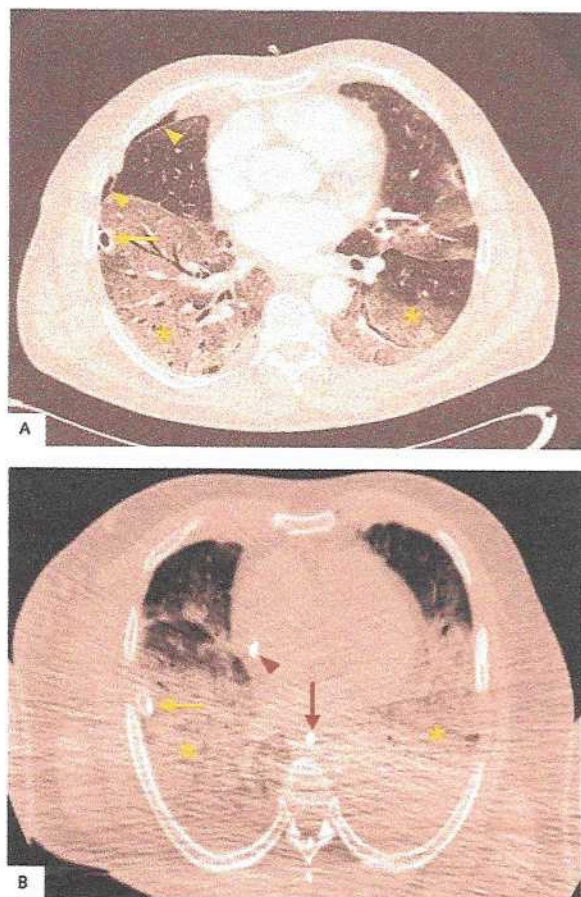
During autopsy, all cases except for case 6 presented with preexisting heart disease, including high-grade coronary artery sclerosis (7 of 12); myocardial scarring, indicating ischemic heart disease (6 of 12); and congestive cardiomyopathy. Mean heart weight was 503 g (median, 513 g). In addition to this finding, the most common accompanying diseases were pulmonary emphysema (6 of 12) and ischemic enteritis (3 of 12). Often these conditions were known to the treating physician before death (compare columns 4 and 10 of Table 1). The macroscopic autopsy findings are presented organ by organ in Appendix Table 2 (available at [Annals.org](#)) and the lung findings in Table 1.

A clear trend toward obesity was observed among the cases (mean body mass index, 28.7 kg/m<sup>2</sup>; median, 28.7 kg/m<sup>2</sup>). However case 9, involving a patient with known neuroendocrine tumor of the lung, presented with severe cachexia (body mass index, 15.4 kg/m<sup>2</sup>). The comorbid conditions found are summarized in Table 1.

### Histology

Histopathology of the lungs showed diffuse alveolar damage, consistent with early acute respiratory distress syndrome in 8 cases. Predominant findings were hyaline membranes (Figure 3, A and B), activated pneumocytes, microvascular thromboemboli, capillary congestion, and protein-enriched interstitial edema. As described by Wang and colleagues (15), a moderate degree of inflammatory infiltrates concurred with clinically described leukopenia in patients with COVID-19 and predominant infiltration of lymphocytes fit the pic-

**Figure 1.** Antemortem versus postmortem computed tomographic imaging (case 3).



Top. Contrast medium-enhanced computed tomography scan demonstrates the antemortem findings: bilateral ground glass opacities in the lower lobes of both lungs (*yellow asterisks*) and a chest tube (*yellow arrow*), which has been introduced to treat a pneumothorax (*yellow arrowheads*). Bottom. Computed tomography scan without contrast medium enhancement demonstrates the corresponding postmortem findings. For technical reasons, the postmortem image has a lower resolution. To protect the staff from potential infection, bodies were scanned in a double-layer body bag with the arms positioned alongside the body. Although the findings correspond to the antemortem images, ground glass opacities in both lower lobes (*yellow asterisks*) and a chest tube (*yellow arrow*) are seen. In addition, a central venous line (*red arrowhead*) and gastric tube (*red arrow*) are visible.

ture of a viral pathogenesis. In later stages, squamous metaplasia was present (Figure 3, C). Long-term changes, such as destruction of alveolar septae and lymphocytic infiltration of the bronchi, were often visible as preexisting conditions. Four cases (6, 8, 9, and 10) showed no diffuse alveolar damage but extensive granulocytic infiltration of the alveoli and bronchi, resembling bacterial focal bronchopneumonia. Histologically, thromboemboli were detectable in cases 1, 3, 4, and 5 (Figure 3, D). Microthrombi were regularly found within small lung arteries, occasionally within the prostate, but not in other organs.

In addition to the lung changes described in Table 1, there were isolated histologic findings that might indicate a viral infection. The pharyngeal mucosa was examined in 7 cases. In 6 of them, hyperemia and alternating dense, predominantly lymphocytic infiltrates were found as signs of chronic pharyngitis. In 1 case (case 3), lymphocytic myocarditis was seen in the right ventricle (Appendix Figure 2, available at [Annals.org](https://annals.org)). The remaining histologic changes were compatible with shock changes in part of the deceased patient (liver, kidneys, intestine) or corresponded to the macroscopically determined virus-independent preexisting pathology (such as ischemic cardiomyopathy).

Apart from findings related to SARS-CoV-2 infection, patients showed other histopathologic findings related to their chronic preexisting conditions, including hypertrophy of myocardial fibers or scarring of the myocardium. The peripheral veins, including those occluded by thrombi, showed no abnormalities on hematoxylin-eosin staining.

### PCR Results

Quantitative reverse transcription PCR detected SARS-CoV-2 RNA in the lungs of all 12 patients (range,  $1.2 \times 10^4$  to  $9 \times 10^9$  copies/mL) and in the pharynx of 9 patients. Six patients showed moderate viremia ( $<4 \times 10^4$  copies/mL). In 5 of these patients, viral RNA was also detected in other tissues (heart, liver, or kidney) in concentrations exceeding viremia. Patients without viremia showed no or a low virus load in the other tissues. Only 4 patients had detectable viral RNA in the brain and saphenous vein.

### DISCUSSION

In this autopsy study of 12 consecutive patients who died of COVID-19, we found a high incidence of deep venous thrombosis (58%). One third of the patients had a pulmonary embolism as the direct cause of death. Furthermore, diffuse alveolar damage was demonstrated by histology in 8 patients (67%).

To our knowledge, this is the first case series summarizing and comparing clinical data of consecutive COVID-19 cases with findings obtained by a full autopsy, supplemented by PMCT, histology, and virology.

The high rate of death-causing pulmonary embolism at autopsy correlates well with the unsuccessful resuscitation of 3 of 4 patients, 2 of whom died out of the hospital. Apart from that, no preclinical evidence had been reported of pulmonary embolism or deep venous thrombosis.

In studies that examined deceased patients with COVID-19 without relying on autopsy, no increased rates of pulmonary embolism were observed clinically. However, it is known that many cases of pulmonary embolism remain clinically overlooked and are often associated with sudden, unexpected death. This may have been aggravated by the method for diagnosing COVID-19 in Germany, which is based on PCR tests rather than computed tomographic imaging because of concerns about infection of medical staff and other

patients. A recent report described clinical features of 85 fatal cases of COVID-19 from Wuhan (16). Besides respiratory failure, the cause of death was multiorgan failure in 16% and cardiac arrest in 9%. No autopsies were performed. The gold standard for identifying cause of death is still the autopsy (17). However, in-hospital autopsy rates have declined worldwide over the past decades. Also, because of pathologists' potential risk for SARS-CoV-2 infection, very few autopsies have been performed worldwide (18). To our knowledge, only 3 case reports have been published on patients with COVID-19 who have undergone complete autopsy and a few more in which only lung tissue was examined (7, 8).

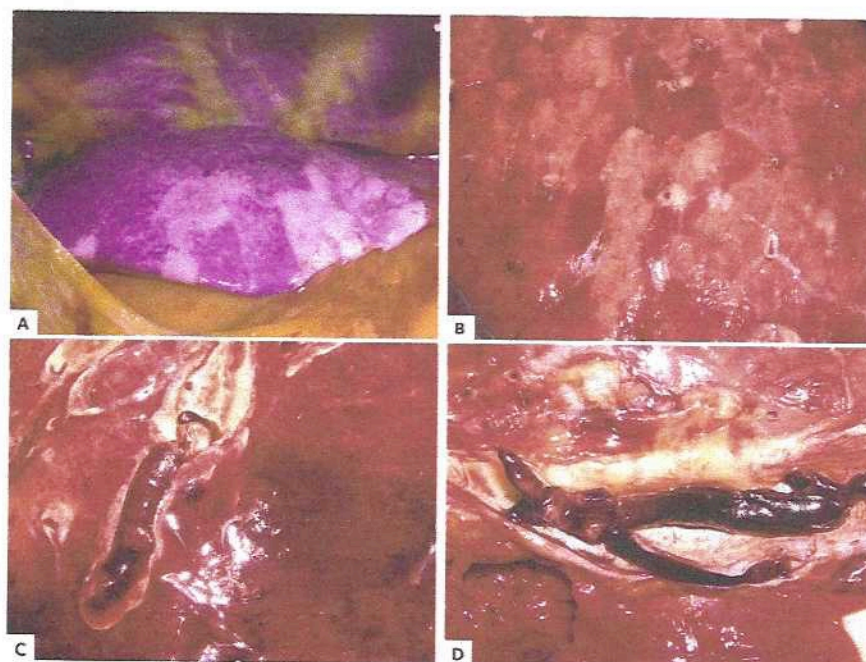
Other researchers have described coagulopathy as a common complication in patients with severe COVID-19 (5, 6, 19). In a recent study of 191 patients with COVID-19, 50% of those who died had coagulopathy, compared with 7% of survivors. D-dimer levels greater than 1000  $\mu\text{g/L}$  were associated with a fatal outcome (6).

COVID-19 may predispose to venous thromboembolism in several ways. The coagulation system may be activated by many different viruses, including HIV, dengue virus, and Ebola virus (20, 21). In particular, coronavirus infections may be a trigger for venous thromboembolism, and several pathogenetic mechanisms are involved, including endothelial dysfunction, characterized by increased levels of von Willebrand factor; systemic inflammation, by Toll-like receptor activation; and a procoagulatory state, by tissue factor pathway activa-

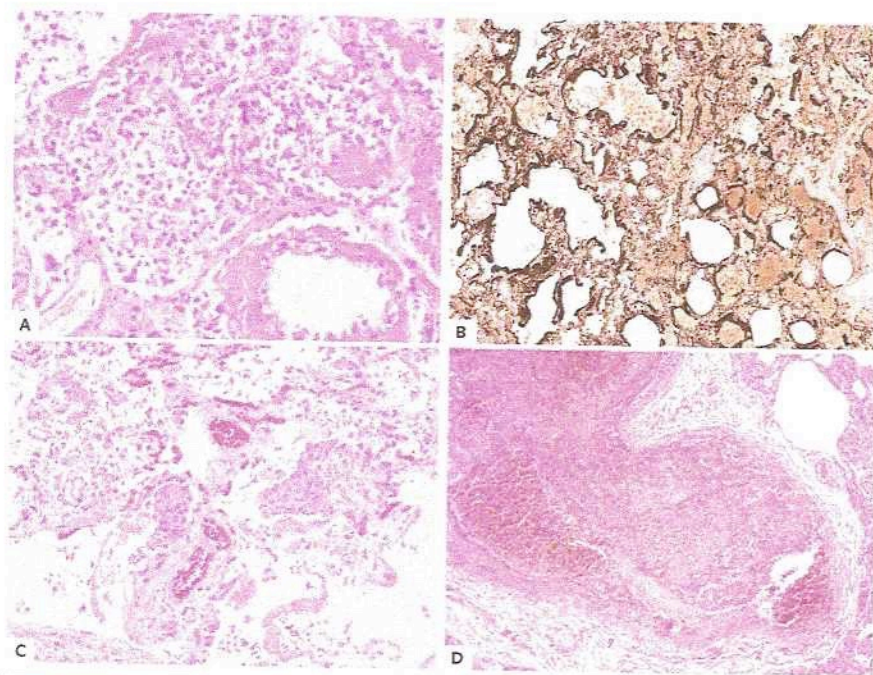
tion (22). In a subgroup of patients with severe COVID-19, high plasma levels of proinflammatory cytokines were observed (23). The direct activation of the coagulation cascade by a cytokine storm is conceivable. With COVID-19, severe hypoxemia develops in some patients (24). Thrombus formation under hypoxic conditions is facilitated both in animal models of thrombosis and in humans. The vascular response to hypoxia is controlled primarily by the hypoxia-inducible transcription factors, whose target genes include several factors that regulate thrombus formation (25). Lastly, indirect causes, such as immune-mediated damage by antiphospholipid antibodies, may partially contribute, as speculated by Zhang and colleagues (26).

The macroscopic findings in our autopsy series—with rather heavy, consolidated, friable, basically air-free lungs in most of the cases—were impressive and explain the difficulties in sufficiently ventilating some of these patients. The histopathologic changes in most of our cases with diffuse alveolar damage as the main finding resemble those described by Xu and colleagues (7) and Barton and colleagues (8), who reported single cases; Zhang and colleagues (26), who reported on lung biopsy in a patient with SARS-CoV-2 positivity; and Tian and colleagues (27), who described macroscopic and histologic pulmonary findings in 2 patients with lung cancer who received positive results on SARS-CoV-2 testing. However, the full-blown picture of diffuse alveolar damage seems to be more prevalent in younger patients with fewer preexisting diseases and longer survival, whereas older patients with more co-

**Figure 2.** Macroscopic autopsy findings.



A. Patchy aspect of the lung surface (case 1). B. Cutting surface of the lung in case 4. C. Pulmonary embolism (case 3). D. Deep venous thrombosis (case 5).

**Figure 3.** Histopathologic findings.

A. Diffuse alveolar damage with hyaline membranes (case 4) (hematoxylin-eosin [H&E] stain; original magnification,  $\times 50$ ). B. Hyaline membranes (case 4) (cytokeratin AE1/AE3 stain, original magnification  $\times 50$ ). C. Squamous metaplasia in the lung (case 5) (H&E stain; original magnification,  $\times 100$ ). D. Pulmonary embolism (case 1) (H&E stain; original magnification,  $\times 100$ ).

morbid conditions tend to die in the early stages of the disease.

In line with clinical, macroscopic, and histopathologic findings, PCR detected the highest concentration of SARS-CoV-2 RNA in lung and pharyngeal tissue. Of interest, in most patients with disease, high titers of RNA were also detected in postmortem samples. The clinical relevance of this is not yet clear. Clearance of viral RNA from blood 7 days after transfusion of COVID-19 convalescent plasma was associated with substantial clinical improvement, but studies have not shown a correlation between viremia and acute respiratory distress syndrome in patients with severe COVID-19 (28, 29). As in patients with SARS-CoV-1, in whom viral replication could be detected in other organs, including the liver, kidney, spleen, and cerebrum (30), we detected viral RNA at high titers in other organs (liver, kidney, and heart) in 5 patients. These data suggest that SARS-CoV-2 may spread via the bloodstream and infect other organs. To prove this, replication intermediates must be detected.

The current study had some limitations: First, the sample size was small, possibly leading to overestimation of the rate of pulmonary embolism. However, both the clinical and postmortem observations agree well with the current knowledge about SARS-CoV-2 pathology. This includes the sex and age distribution as well as the preexisting conditions among the patients, but also the histologic findings. Second, although viral titers in swabs (pharynx) taken longitudinally up to 7

days after death remained similar, we lack data on how postmortem processes affect viral titers and dynamics in different tissues and body fluids. Moreover, the quantitative PCR assay used cannot discriminate between genomic and subgenomic RNA. As stated earlier, to prove viral replication, detection of replication intermediates or antigenomic RNA would be necessary.

In conclusion, we found a high incidence of thromboembolic events in patients with COVID-19. When hemodynamic deterioration occurs in a patient with COVID-19, pulmonary embolism should always be suspected. That patients with COVID-19 who have increased D-dimer levels, a sign of coagulopathy, may benefit from anticoagulant treatment seems plausible (31). As demonstrated in our cohort, this might be important for hospitalized patients and outpatients. In this context, some professional societies have already made recommendations for antithrombotic therapy for patients with COVID-19 (32). Robust evidence, however, remains scant, and further prospective studies are urgently needed to confirm and validate these results.

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**Reproducible Research Statement:** *Study protocol:* Available with approval through written agreement with Dr. Wichmann (e-mail, [d.wichmann@uke.de](mailto:d.wichmann@uke.de)). *Statistical code:* Available from Dr. Kluge (e-mail, [s.kluge@uke.de](mailto:s.kluge@uke.de)). *Data set:* Not available.

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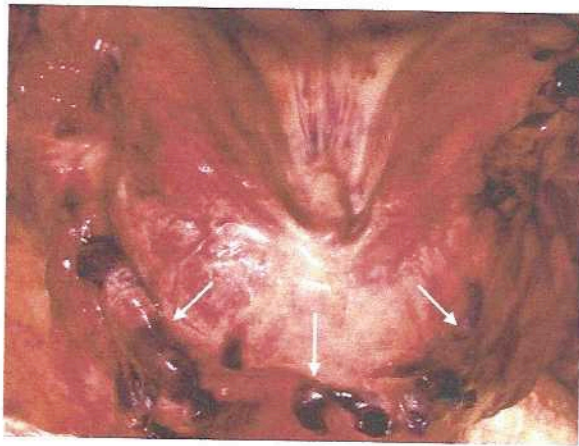
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**Appendix Figure 1.** Thrombosis of the prostatic vein (case 1) (arrows).



**Appendix Table 1.** Weights of Individual Organs, in Grams, for All Cases\*

Case Number	Brain	Heart	Lung (Right)	Lung (Left)	Liver	Kidney (Right)	Kidney (Left)	Spleen
1	1520	660	1135	940	3880	215	305	500
2	1430	515	1220	1030	2030	155	155	355
3	1665	510	1280	1445	1930	240	240	280
4	1435	605	1370	1100	2180	210	210	240
5	1450	360	955	845	1645	180	165	310
6	950	250	275	275	890	80	90	90
7	1210	415	690	655	1380	105	120	95
8	1170	575	1160	940	1610	145	160	260
9	1080	355	480	410	715	100	35	50
10	1350	390	730	630	945	115	145	135
11	1400	650	1880	1540	1450	225	240	240
12	1460	745	1580	1290	2265	270	220	360

\* Weights are rounded to 5 g. Standard weights for men and women (adopted from Molina and DiMaio [13, 14]), respectively, are as follows (the dependence of standard organ weights on body weight was not considered here): brain, 1401 g and 1233 g; heart, 331 g and 245 g; lung (right), 445 g and 340 g; lung (left), 395 g and 299 g; liver, 1561 g and 1288 g; kidney (right), 129 g and 108 g; kidney (left), 137 g and 116 g; and spleen, 139 g and 115 g.

Appendix Table 2. Macroscopic Autopsy Findings in Organs Other Than the Lung in Patients Dying of COVID-19\*

Case Number	Heart	Liver	Kidneys	Spleen	Prostate	Veins	Bowel	Pharynx	Adrenal Glands	Arteries	Miscellaneous
1	Eccentric hypertrophy of both ventricles	Hepatomegaly	Shoek kidneys	Splenomegaly	Thrombosis	Thrombosis	Ichemic enterocolitis	Normal	Normal	Atherosclerosis	—
2	CHD with stenosis, status post MI, cardiac aneurysm, hypertrophy	Shoek liver	Normal	Enlarged	Status post prostatectomy	Normal	Normal	Normal	Normal	Atherosclerosis	—
3	Biventricular hypertrophy, moderate CHD	Shoek liver	Normal	Normal	Thrombosis, benign hypertrophy	Thrombosis, phlebosclerosis	Diverticulosis, ischemic enterocolitis	Normal	Micronodular hyperplasia	Atherosclerosis	Status post VATS (resection of right upper lung lobe)
4	Left ventricular hypertrophy	Shoek liver	Normal	Normal	Thrombosis	Thrombosis	Ichemic colitis	Normal	Normal	Slight atherosclerosis	Struma colloidosa nodosa
5	CHD, status post MI	Normal	Normal	Normal	Thrombosis	Thrombosis	Status post abdominal surgery	Normal	Normal	Slight atherosclerosis	—
6	Normal	Normal	Old infarctions/chronic inflammation, nephrolithiasis	Normal	—	Normal	Adenoma of the duodenum	Normal	Adenoma	Normal	Trisomy 21, PEG tube, cholelithiasis, small brain,
7	CHD, moderate hypertrophy, calcification of the mitral ring, status post MI, pacemaker, lipomas of cordis	Normal	Normal	Normal	—	Normal	Normal	Normal	Micronodular hyperplasia	Atherosclerosis	Anasarca, umbilical hernia, cholelithiasis
8	CHD, status post bypass surgery, status post MI, cardiac aneurysm, global hypertrophy	Chronic congestion	Cysts	Chronic congestion	Thrombosis, benign hypertrophy	Thrombosis	Diverticulosis of the small bowel	Normal	Normal	Atherosclerosis	Cerebral sclerosis, status post cholecystectomy
9	Left atrial dilatation, CHD, status post MI	Fatty change	Shrinkage (left kidney)	Nonspecific acute splenitis	—	Normal	Pseudomembranous colitis	Normal	Normal	Atherosclerosis	NET of the lungs, osteoporosis
10	CHD, status post MI	Normal	Atherosclerosis, atrophy, cysts	Nonspecific acute splenitis	Benign hypertrophy	Normal	Normal	Normal	Normal	Slight atherosclerosis	Suspected septic encephalomalacia (brain dissection pending), pancreatic fibrosis, cholelithiasis
11	CHD, status post aortic valve replacement, biventricular hypertrophy	Normal	Normal	Normal	Status post prostatectomy	Thrombosis	Normal	Normal	Micronodular hyperplasia	Atherosclerosis	—
12	CHD, hypertrophy	Chronic congestion, fatty changes	Multiple cysts	Chronic congestion	Benign hypertrophy, thrombosis	Thrombosis	Normal	Normal	Micronodular hyperplasia	Atherosclerosis	Cerebral sclerosis, hemorrhagic cystitis

CHD = coronary heart disease; MI = myocardial infarction; NET = neuroendocrine tumor; PEG = percutaneous endoscopic gastrostomy; VATS = video-assisted thoracoscopic surgery.  
 \* No abnormal findings were present in the testes or ovaries of any patient.

**Appendix Figure 2.** Mononuclear infiltrations consisting of lymphocytes (*arrows*) in the myocardium of the right ventricle (case 3) (hematoxylin-eosin stain; original magnification,  $\times 100$ ).

