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Women in science - hematology 2023

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Women in science - hematology 2023

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Editorial: Women in science - hematology 2023

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Editorial on the Research Topic
Women in science - hematology 2023

This Research Topic is part of the Women in Science 2023 series. Other titles in the series include Women in Science—Gastroenterology 2023, Women in Science—Regulatory Science 2023, and Women in Science—Rheumatology 2023. Building on the success of Women in Science—Hematology 2021 (1), we are pleased to present a new volume for 2023 of this Research Topic. While the proportion of women and men in science, technology, engineering, and mathematics (STEM) at undergraduate levels is relatively equal, there is a noticeable lack of representation of women in senior positions in public health. According to 2016 data from the UNESCO Institute for Statistics (UIS) data, women represent <30% of researchers in STEM and <4% of Nobel Prizes for science. In Hematology, many highly influential and successful women are contributing to the field and tackling important questions. Nevertheless, female scientists are still underrepresented in various aspects of academic life. Several initiatives have been recently created to increase the visibility of women in science, such as awards for women in STEM. However, evidence indicates that gender bias is still present throughout many scientific disciplines.

This Research Topic aims to highlight female contributions to medicine, specifically in the field of Hematology and will therefore welcome:

- General perspectives on a specific field of research inspired, started or sparked by a woman.
- Articles celebrating outstanding female researchers and their contributions to computer science and public health.
- Public Health studies led by women researching technology and health.

All articles considered for this Research Topic were written by female scientists as first or last authors. Early career scientists were encouraged to team up with senior female colleagues After a rigorous peer review process, seven articles were published.

(i) Palomo et al. have reviewed mechanisms and suggested biomarkers of endothelial damage. A better understanding of endothelial injury is necessary for future preventive or therapeutic strategies.

(ii) In the context of paroxysmal nocturnal hemoglobinuria (PNH), Du et al. have reported a rare case of hemorrhagic esophageal varices with portal vein thrombosis that was treated with transjugular intrahepatic portosystemic shunt (TIPS).

(iii) In a collaboration of first and last female authors, Kalamara et al. have performed a meta-analysis of observational studies, highlighting the significant association of splenectomy with thrombosis but not with pulmonary hypertension in patients with transfusion-dependent thalassemia.

(iv) In the life-threatening field of thrombotic microangiopathies, Gavriilaki et al. reported the safety and efficacy of caplacizumab in immune thrombotic thrombocytopenic purpura (TTP), including cases refractory to plasma exchange, re-administration, and cases without previous plasma exchange treatment.

(v) In an interesting review, Kaddoura et al. provided a practical guide to the management of cardiopulmonary toxicities related to tyrosine kinase inhibitors in patients with chronic myeloid leukemia.

(vi) From the nursing perspective, Yang et al. reviewed the management of treatment-related venous thromboembolism in multiple myeloma. Through this retrospective analysis, the researchers identify the necessity for effective risk assessment models to provide a more accurate prediction of thrombosis.

(vii) Xu et al. (2) demonstrate how the addition of two condition-specific bolt-on items can increase performance on the EQ-5D-5L in patients with hemophilia.

(viii) Last but not least, Shafqat et al. summarized the role of neutrophil extracellular traps in diabetes mellitus complications and highlight the importance of clinical trials to translate the results of these studies.

Considering the multi-disciplinary character of this Research Topic, we hope that it will inspire female researchers and clinicians to continue their explorations into novel advances in their fields.

Author contributions

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Conflict of interest

EG has received honoraria from Alexion, Gilead, Sanofi, Sobi, and Omeros Pharmaceuticals.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Emerging role of neutrophil extracellular traps in the complications of diabetes mellitus

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Immune dysfunction is widely regarded as one of the central tenants underpinning the pathophysiology of diabetes mellitus (DM) and its complications. When discussing immunity, the role of neutrophils must be accounted for: neutrophils are the most abundant of the circulating immune cells and are the first to be recruited to sites of inflammation, where they contribute to host defense *via* phagocytosis, degranulation, and extrusion of neutrophil extracellular traps (NETs). NETs are composed of DNA associated with nuclear and cytosolic neutrophil proteins. Although originally reported as an antimicrobial strategy to prevent microbial dissemination, a growing body of evidence has implicated NETs in the pathophysiology of various autoimmune and metabolic disorders. In these disorders, NETs propagate a pathologic inflammatory response with consequent tissue injury and thrombosis. Many diabetic complications—such as stroke, retinopathy, impaired wound healing, and coronary artery disease—involve these mechanisms. Therefore, in this review, we discuss laboratory and clinical data informing our understanding of the role of NETs in the development of these complications. NET markers, including myeloperoxidase, citrullinated histone H3, neutrophil elastase, and cell-free double-stranded DNA, can easily be measured in serum or be detected *via* immunohistochemical/immunocytochemical staining of tissue specimens. Therefore, NET constituents potentially constitute reliable biomarkers for use in the management of diabetic patients. However, no NET-targeting drug is currently approved for the treatment of diabetic complications; a candidate drug will require the outcomes of well-designed, robust clinical trials assessing whether NET inhibition can benefit patients in terms of morbidity, quality of life, health expenditures, and mortality. Therefore, much work remains to be done in translating these encouraging pieces of data into clinical trials for NET-targeting medications to be used in the clinic.

KEYWORDS

diabetes mellitus, neutrophil extracellular traps, atherosclerosis, thrombosis, macrovascular and microvascular complications

Introduction

Diabetes mellitus (DM)—both type 1 (T1DM) and type 2 (T2DM)—is characterized by hyperglycemia, which chronically leads to several vascular complications. The global prevalence of DM is estimated to be 9.3% (463 million people) and is projected to rise to 10.9% (700 million people) by 2045. The healthcare and economic burden of DM is proportionately massive: the 10th edition Atlas of the International Diabetes Federation estimates the number of deaths caused by diabetes in 2021 at 6.7 million.

Diabetes mellitus is a major cause of cardiovascular disease, including strokes and myocardial infarction (MI), and is the leading cause of non-traumatic limb amputations, end-stage renal disease (ESRD), and adult blindness (1). This makes diabetes mellitus a major threat to public health and a risk factor for premature death. To understand these complications better and provide a foundation for the development of interventions, it is essential to study the potential mediators of these effects. In this regard, autoimmune destruction of pancreatic β-cells is central to the development of T1DM (2). Similarly, although T2DM is underpinned by peripheral insulin resistance, it features chronic low-grade sterile inflammation in which the role of adaptive immunity has been extensively studied (3).

Neutrophils are important to acknowledge when studying inflammation; they are the most abundant of all immune cells and the first to be recruited to sites of acute inflammation. Neutrophils contribute to host defense by phagocytizing microbes and degranulating to release antimicrobial effectors. In 2004, Brinkmann et al. described a novel neutrophil function called NETosis, which involved the release of DNA decorated with cytosolic and granular proteins as web-like structures called neutrophil extracellular traps (NETs) (4).

The beneficial roles of NETs remain best characterized in the context of infections (5). Generally speaking, neutrophils release NETs when microbes overwhelm other neutrophil functions. For instance, neutrophils undergo NETosis in fungal infections, as fungi are too large for neutrophils to phagocytose (5, 6). This is underscored by studies in humans and mice, which show that defective NET production predisposes to severe and recurrent fungal infections (6, 7). This is not the case for bacteria, which can be phagocytized and eliminated *via* the respiratory burst. Therefore, NET-mediated protection for bacterial infections is more selective. For instance, Brinkmann et al.'s study and other reports have shown that NETs bind gram-positive and gram-negative bacteria, preventing their dissemination—which is essential in combating septic shock—accompanied by the degradation of virulence factors by NET-associated proteases (4, 8). Furthermore, interestingly, impaired killing of *Shigella flexneri and* Group A Streptococci is seen in NET-deficient mice, suggesting that perhaps NETs exert unique, non-redundant antibacterial functions in these infections (9). By contrast, data on NETs as mediators of protection in parasitic infections is inconclusive, whereas they are primarily considered pathologic in viral infections such as influenza and SARS-CoV-2 (5).

However, NET production is mainly inappropriate in other diseases, including autoimmune and metabolic disorders and cancers. Indeed, NET production is a significant driver of a whole host of non-infectious pathologies. In keeping with the focus of this review, NET markers—MPO-DNA, cit-H3, and NE—are elevated in diabetic mice and humans who develop these complications, thereby paving the way for clinical studies evaluating the robustness of NET markers as prognostic markers and therapeutic targets. Therefore, in this review, we survey the literature on the role of NETs in the complications of DM and suggest how future research can expand upon these findings.

NETosis, NET formation, and methods of detection

NETosis is a unique form of cell death (suicidal NETosis) that produces NETs. Neutrophils can also produce NETs while still retaining membrane integrity and effector functions, *via* a mechanism called vital NET production. Another form of NETs contains largely mitochondrial DNA (mtDNA) and is termed mitochondrial NET production. Therefore, the term "NETosis" specifically refers to NET release accompanied by lytic neutrophil death and does not include vital and mitochondrial NET formation (Figure 1).

NETosis begins with disruptions to the characteristic lobular nuclear architecture of neutrophils and chromatin decondensation (10). This is followed by the disintegration of the nuclear membrane with the release of DNA and histones into the cytosol. Lastly, the plasma membrane ruptures occur, thereby extruding NETs into the extracellular space (5). From a mechanistic standpoint, these cellular pathways are predicated on the activation of NADPH oxidase-2 (NOX2), which produces reactive oxygen species (ROS) *via* a respiratory burst. It is important to note that protein kinase C (PKC) isoforms and mitogen-activated kinases (MAPK) regulate NOX-2 to influence NETosis and NET formation. ROS trigger the release of myeloperoxidase (MPO) and neutrophil elastase (NE) from azurophilic granules, both of which translocate to the nucleus and synergize to partially degrade histones and thereby loosen their association with DNA—resulting in chromatin decondensation (11, 12). Importantly, DNA inhibits proteases, meaning this close association between MPO, NE, and DNA allows both enzymes to retain their enzymatic activity in a highly proteolytic environment, which later enables them to exert protective or pathologic effects extracellularly. Chromatin decondensation is augmented by peptidylarginine deaminase 4 (PAD4)—also ROS-dependent—which citrullinates histones to decrease their positive charge, hence reducing their electrostatic attractions between DNA and loosen the chromatin (13, 14).

This is followed by disassembly of the nuclear envelope and release of chromatin associated with histones, NE, and MPO into the cytosol. Finally, pores in the plasma membrane form, through which NETs are extruded into the extracellular space, resulting in NETosis (15).

Neutrophils can alternatively release NETs without lysing, a process termed vital NET formation. This allows neutrophils to retain their effector functions, such as chemotaxis, phagocytosis, and degranulation. Nonlytic NET formation has particularly been studied in the context of infection, where live neutrophils have been visualized as releasing NETs during crawling but without lysing (16, 17). Importantly, nonlytic NET production occurs independently of NADPH-oxidase. Mechanistically, lipopolysaccharides in the cell wall of gram-negative bacteria can activate platelets *via* Toll-like receptor 4 (TLR4). Activated platelets can adhere to neutrophils *via* P-selectin and stimulate NET production through platelet-derived high mobility group box protein B1 (HMBG1) binding to receptors for advanced glycation end-productions (RAGE) on neutrophils (18, 19). In vital NET production, activated PAD4—a step that occurs independently from NADPH oxidase—translocates to the nucleus to citrullinate histones, followed by extrusion of decondensed chromatin, histones, and other embedded protein in blebs of nuclear membrane, which is then resealed (20). Notably, Yousefi et al. reported ROS-dependent nonlytic NET production containing mtDNA and not nuclear DNA, termed mitochondrial NET formation (21). In terms of upstream regulators, activation of TLR4 and complement factor 5a (C5a) receptor after the application of GM-CSF result in mitochondrial NET formation (21).

Collectively, this is compelling evidence for the heterogeneity of NETs, depending on the type of the activating stimulus. Importantly, NETs heterogeneity could have important clinical implications as different stimuli may confer differential NET compositions. For instance, NETs containing mitochondrial DNA would not contain histones. How these varying compositions translate into differing protective and pathologic functions of NETs remains unclear. In the context of diabetic complications, studying the inducers of NETosis and NET production as will be discussed later herein may provide clues as to the types of NETs present in these circumstances. Numerous lines of evidence have shown NET production and NET markers to be elevated in DM patients. From a mechanistic perspective, hyperglycemia activates NOX2, evidenced by heightened ROS generation by neutrophils after high glucose stimulation, and its abrogation following treatment with NOX inhibitors (22). However, the interplay between or independent contributions of comorbidities and lifestyle habits that frequently accompany diabetes—such as obesity, hypertension, smoking, and aging—to NET production in addition to hyperglycemia remain unexplored. In this regard, whereas obesity has been reported to reduce neutrophil NET-producing capacity, all of the other aforementioned factors promote NET production or feature NET contributions in their pathophysiology (23–26). Therefore, in a clinical context, these factors may represent important variables to control for when assessing NETs in diabetics.

There is currently no gold standard modality for detecting NETs. For this reason, studies have advocated using combinations of detection methods rather than a single method (27). For instance, markers of NET production include MPO, citrullinated histone-3 (cit-H3), NE, and extracellular DNA, which have been measured in the serum of patients with inflammatory illnesses. Other techniques, such as flow cytometry, immunocytochemistry, and immunohistochemistry are also commonly used to detect NETs (28, 29). Investigators caution that DNA and histones are also released by necrosis and tissue death, underscoring the importance of objectively defining what constitutes NETs. In this regard, most studies identify NETs by colocalizing at least three NET components, most commonly, DNA, histones, NE, and/or MPO. Lastly, live cell imaging and intravital imaging may allow real-time observations of NETs *in vivo* (30, 31).

Role of NETs in the complications of diabetes

The so-called macrovascular complications of diabetes are related to the development of atherosclerosis, chronically resulting in coronary artery disease (CAD), MI, strokes, and peripheral vascular disease. Microvascular complications of diabetes include retinopathy, nephropathy, and neuropathy. The following section summarizes and interprets basic science and clinical data associating NETs with the development and outcomes of the aforementioned complications.

Coronary artery disease

Diabetes is associated with an increased risk of atherosclerosis, with coronary artery disease (CAD) remaining the most common cause of mortality in diabetics (32). CAD occurs secondary to the development of atherosclerotic plaques, which progressively occlude coronary circulation, causing stable angina. Acute coronary syndrome (ACS) is the major complication of CAD and is typically the result of the rupture of an atherosclerotic plaque, upon which a thrombus is formed. The pathogenesis of atherosclerosis begins with endothelial cell dysfunction, with resultant monocyte migration into the tunica intima, where the monocytes eventually transform into foam cells and drive plaque development. T-cells also comprise a major portion of the immune cell landscape of both intact and complicated atherosclerotic

plaques. However, studies have also investigated (1) the presence of NETs in atheromas, (2) whether NETs contribute to plaque development and/or complications, and (3) if NET markers correlate with the severity of CAD or outcomes of ACS.

Megens et al. initially demonstrated the presence of NETs in both mice and human atherosclerotic plaques (33). Subsequent immunohistochemical staining for NETs in human atherosclerotic plaques showed them to be concentrated at sites of superficial erosions next to apoptotic endothelial cells, a process that results in thinning of the overlying fibrous cap making the plaque more prone to rupture and subsequent thrombosis (34). Histological analyses conducted on plaque specimen samples from the human carotid artery reveal neutrophils and NETs to be localized in plaques with a large lipid and low fibrous content, which are indicators of plaque instability (35). These results suggest that NETs play a role in plaque destabilization or complications. A recent study, attempting to explore the mechanisms underlying this observation, revealed that citrullinated histone H4 (cit-H4) kills smooth muscle cells and endothelial cells to cause fibrous cap thinning (36, 37).

Accordingly, immunohistochemical staining of human coronary artery atheromas reveals the presence of NETs in complicated plaques (including intraplaque hemorrhage, eroded plaques, or fibrous cap ruptures) but not in intact plaques (38). This is backed by clinical data suggesting that neutrophils may contribute to plaque complications: Borissoff et al. showed NET components—dsDNA, nucleosomes, cit-H4, and MPO-DNA complexes—to be significantly higher in patients with severe CAD than in healthy controls and to be associated with the severity of luminal stenosis and occurrence of major cardiac events, including ACS, percutaneous coronary intervention, coronary artery bypass grafting and cardiac death (39). NETs may also impair plaque resolution: Josefs et al. showed NETs impairing plaque resolution in mice by promoting macrophage-mediated plaque inflammation, evidenced by elevated levels of anaerobic glycolysis and inflammasome (40). Furthermore, NETs decline in resolving plaques, and treatment with exogenous DNAse-1 reduced atheroma NET content and macrophage inflammation, promoting plaque resolution when given adjunctively to lipid-lowering therapy (40).

However, despite these encouraging preclinical findings and intriguing hypotheses, the clinical utility of NET markers in diabetics at risk of CAD has not been proven. Conversely, a recent paper studying NET markers in T1DM and their link to CAD did not find any significant differences in NET markers between T1DM patients and age-matched healthy controls. Furthermore, NETs did not differ significantly in type 1 diabetics according to the presence of CAD.

Myocardial infarction

Crucially, NETs are well-recognized mediators of thrombosis: NETs interact with platelets to promote thrombogenesis, NETs promote the generation of thrombin, and NET constituents such as tissue factor, factor XII, histones H3 and H4, cell-free DNA, and fibrinogen, are all prothrombotic (37). Therefore, researchers have investigated the contribution of NETs to coronary thrombosis and MI in depth.

Indeed, higher circulating NET markers (MPO-DNA, NE-DNA, cit-H3) and platelet activation markers (soluble P-selectin) confer an increased risk of major cardiovascular events (MACEs) 12 months after MI (41). NETs are found in coronary artery thrombi, particularly in fresh thrombi rather than older, organized thrombi (42). Another study used colocalization of NE and extracellular dsDNA to identify NETs in 25% of coronary stent thrombi (43). Importantly, Cui et al. reported higher dsDNA levels in ACS patients than in stable angina (SA) patients and healthy controls, informing the potential utility of dsDNA as a biomarker in this setting (44). Furthermore, in the ACS group, significant differences were shown in dsDNA levels observed between unstable angina (UA), non-ST elevation myocardial infarction (NSTEMI), and ST-elevation myocardial infarction (STEMI) patients (44). NET markers dsDNA, MPO-DNA, and cit-H3 reveal a higher NET burden in STEMI patients at the culprit site lesion as compared to other areas such as the femoral artery (45). Furthermore, NET burden at the culprit site correlates positively with infarct size, assessed by cardiac enzyme elevation and cardiac MRI, and left ventricular dysfunction, assessed by wall-motion score index at a year follow-up (45). Circulating MPO and NE levels significantly decrease after MI treatment (46).

Neutrophils also play a role in acute cardiac fibrosis post-MI, as necrosis is followed by inflammation and healing by fibrosis, but crucially release NETs that contribute to chronic cardiac fibrosis and remodeling after MI (47). Pathologic cardiac remodeling increases the risk of ventricular aneurysm formation, which may be lethal. In this regard, NETs are detected in ventricular aneurysms in both humans and mice. NETs are also elevated in the peripheral blood of patients that develop ventricular aneurysms (47).

Importantly, DNAse-1, an enzyme that degrades NETs, correlates negatively with infarct size and positively with ST-segment resolution, and *ex vivo* administration of DNase-1 accelerates lysis of coronary thrombi (45). In mouse models of MI, administering a PAD4 inhibitor GSK484 intraperitoneally reduces infarct size and improves cardiac function (48). DNAse-1 administration also abrogates NET-induced cardiac fibrosis both *in vivo* and *in vitro*, suggesting a prognostic and therapeutic role of NETs in pathologic cardiac fibrosis and secondary ventricular aneurysms (47).

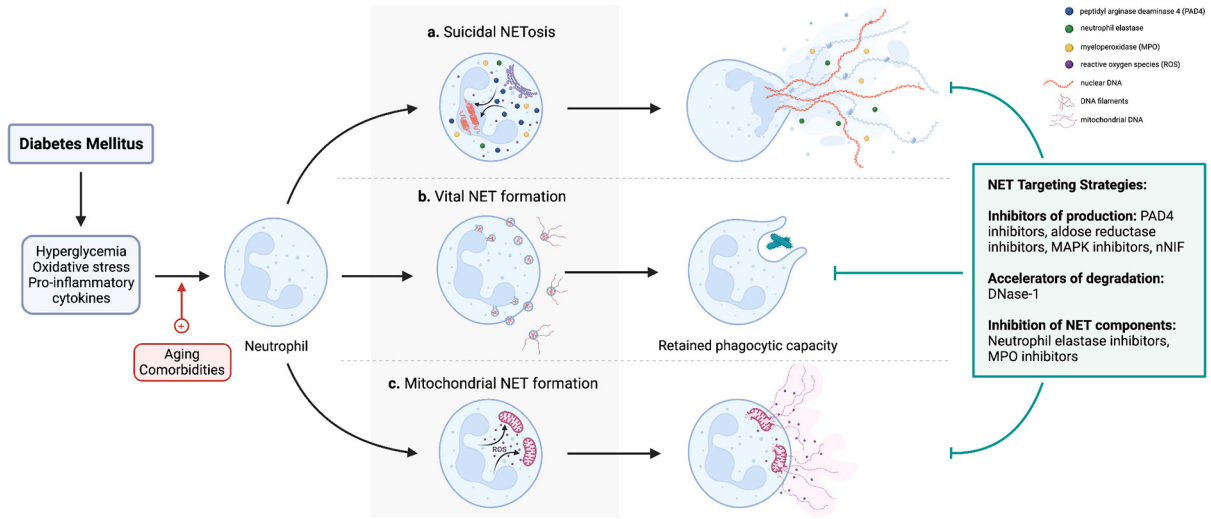

FIGURE 1
A diagrammatic representation of the different pathways of NETosis and NET production. Importantly, whereas (a) NETosis entails release of NETs with concomitant neutrophil apoptosis, neutrophils retain viability and effector functions after (b) vital and (c) mitochondrial NET production. In the context of diabetes, hyperglycemia activates NADPH oxidase, and oxidative stress and elevated pro-inflammatory cytokines consequent to a chronic sterile inflammatory state induce NETosis and NET production. However, if and how age and co-existing cormorbidities such as obesity contribute to these pathways are unclear. Since numerous studies have implicated NETs in the pathogenesis of diabetic complications, strategies to mitigate the effects of NETs include preventing their production (e.g., PAD4 inhibitor, NE inhibitors), accelerating their degradation (DNAse-1), or inhibiting NET components to ameliorate their respective pathologic effects (NE inhibitors, other protease inhibitors e.g., MMP-9). However, it should be noted that no drug currently targeting NETs is approved for prevention or treatment of diabetic complications.

Stroke

Ischemic stroke is a major macrovascular complication of diabetes. Similar to CAD, the role of NETs in plaque destabilization and thrombosis is highly relevant in ischemic stroke. NETs indeed are detected in almost all thrombi retrieved from ischemic stroke patients analyzed by immunohistochemical staining (49). Hyperglycemia is associated with poor outcomes in ischemic stroke patients. To explain this, hyperglycemia induces NETosis; NET infiltration is more extensive in ischemic stroke thrombi retrieved from hyperglycemic patients compared to normoglycemic patients (50). Importantly, blocking NET formation with Cl-amidine (a PAD4 inhibitor) reduced brain infarction volume and alleviated neurologic deficits in diabetic and wild-type (WT) mice (50).

Interestingly, in contrast to coronary thrombi, NETs are predominantly present in older ischemic stroke thrombi rather than fresh thrombi (49). In murine models of ischemic strokes, dense neutrophilic infiltration and NETs are present throughout the brain tissue (51). Mechanistically, platelet-induced NET formation *via* high-mobility group box 1 protein (HMGB1) expression was found to be the dominant mechanism of NET formation in these models, exemplified by HMGB1-depleted mice showing significantly lower plasma NET levels after stroke with greatly improved clinical outcomes (51).

Importantly, NETs may increase resistance to tissue plasminogen activator (tPA) therapy; NET content cerebral thrombi correlated significantly with endovascular therapy procedure length and the number of thrombectomy device passes performed to achieve successful recanalization (52). Importantly, combining DNase-1 with conventional tPA more effectively lysed patient thrombi as compared to tPA alone (49, 52). However, DNase-1 alone was not effective in *ex vivo* clot lysis, which is in contrast to findings from coronary thrombi (52). To explain this, Farkas et al. compared NET content in ischemic stroke clots, coronary thrombi, and peripheral artery disease (53). NET content was lowest in ischemic stroke thrombi, perhaps explaining why DNAse-1 alone was unable to effectively degrade these thrombi. Other than accelerating NET degradation *via* DNAse-1, inhibiting NET formation by targeting PAD4 is a feasible strategy: PAD4 overexpression impairs revascularization and vascular remodeling in stroke, while PAD4 inhibition restores angiogenesis (54). Lastly, treating mice with neonatal NET-inhibitory factor (nNIF) reduced the size of brain infarcts, improved long-term neurological and motor function, and enhanced survival after stroke (51). Another crucial finding from this study was that nNIF improved stroke outcomes in diabetic mice, even when administered 1 h after stroke onset (51).

These findings warrant investigations into NETs as prognostic markers for stroke patients including diabetics. Furthermore, clinical trials evaluating a combination of DNAse-1 and tPA as stroke therapy vs. conventional tPA alone are needed to confirm the utility of NETs as therapeutic targets in stroke. The same applies to nNIF, which, as mentioned above, has recently shown encouraging results as a stroke therapy.

Diabetic retinopathy

Diabetic retinopathy (DR) is the most common microvascular complication of diabetes, being more common in T1DM than T2DM, and is the leading cause of adult blindness globally (55, 56). A third of diabetic patients suffer from DR, of which a third is vision-threatening (57). DR is divided into two pathophysiologically distinct stages based on fundoscopic findings: an early non-proliferative (NPDR) stage and a later proliferative (PDR) stage. NPDR is characterized by pericyte and endothelial cell dysfunction with a consequent increase in capillary permeability and capillary occlusion, manifesting on fundoscopy as micro-aneurysms, hard exudates, and hemorrhage. NPDR is often clinically asymptomatic (58). PDR, on the other hand, represents an advanced stage, occurring secondary to angiogenesis mediated by vascular endothelial growth factor (VEGF). It manifests as neovascularization on fundoscopy but is also sometimes accompanied by vitreous hemorrhage and/or retinal detachment. PDR often results in pronounced visual impairment. More importantly however is diabetic macular edema (DME), which can occur at any stage of DR and is the most common cause of blindness in diabetic retinopathy (58).

Hyperglycemia is a major player in the pathogenesis of DR. The retinal blood vessels dilate in response to the high blood glucose, increasing local blood flow (59). Hyperglycemia also induces apoptosis of pericytes, increasing capillary permeability but also causing outpouching of these segments of the capillary wall to form micro-aneurysms. Apoptosis of endothelial cells also occurs, collectively leading to hypoxia and induction of hypoxia-inducible factor-1α (HIF-1α), the transcription factor which activates the expression of vascular endothelial growth factor (VEGF) (58).

Inflammation also contributes to the pathogenesis of DR. For instance, leukostasis is a central part of occlusion of the retinal microvasculature. Furthermore, pro-inflammatory cytokines such as IL-1β, TNF-α, IL-6, and IL-8 are significantly increased in diabetic patients and correlate positively with disease severity (60). Many of these cytokines attract neutrophils and induce NETosis/NET production. DR patients present with a high neutrophil-to-lymphocyte ratio, which correlates with disease severity, establishing its potential utility as a biomarker of DR severity (61). In addition, murine models of DR exhibit dense retinal neutrophilic infiltrates which adhere to endothelial cells, causing leukostasis (22). Circulating DNA-histone complexes and NE were significantly elevated in diabetic patients with retinopathy compared to those without retinopathy. These markers were significant independent risk factors of retinopathy (62, 63). In agreement with these findings, a later study demonstrated that injecting IL-8 or TNF-α intravitreally into the eyes of mice resulted in infiltration of neutrophils producing NETs, as evidenced by positive immunohistochemical staining for NE, MPO, and cit-H3. These markers were also elevated in vitreous samples of patients with PDR and, also, correlated significantly with its severity (64).

Taken together, these findings suggest that chronic hyperglycemia disrupts the inner blood-retinal barrier, resulting in non-proliferative diabetic retinopathy (65). This disruption also can result in neutrophil migration into the retina, causing leukostasis and inflammation, two events crucial for DR progression (65). Subsequently, the pro-inflammatory cytokines listed above may be responsible for NET production. Hyperglycemia itself also induces NETosis in a dose-dependent manner (22, 62). Regarding the specific contribution of NETs in DR pathogenesis, the NET component NE has been shown to contribute to vascular damage in DR (66). Another important neutrophil protein is lipocalin-2 (LCN2), which is elevated in cases of PDR and has been shown to activate HIF-1α (67–69). LCN2 may also inhibit the degradation of matrix metalloproteinase 9 (MMP9) to enhance apoptosis of retinal capillary cells and promote angiogenesis and neovascularization (70).

From a clinical standpoint, DNAse-1 abrogates NETs in the anterior and posterior chambers of mice eyes (64). Seeing as NE may contribute to the pathogenesis of DR, future research should assess if inhibiting this enzyme brings any benefits in ameliorating the severity of DR. LCN2 also warrants extensive analysis to fully uncover its use as a potential biomarker for DR severity and as a therapeutic target in this setting. In this regard, inhibiting serine/threonine kinase AKT2, an upstream regulator of LCN2, has been utilized in the early stages of age-related macular degeneration to reduce inflammation, neutrophil infiltration, and activation of retinal glial cells, but it has yet to be tested in models of DR (71).

Other than LCN2, numerous studies have shown the benefit of mitogen-activated protein kinase (MAPK) inhibitor annexin-1 in attenuating the microvascular complications of diabetes, namely nephropathy and cardiomyopathy, but their use in DR remains unknown (72). Another potentially useful class of such molecules is the lipidic pro-resolving mediators called lipoxins, specifically lipoxin-A4 (LXA4). LXA4 is synthesized in the inner retina and its levels decrease following eye injury (73). Both NPDR and PDR patients have lower serum levels of LXA4 compared to diabetics without retinopathy; lipoxin-A4 levels were also lower in the vitreous of PDR patients (74). LXA4 reduces the production of proinflammatory cytokines, including VEGF. Because this effect is deficient in DR, assessing the

potential benefit of exogenous LXA4 administration in DR could prove fruitful (75).

Lastly, the use of purinergic receptor blockers in age-related eye disorders, including DR, is informed by the high amount of ATP released in these conditions, which bind to $P2X_7$ receptors. Stimulation of $P2X_7$ receptors positively regulates a HIF-1α-mediated expression of VEGF, thereby propagating angiogenesis and neovascularization in PDR (76). In this context, blocking the $P2X_7$ receptor has been shown to reduce inflammation in rat models of DR (77). Two different $P2X_7$ antagonists, A740003 and AZ10606120, have recently been shown to reverse increased vascular permeability and VEGF expression in a model of streptozotocin-induced hyperglycemic retinopathy in rats. The production of many cytokines, such as IL-1β and TNF-α, is also abolished by $P2X_7$ inhibition. These cytokines, as discussed above, activate NETosis, suggesting that elimination of NETosis may be one of the pathways by which $P2X_7$ antagonists exert their beneficial effects. The specific pathways activated by $P2X_7$ and its utility as a therapy for DR were recently described in a review (76).

An excellent review by Martínez-Alberquilla et al. detailed the specific effects of these medications in age-related eye disorders, including DR (65). This paper also raised an important point with regards to NET markers: future comparative studies should evaluate whether NET markers in tear film and on the ocular surface are reliable predictors of DR compared to blood and intra-ocular samples, as they are much easier and less invasive to attain. The use of such techniques is suggested as a potential avenue to monitor ocular and neurodegenerative diseases, with LCN2 being suggested as a biomarker and therapeutic target in both conditions (78). Future comparative studies assessing blood, intra-ocular, and tear film NET markers as independent and combined predictors of DR severity and progression will undoubtedly shed some light on what is the most accurate sampling method to measure these biomarkers.

Diabetic nephropathy

Diabetic nephropathy (DN) is a form of chronic kidney disease (CKD) that occurs in 20–40% of long-standing diabetics (79, 80). The progression of diabetes-associated CKD to end-stage renal disease (ESRD) is drastic, with 50% of T1DM patients developing ESRD within 10 years of onset of their kidney disease symptoms, specifically proteinuria. This makes DM the leading cause of ESRD globally (81). While chronic hyperglycemia is the key player in the pathogenesis of diabetic nephropathy, it alone does not explain all the pathologic changes which occur in this disease. In this context, recent evidence has considered inflammation crucial in the progression of diabetic nephropathy.

The pathophysiological hallmarks of diabetic nephropathy culminate in glomerular endothelial cell injury, resulting in the expression of adhesion molecules P- and E-selectin on endothelial cells that attract immune cells such as neutrophils. Furthermore, NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3) inflammasome activation is recognized to underpin DN pathogenesis and proinflammatory cytokines such as IL-6, IL-8, TNF-α, IL-17 (a neutrophil chemoattractant), and IL-18 are significantly elevated in the serum and urine of diabetic patients (82). Neutrophil adhesion and homing are significantly increased in diabetic patients with overt proteinuria compared to diabetic normoalbuminuric patients and healthy, non-diabetic controls (83). Neutrophils in diabetics also show an activated phenotype with increased ROS production, which may damage endothelial cells to cause DN progression (84). Thus, the data suggest that neutrophil homing into DN kidneys may, at least in part, contribute to glomerular inflammation with subsequent scarring.

NETosis is a ROS-dependent process, and the inflammasome-dependent cytokines IL-1β and IL-18 are well-established inducers of NETs. Hyperglycemia itself, which is the major factor underpinning DN pathogenesis, also induces NETosis. There is also a wealth of data linking NETs to inflammatory kidney disorders, including acute kidney injury, systemic lupus erythematosus, and the ANCA-positive vasculitides (85–88). Judging by these observations, it can be assumed that NETs play a role in DN, albeit a direct link between NETs and diabetic nephropathy remains yet to be demonstrated. A study demonstrated significantly higher levels of circulating cell-free DNA in T2DM patients with nephropathy compared to those without nephropathy. Furthermore, T2DM patients in general showed higher levels of NET markers, including nucleosomes, dsDNA, and NE (89). Consistent with this study, Miyoshi et al. demonstrated that higher MPO-DNA levels in T2DM patients correlated positively with clinical and laboratory parameters regarded as risk factors (e.g., prolonged disease duration, elevated BMI, albuminuria, and elevated liver function tests) for microvascular complications including DN (90).

The same study showed that hyperglycemia induces NETosis *via* the polyol pathway (90), which has been supported by other studies (91). Accordingly, treatment with ranirestat—an aldose reductase inhibitor (ARI) acting upstream of the polyol pathway by inhibiting sorbitol production—eliminates NET formation (90). Contrastingly, Sotrastaurin, an inhibitor that acts more downstream in the polyol pathway, only partially mitigates NETosis (90). ARIs are used clinically for the treatment of diabetic neuropathy and retinopathy, but numerous studies have also tested their use in DN. For instance, Tolrestat was found to prevent glomerular hypertrophy, mesangial expansion, glomerular basement membrane thickening, mesangial expansion and hypocontractility, and progression of albuminuria in streptozotocin-induced diabetic rat models (92). Epalrestat, another ARI, significantly improved renal arterial blood flow, which plays a protective role in early-stage DN (93). Furthermore, it was recently demonstrated that epalrestat

significantly reduced albuminuria, the fusion of podocyte foot processes, and interstitial fibrosis in the kidneys of *db/db* mice (a widely used mouse model of T2DM) (94). In agreement with these findings, treating microalbuminuric T2DM patients with epalrestat for 5 years prevented significant increases in urinary albumin excretion (95). These results suggest that inhibition of the polyol pathway is a potential strategy for the treatment of DN.

Seeing as inhibiting aldose reductase significantly abolishes NETosis, perhaps NET inhibition could account for one of the pathways by which ARIs achieve their therapeutic effect in diabetes. However, serum levels of NET markers may not be reflective of potential renal NET infiltration. NETs also have not yet been physically visualized in the kidneys of DN. In this regard, assessing for the presence of NETs by immunofluorescence and quantifying their presence on renal biopsy specimens would directly implicate NETs in DN pathogenesis, as has been done in the case of lupus nephritis (96). Therefore, questions for future research include: are NETs physically present in DN kidneys? Do NETs presence in DN kidneys or elevated circulating NET markers correlate with clinical and laboratory markers of DN severity? Does inhibiting NET formation or accelerating NET degradation clear NETs within kidneys? And does NET inhibition result in clinical improvement in renal function?

Impaired wound healing

Diabetic foot ulcers (DFUs) are ulcers or wounds commonly located at the bottom of the foot that occur due to both atherosclerotic peripheral artery disease and peripheral neuropathy. DFUs are responsible for more diabetes-related hospitalizations than any other diabetic complication (97). But more importantly, 40% of patients presenting with the diabetic foot will require amputation, making diabetes mellitus the leading cause of non-traumatic amputations worldwide (98); Zhang et al. identified the prevalence of DFUs to be as high as 13% in North America (99).

Physiologic wound healing comprises 4 stages: hemostasis, inflammation, proliferation, and remodeling. Many conditions disrupt one or more of these steps to impair wound healing. These conditions include either local (e.g., ischemia, foreign bodies, infection) or systemic perturbations (e.g., DM, obesity, medications, hypothyroidism, etc.) (100). On a mechanistic level, many of these factors share common pathophysiology in that they propagate an inappropriate inflammatory response detrimental to wound healing.

As neutrophils are the first cells to be recruited to sites of acute inflammation, their contribution to physiologic and pathologic wound healing has garnered much interest. Mechanistically, neutrophilic migration into wounds may be mediated by factor XII (FXII) (101). Indeed, FXII-deficient mice show reduced neutrophil infiltration into wounds compared to WT mice. FXII is synthesized by neutrophils themselves and mediates the trafficking of neutrophils into sites of inflammation (102). This effect is mediated by FXII binding to urokinase plasminogen activator receptor (uPAR) that downstream induces expression of integrin. Importantly, activation of uPAR by FXII results in increased intracellular calcium and NETosis. FXII-deficient mice showed decreased inflammation and faster wound resolution than WT mice, consistent with a detrimental role of NETs in diabetic wound healing (102).

As mentioned above, hyperglycemia causes NET production, which predisposes diabetics to higher levels of NETosis and consequently impaired wound healing (27). NETs are abundant in mice wounds and are absent in unwounded skin (103). Proteomic analysis of non-healing vs. healing DFUs showed enrichment of NET-related proteins, including NE, histone H4, and neutrophil proteinase, in the non-healing group (104). A recent study demonstrated PAD4 overexpression at surgical sites after total joint arthroplasty in insulin-resistant subjects compared to insulin-sensitive subjects, which is associated with delayed surgical wound healing (105). Wounds in PAD4 knockout mice heal significantly faster than in WT PAD4-positive mice; on day 14 after the mice were subject to excisional skin wounds, 80% of all wounds in PAD4-depleted mice had healed, whereas only 25% healed in PAD4-positive mice (103). Yang et al. recruited a cohort of diabetic patients with active foot ulcers, calculated clinical scores of DFU severity, and evaluated for a potential correlation between NET-specific markers and the clinical severity of DFUs (106). Indeed, NET-specific markers were significantly in DFU patients than in diabetics without DFU or in healthy controls and correlated positively with diabetic ulcer severity score (DUSS) and the wound, ischemia, and foot infection (WIFI) DFU severity scores. Furthermore, DFU tissue NE levels were significantly higher in DFU cases that became infected and experienced delayed healing (106). This was supported by Fadini et al., who not only reported higher levels of NE but also proteinase-3 to positively correlate with the chances of wound infection (104). Lastly, cit-H3 was identified as an independent risk factor for impaired wound healing and amputation (106). Therefore, NE, proteinase-3, and cit-H3 may constitute biomarkers for the risk stratification of DFU patients.

To explain these findings, NE degrades the extracellular matrix (ECM) to delay wound healing (107). On the other hand, secretory leukocyte protease inhibitor (SLPI) degrades NE to counteract its pathologic effects. Accordingly, administering SLPI or other NE inhibitors accelerated wound healing (108, 109) while depleting SLPI has the reverse effect (110). MMPs in NETs also degrade the ECM. Chronic wounds indeed feature elevated protease levels (111). Tissue inhibitors of matrix metalloproteinase (TIMP) inhibit MMPs, but overproduction

of MMPs in NETs overwhelms this mechanism; indeed, a higher MMP/TIMP ratio predicts poor wound healing (112). MPO stabilizes NETs in wounds and exerts pro-inflammatory effects *via* oxidative stress (113). Extracellular histones activate complement, endothelial cells, and platelets to endothelial cells to promote local inflammation and hypercoagulability, reducing blood flow to wound areas delaying clearance of dead tissue, and impairing wound healing (114–116). NET components interact with many of the cellular constituents of wounds, including endothelial cells, macrophages, keratinocytes, and fibroblasts, a topic which has been covered extensively by Zhu et al. (27).

Specifically in diabetic wounds, excessive NET production activates the NLRP3 inflammasome in macrophages to increase IL-1β production, which in turn induces NETosis. The impact of this positive feedback loop is underscored by the elimination of NETs, significantly benefiting wound healing by reducing NLRP3 levels and mitigating the development of a pro-inflammatory M1 macrophage phenotype (117). Hence, future studies should further explore the clinical benefit of targeting this pathway. In this regard, milk fat globular epidermal growth factor VIII (MFG-E8) attenuates the NLRP3-NET axis to promote inflammation resolution and wound healing (118). Consistently, MFG-E8-deficient mice display dense neutrophilic infiltration into wounds and subsequent excessive NET production, associated with delayed wound closure (118). Another protein, leucine-rich alpha-2-glycoprotein 1 (LRG-1), although essential for timely wound closure, is elevated in diabetic patients and mice, where it causes NETosis which, when excessive such as in diabetics, actually impairs normal wound healing. Indeed, depleting LRG1 in mice is protective against impaired wound healing, acting partly through dampening inflammation by reducing NET overproduction (119).

Protein Kinase C βII (PKC βII) is another mediator of NET production; administering a PKC βII inhibitor such as ruboxistaurin reduces NET production, increases local perfusion to wounds, and increases endothelial cell proliferation, all encouraging physiologic wound healing (120). Gonadotropin-releasing hormone (GnRH) can bind to GnRH receptors on neutrophils to induce NETosis to impair wound resolution while administering GnRH antagonists reduces NET formation and wound size (121). Like in normoglycemic wounds, PAD4 depletion or inhibition by Cl-amidine in diabetic wounds reduces NET markers and accelerates wound healing (103). Similar to the MAPK inhibitor annexin-1 which has shown benefit in preventing the microvascular complications of diabetes, investigators have utilized Na_2S to inhibit MAPK, which dampens NETosis with a corresponding acceleration in wound healing (122). In summary, all these mediators constitute potential therapeutic targets, the inhibition of which could be of significant clinical benefit in the management of DFUs.

Metformin and NETs

Current guidelines—as per the American Diabetes Association—recommend Metformin as the drug of choice for the initial management of T2DM patients at the time of diagnosis (123, 124). This recommendation is underpinned by various clinical trials showing Metformin to be an effective agent in lowering plasma glucose and HbA1C levels but without the risk of weight gain or severe hypoglycemia. Clinical trials have also demonstrated long-term metformin use to reduce the incidence of MACEs, cardiovascular mortality, and all-cause mortality in T2DM patients (125–127). Metformin may also reduce ischemia-reperfusion injury and thereby infarct size of MI in diabetic and non-diabetic mouse models (128).

However, although these beneficial clinical effects of Metformin have been intensively studied and characterized, the mechanisms of its effects remain investigational. The well-known glucose-lowering effect of Metformin is secondary to the suppression of hepatic gluconeogenesis and enhanced skeletal muscle glucose uptake (129). However, Metformin is also under investigation for use in treating cardiovascular disease irrespective of diabetes status, underscoring its yet-to-be understood glucose-independent mechanisms. This is further reinforced by the apparent beneficial effects of Metformin in aging, COVID-19, and various types of cancers, conditions unrelated to blood glucose changes (130).

In this context, Metformin reduces neutrophil-to-lymphocyte ratios in patients with diabetes (131). Furthermore, Metformin reduces levels of NETosis in neutrophils of T2DM patients (132). A subsequent study by Menegazzo et al. reported metformin treatment as reducing serum NE, proteinase-3, citrullinated histone, and dsDNA levels (133). Metformin blunted NETosis in neutrophils after exposure to classical NET-inducing stimuli, and this effect was independent of the anti-hyperglycemic effect of Metformin (133). The potential clinical significance of the inhibition of neutrophils and NETosis by Metformin has also been studied. Lipocalin—a NET protein discussed above in the context of DR—is found in carotid artery atheromas in diabetic patients, particularly in complicated plaques (134). In agreement with this, higher lipocalin levels in plaques were associated with an increased risk of cerebral embolization. Metformin significantly reduced leukocyte recruitment into carotid plaques of diabetes and decreased lipocalin levels (134). Lastly, a study on rat models reported that treatment with Metformin before MI significantly attenuates pathologic cardiac remodeling and fibrosis by mitigating neutrophil recruitment and NET production, evidenced by lower neutrophilic infiltration into the infarcted myocardium on histopathological examination and reduced MPO activity (135). In summary, Metformin has been shown to reduce the risk of adverse cardiovascular outcomes in diabetic patients

via mechanisms unrelated to glycemic control and potentially involving the inhibition of NETosis.

Severe COVID-19 also features dysregulated neutrophil responses with increased NET production, contributing to the systemic inflammatory response syndrome and immunothrombosis in these patients (136, 137). DM patients are known to be predisposed to severe COVID-19. Given that NETs play essential roles in the immunologic and clinical phenotypes of severe COVID-19 and that many diabetic patients are prescribed Metformin, studies looked into the potential benefit of Metformin in people with diabetes who developed COVID-19. Metformin reduced in-hospital mortality of diabetic patients who contracted COVID-19 (138, 139). Cancer is another disease in which NETs are involved; NETs promote the acquisition of various hallmarks of carcinogenesis, including invasion, angiogenesis, immune evasion, and metastasis (140, 141). A recent study demonstrated high levels of mitochondrial NET formation in circulating neutrophils of hepatocellular carcinoma (HCC) patients, which promote the expression of metastasis-promoting inflammatory mediators. Metformin ameliorated mitochondrial NET production and mitigated the metastatic capacity of HCC cells (142). Therefore, the NET-targeting mechanism of Metformin broadens its clinical indication beyond just glycemic control in T2DM patients to conditions such as COVID-19 and cancer.

Regarding diabetes complications, numerous mechanistic studies show Metformin to reduce the development of DR (143) and accelerate wound healing (144, 145), but unfortunately, clinical data on the latter is limited (146). Therefore, observational studies comparing the incidence of impaired wound healing in diabetic patients receiving Metformin with controls are needed. Nevertheless, whether NET inhibition partially contributes to this beneficial effect remains unknown. Future investigations into this topic may rationalize novel drug combinations, whereby Metformin and other NET-targeting medications may synergize to benefit diabetic patients at risk of these complications.

Discussion

In this review, we set out to summarize the important laboratory and clinical studies supporting the role of NETs in diabetic complications, in the hopes to inform the focus of future studies on this pertinent topic. However, many questions remain to be addressed, the answers to which will undoubtedly further the discussion surrounding NETs and diabetic complications.

Firstly, NET heterogeneity must be explored further; many different types of NETs exist, including the classic lytic NETs, vital NETs, and mitochondrial NETs. These NETs are produced under different circumstances secondary to distinct stimuli, have varying compositions, may have differential contributions to diabetic complications, and respond differently to anti-NET therapies. The focus of future research should therefore involve elucidating the relative contributions of each of these NETs in each complication. Classifying the different types of NETs at sites of pathology will also provide further insight into the probable NETs-inducing stimuli involved in these pathways. The potential compositional variations between different NETs types may underpin variable clinical responses to NET-targeting medications. Another factor to consider in diabetic complications is the effect of age on the propensity of neutrophils to produce NETs. Indeed, a recent study by Lu et al. showed aged neutrophils to produce more NETs than younger ones (26). Given that age is a major risk factor in the development of diabetes and its complications, perhaps one of the many effects of aging is reflected in neutrophils producing more NETs. Intriguingly, this study found major gender-specific differences in neutrophil gene expression (26). In the context of NETs heterogeneity, it would be interesting if future research decides to explore the composition of NETs which are elaborated by aged neutrophils and any potential sex-specific differences. The study by Lu et al. examined primary bone marrow neutrophils, paving the way for studies evaluating these relationships in blood circulating neutrophils.

Secondly, while we elaborate on numerous ways to inhibit NETs, such as accelerating NET degradation, inhibiting certain NET components, or inhibiting NET production, diabetic patients are immunosuppressed and susceptible to various debilitating infections. Inhibiting an integral neutrophil function could exacerbate this condition. Therefore, the indication and side-effect profile of NET-inhibiting drugs should be clearly defined (which will require large-scale clinical trials), and more local routes of administration, such as topical applications for DFUs or eyedrops for DR, be further explored. Related to this point, it is also important to consider that NETs are at the end of the day a physiologic neutrophil response aimed at protecting the body, and many of its beneficial effects have been identified. Therefore, when exactly do NETs switch from being physiologic to pathologic is an interesting topic for future work to address.

Thirdly, investigators must continue to describe the precise mechanism by which NET components—such as histones, extracellular DNA, and proteases such as MMPs—contribute to diabetes complications. A reason for this is that histones, DNA, and MMPs are not always present in tissues as NET components; for instance, DNA and histones are released during necrosis of tissue-resident cells. Furthermore, whether DNA-bound or free histones exert different local effects remains unclear. Cellular senescence is a hot topic that reportedly plays a role in the pathogenesis of various age-related diseases, including diabetes. In the context of wound healing, transient senescence is critical to physiologic wound healing, but chronic senescence conversely impairs wound healing (147). In diabetic wounds, levels of senescent cells are higher, and senescent macrophages

secrete abundant C-X-C motif chemokine receptor 2 (CXCR2) ligands as part of secretory phenotype (SASP) (148, 149). Since neutrophil homing into diabetic wounds with subsequent NET production has been demonstrated, and given that CXCR2 is well-recognized as a neutrophil chemokine receptor, an interplay between elevated senescent cell burden and NET production may be reasonable to investigate. Additionally, senescent cells release MMPs which degrades the ECM to drive age-related loss of skin elasticity (150). As described above, NET-derived MMPs also contribute to impaired wound healing. Therefore, dissecting the differential contributions of senescence and NET-derived MMPs and their relative importance to chronic diabetic wounds may inform future strategies to target certain mechanisms over others to improve wound healing in diabetics.

Lastly, no drug specifically targeting NETs is currently approved for clinical use. In Figure 1 we describe different NET-targeting strategies, which preclinical and clinical data have substantitated in the context of diabetes. However, it should be noted that DNAse-1—despite its apparent benefits in accelerating the breakdown of coronary and cerebral thrombi—has in cases been harmful to bronchiectasis patients in one clinical trial (151). The mechanism underpinning may involve the release of DNA-bound NE and other proteases into tissues and circulation, where they aggravate tissue injury; DNase treatment can even increase NE activity (152). Nevertheless, randomized clinical trials showed that DNase-1 administration significantly improved oxygen saturation and lung compliance in severe COVID-19 pneumonia patients compared to controls who did not receive DNase-1 (153). This was presumably through degrading lung NETs, as reductions in BALF MPO levels were noted in treated patients (153). This further underscores the point of specifying the clinical indications of NET-targeting drugs, which necessarily will require the outcomes of well-designed, robust clinical trials. Therefore, current focus should be on translating important benchwork into clinical data. An interesting recent study revealed disulfiram to ameliorate NETs to ameliorate the pathology of transfusion-related acute lung injury in mice and also COVID-19 lung injury in golden Hamsters (154). Therefore, testing disulfiram in other NET-related disorders, including diabetes complications, should be explored by future studies. But again, the heterogeneity of NETs in diabetic complications—in terms of composition and stimuli—remains unexplored. Studying the intrinsic mechanisms behind NETosis and potential inducers in this context will not only possibly reveal novel therapeutic targets but also better specify the precise clinical indications of already existing drugs that could be repurposed to prevent or treat diabetic complications *via* their NET-targeting mechanism.

In summary, we believe NETs are potentially significant executors in the pathogenesis of diabetic macrovascular and microvascular complications. Future research expectedly will continue to expand upon the association between NETs and major complications to rationalize clinical practice-changing trials that will benefit diabetic patients and the healthcare system.

Author contributions

AS and AY: conceptualization. AS, SAb, OA, and SAl: writing—original draft preparation. AS, AA, JK, KA, and AY: writing—review and editing. JK, KA, and AY: supervision. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Nursing management of treatment-related venous thromboembolism in patients with multiple myeloma

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Objectives: Venous thromboembolism (VTE) is a common complication among patients with newly diagnosed multiple myeloma (NDMM). Therefore, this study aimed to analyze the incidence and risk factors associated with VTE in the current era of thromboprophylaxis and to propose appropriate nursing measures.

Methods: A total of 1,539 NDMM patients were retrospectively analyzed. All patients underwent VTE risk assessment and received aspirin or low molecular weight heparin (LMWH) to prevent thrombosis, followed by appropriate care based on their individual thrombosis risk. The incidence of VTE and its related risk factors were then analyzed.

Results: All patients received at least four cycles of therapy containing immunomodulators (IMiDs) and/or proteasome inhibitors (PIs). We assigned 371 patients (24.1%) to the moderate-risk thrombosis group, who received daily aspirin (75 mg) for thrombosis prevention and 1,168 patients (75.9%) to the high-risk group, who received daily low molecular weight heparin (3,000 IU) for thrombosis prevention two times a day. Among all the patients, 53 (3.4%) experienced lower extremity venous thromboembolism events, with three of those patients experiencing a concurrent pulmonary embolism. A multivariate analysis indicated that bed rest lasting more than 2 months and plasma cells of ≥60% were independent factors associated with thrombosis.

Conclusion: More effective risk assessment models are needed to predict thrombosis accurately. In addition, nurses involved in the treatment and management of thrombosis should continually engage in professional development to enhance their knowledge and skills.

KEYWORDS

multiple myeloma, nursing, venous thromboembolism, management, treatment

1. Introduction

Venous thromboembolism (VTE) is a common complication among patients with multiple myeloma (MM). The cause is associated with increased levels of coagulation-stimulating factors and monoclonal gamma globulin in MM patients, as well as the increased use of immunomodulators (IMiDs) such as thalidomide, lenalidomide, and pomalidomide, and proteasome inhibitors (PI) such as carfilzomib and dexamethasone (1–4).

The prevention of VTE is of utmost importance, and nursing plays an important role in its prevention, even more so than treatment. Clinical research has placed great importance on the prevention of VTE (5). However, nurses' understanding of VTE remains suboptimal, especially in relation to disease-specific and drug-related prevention measures.

Therefore, continuing education for nurses must include comprehensive project studies and practices to strengthen their understanding of deep vein thrombosis, with a specific emphasis on VTE prevention (6, 7). Accurate VTE risk assessment is critical to the development of appropriate preventive measures for MM patients. Based on nursing work for myeloma, we analyzed risk factors associated with VTE during the course of treatment, providing nurses with a foundation for identifying patients at risk for VTE in clinical practice.

Risk assessment models (RAMs) for thrombosis in MM patients typically stratify risk based on algorithms established by organizations such as the International Myeloma Working Group (IMWG) (8), the European Myeloma Network (EMN) (9), and the National Comprehensive Cancer Network (NCCN) (10) risk stratification algorithms and on the selection of thromboprophylaxis in MM patients (Appendix 1) (11). This study retrospectively analyzed 1,539 patients with newly diagnosed MM (NDMM), admitted to Beijing Chaoyang Hospital from 2011 to 2020, who received thromboprophylaxis in accordance with the ethical guidelines established by organizations mentioned above. Among these patients, 53 patients (3.4%) developed VTE during the first four treatment cycles of induction. Based on clinical nursing work, this study aimed to analyze high-risk factors associated with VTE and propose the corresponding nursing measures.

2. Methods

2.1. Patients

This was a retrospective study. We analyzed the incidence of VTE in all NDMM patients. VTE is diagnosed based on the patient's clinical symptoms, a vascular ultrasound or pulmonary perfusion CT scan, and a D-dimer. The inclusion criteria were as follows: (1) patients with a diagnosis of active MM according to IMWG criteria; (2) patients who received at least four cycles of therapy containing PI (bortezomib, BORT) and/or ImiDs (thalidomide, THAL or lenalidomide, LEN) and dexamethasone (DEX); and (3) patients who agreed to thromboprophylaxis according to the IMWG, EMN, and NCCN risk stratification algorithms (Appendix 1). The exclusion criteria were as follows: (1) patients who had received chemotherapy; (2) patients with relapsed or refractory multiple myeloma (RRMM); (3) patients who had been diagnosed with other active malignant tumors; and (4) patients with a disorder of consciousness or expression. A total of 1,539 NDMM patients who met the inclusion and exclusion criteria and who were treated at Beijing Chaoyang Hospital, Capital Medical University, Beijing, China from 1 January 2011, to 31 December 2020 were enrolled. All patients were followed up for four cycles. This study was approved by the Medical Ethics Committee of Beijing Chaoyang Hospital.

2.2. Thrombosis prevention and nursing measures

All patients received thrombosis prevention measures and follow-up in accordance with the guidelines established by organizations such as the International Myeloma Working Group (IMWG), the European Myeloma Network (EMN), and the National Comprehensive Cancer Network (NCCN) (8, 9). These measures included the administration of either aspirin or low molecular weight heparin (LMWH) for thrombosis prevention, as well as related nursing measures. Nursing measures were taken according to the consensus of the Oncology Nursing Society (12). If a patient showed signs or symptoms of VTE during induction therapy, a vascular ultrasound or CT scan was performed, and the patient was re-evaluated for VTE.

2.3. Statistical analysis

All data were expressed as median ± standard deviation (SD). A multivariate Cox proportional hazard regression analysis was performed for VTE-related factors, and the results were reported as hazard ratios (HRs) with a 95% confidence interval (95%). SPSS 23.0 software (SPSS Institute) was used for statistical analysis. A p-value of < 0.05 was statistically significant, and all tests were bilateral.

3. Results

3.1. Patients

A total of 1,539 NDMM were retrospectively analyzed. The baseline patient characteristics were as follows: age range of 28–84 years, with a mean age of 59.8 years, male gender was predominant with 897 cases (58.3%) than female gender with 642 cases (41.7%), and the MM subtypes of IgG being 42.9%, IgA being 26.7%, IgD being 8.2%, light chain being 20.4%, and non-secretory being 1.8% of cases. The International Staging System (ISS) was classified as stage I at 15.4%, stage II at 36.2%, and stage III at 48.4%. The Revised International Staging System (R-ISS) stage was stage I in 16.9%, stage II in 56.7%, and stage III in 26.4%. Serum albumin levels were ≥35 g/L in 60.5% of patients and <35 g/L in 39.5%. Serum β2-microglobulin levels were <3.5 mg/L in 25.2% of patients, ≥3.5 mg/L and <5.5 mg/L in 26.4%, and ≥5.5 mg/L in 48.4%. Hemoglobin levels were <100 g/L in 56.5% of patients, serum creatinine levels were ≥177 umol/L in 32.3%, corrected serum calcium levels were ≥2.75 mmol/L in 15.4%, and lactate dehydrogenase (LDH) levels were above the upper limits of normal in 18.4% of patients. Cytogenetic abnormalities by FISH included $t_{(4;14)}$ in 18.2% of patients, $t_{(11;14)}$ in 16.7%, $t_{(14;16)}$ in 2.8%, Del (17p) in 8.5%, and 1q21 gain or 1q21 amplification in 48.8%.

All of the patients received at least four cycles of treatment as part of one of the following regimens: BORT-LEN-DEX (BORT 1.3 mg/m^2, d1, 4, 8,11; LEN 25 mg, d1–21; DEX 20 mg, d1, 2, 4, 5, 8, 9, 11, 12; 21 d/cycle); BORT-THAL-DEX (BORT 1.3 mg/m^2, d1, 4, 8,11; THAL 100 mg, d1–21; DEX 20 mg, d1, 2, 4, 5, 8, 9, 11, 12; 21 d/cycle); BORT-Cyclophosphamide (CTX)-DEX (BORT 1.3 mg/m^2, d1, 4, 8, 11; CTX 300 mg/m^2, d1–4; DEX 20 mg, d1, 2, 4, 5, 8, 9, 11, 12; 21 d/cycle); BORT-DEX (BORT 1.3 mg/m^2, d1, 4, 8, 11; DEX 20 mg, d1, 2, 4, 5, 8, 9, 11, 12; 21 d/cycle); and LEN-DEX (LEN 25 mg, d1–21; DEX 20 mg, d1, 2,

8, 9, 15, 16, 22, 23; 28 d/cycle). BORT-LEN-DEX, BORT-THAL-DEX, or BORT-CTX-DEX was used for the treatment of autologous transplant-eligible patients, and BORT-DEX or LEN-DEX was used for the treatment of transplant-non-eligible patients. A total of 826 patients (53.7%) received BORT-LEN-DEX treatment, 161 patients (10.5%) received BORT-THAL-DEX, 257 patients (16.7%) received BORT-CTX-DEX, 187 patients (12.1%) received BORT-DEX, and 108 patients (7%) received LEN-DEX. The selection of the regimens was based on age, organ function, and geriatric assessment (GA) score.

3.2. Venous thromboembolism prophylaxis and events

Before the induction treatment, all of the patients underwent the thrombus risk assessment according to the RAM (Appendix 1). The VTE prophylaxis adapted the IMWG, EMN, and NCCN risk stratification algorithms (8–10). In total, 371 patients (24.1%) were assessed as having intermediate risk and received thromboprophylaxis with aspirin at a dose of 75 mg per day, 1,168 patients (75.9%) were assessed as having high risk and received thromboprophylaxis with LMWH at a dose of 3,000 IU, administered every 12 h. Even though 53 VTE events (3.4%) occurred, all of the venous thrombosis occurred in the lower limbs. Among them, three patients issued pulmonary embolisms at the same time. All of the VTE events occurred during the first two cycles of induction treatment. The 53 patients were at a high risk of RAM and were treated with low-dose LMWH (3,000 IU, q12h) to prevent thrombosis. When thrombosis occurred, the dose of LMWH was increased to 6,000 IU, q12h. After 2 weeks of LMWH treatment, the thrombus disappeared, including the pulmonary embolism. No patient died due to the thrombus.

3.3. The role of nurses

The patient's performance status was one factor in thromboembolism risk. In the prevention of thromboembolism, the nurse's role was to educate patients and their caregivers on the patient's risk factors as well as signs and symptoms, including pain, swelling, erythema, and/or warmth of the affected extremity (12–14). Venous lower limb thrombosis may be traveling from an extremity into the lung, resulting in a pulmonary embolism. Signs and symptoms of pulmonary embolism include tachypnea, difficulty breathing, cough, hemoptysis, pleuritic pain, and cyanosis. The nurse must routinely monitor for specific signs and symptoms and seek immediate medical care if any of those signs or symptoms appears.

For MM patients experiencing bone involvement or compression fractures, bone pain is a major complaint. Nurses should encourage patients to roll over every 2 h to avoid pressure sores and lower limb thrombosis. If the patient could not roll over due to the pain, the patient was asked to lie on an air cushion and use an antithrombotic pressure pump in the lower limbs.

3.4. High-risk factors for thrombosis

Despite the aforementioned measures, 53 patients (3.4%) developed venous thrombosis, and three of the patients experienced pulmonary embolisms. All 53 venous thrombosis events occurred during the first two cycles of treatment. The univariate analysis revealed that staying in bed for more than 2 months, a pelvis fracture, diabetes mellitus, obesity (Body mass index >25), plasma cells ≥60%, and accepting IMiDs in combined chemotherapy were the high-risk factors for thrombosis. Multivariate analysis suggests that, by staying in bed for more than 2 months, having plasma cells of ≥60% was the independent related factor of thrombosis (Table 1). Nurses should pay more attention to this kind of patient.

4. Discussion

Multiple myeloma is one of the most common hematologic malignancies in China. Hypercoagulation in MM patients due to hyperimmunoglobulinemia, combined with the use of conventional therapeutic drugs such as immunomodulators and high-dose dexamethasone and immobilization due to surgery or pain, increases the possibility of venous thromboembolism occurring more in MM patients than in other tumor patients. The incidence range of VTE in MM patients treated with IMID-based combination chemotherapy without preventive intervention was as high as 10–34%. The occurrence of VTE not only restricts the choice of drugs but also seriously affects patients' quality of life. In severe cases, it may cause disability or even threaten the lives of patients.

Of all the 1,539 patients, 53 (3.4%) had a VTE event. All 53 patients received low molecular weight heparin (3,000 IU, q12h) for VTE prevention and routine nursing. Most patients developed pelvic fractures and bone pain, and some patients developed tumor lysis syndrome due to a high tumor burden. The multivariate analysis showed that bed rest for more than 2 months and plasma cells of ≥60% were independent factors associated with fracture and hemolysis-associated thrombosis.

For the 1,539 NDMM patients in our study, we assessed the risk of thrombosis and provided corresponding treatment according to the thrombosis risk stratification. Every patient accepted baseline risk stratification and the use of aspirin for low-risk patients and LMWH as a prophylactic dose for higher-risk patients. Even though 53 (3.4%) VTE events occurred, all 53 patients received low molecular weight heparin (3,000 IU, q12h) for VTE prevention and routine nursing. Most patients developed pelvic fractures and bone pain, and some patients developed tumor lysis syndrome due to a high tumor burden. A multivariate analysis showed that bed rest for more than 2 months and plasma cells of ≥60% were independent factors associated with fracture and hemolysis-associated thrombosis. The results might indicate that the guidelines still have limited power for VTE risk stratification and that more accurate RAM is needed (15).

In recent years, there have been several clinical scores for thrombosis risk stratification in MM patients. The IMPEDE VTE score (Appendix 2) was a widely applied risk prediction tool for VTE in MM (16), which was developed by Fotiou et al. (11) and

TABLE 1 Risk factors of thrombosis of newly diagnosed multiple myeloma under the thromboprophylaxis according to the risk assessment model (RAM).

Clinical parameters	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Gender: Male vs. female	1:0.42 (0.12–1.21)	0.097	1:0.52 (0.22–1.34)	0.121
Age: ≤60 vs. >60 years	1:1.21 (0.78–4.23)	0.833	1:1.08 (0.66–3.47)	0.845
Subtype: IgG vs. IgA vs. IgD vs. light chain vs. non-secretory	1:1.12:1.24:1.01:0.85 (0.88–1.43)	0.927	1:1.03:1.28:0.98:0.79 (0.91–2.08)	0.876
ISS stage: I vs. II vs. III	1:1.12:2.03 (0.56–2.51)	0.241	1:1.99:2.41 (0.49–2.23)	0.351
Hemoglobin: ≥100 vs. <100 g/L	1:0.92 (0.45–3.73)	0.879	1:1.12 (0.44–3.71)	0.876
Serum albumin: ≥35 vs. < 35 g/L	1:1.83 (0.38–5.73)	0.078	1:2.15 (0.32–4.29)	0.054
Serum β2-microglobulin: <35 g/L vs. ≥3.5 mg/L and <5.5 vs. ≥5.5 mg/L	1:1.21:1.53 (0.99–1.87)	0.878	1:1.34:2.01 (0.78–2.56)	0.128
Serum creatinine: <177 vs. ≥177 umol/L	1:2.31 (0.32–3.12)	0.211	1:2.13 (0.44–3.01)	0.062
Cytogenetic abnormalities by FISH: Standard risk vs. high risk	1:1.17 (0.17–1.43)	0.177	1:1.29 (0.21–1.44)	0.157
Serum calcium: <2.75 vs. ≥2.75 mmol/L	1:2.03 (0.86–5.46)	0.075	1:1.98 (0.81–3.20)	0.129
Lactate dehydrogenase (LDH): ≤ ULN vs. >ULN	1:2.58 (0.22–6.51)	0.062	1:2.34 (0.32–5.42)	0.075
Stay in bed: ≤2 vs. >2 months	1:4.27 (1.21–7.82)	0.011	1:4.67 (1.44–7.54)	0.002
Pelvis fracture: No vs. yes	1:2.69 (2.11–6.10)	0.047	1:2.54 (2.00–5.43)	0.098
Diabetes mellitus: No vs. yes	1:3.58 (0.89–6.20)	0.039	1:2.38 (0.81–4.22)	0.075
Obesity: BMI≤25 vs. >25	1:2.59 (0.15–1.21)	0.042	1:1.72 (0.24–1.34)	0.219
Plasma cells in bone marrow:<60% vs. ≥ 60%	1:3.62 (1.23–7.01)	0.020	1:4.02 (1.11–7.21)	0.039
MiDs in combined chemotherapy: No vs. yes	1:4.58 (1.51–4.32)	0.041	1:2.45 (1.31–4.98)	0.066

Cytogenetic abnormalities by FISH: risk stratification according to mSMART 3.0.
ULN, Upper limit of normal; BMI, Body mass index.

validated by a series of clinical studies in MM patients (17). We used the IMPEDE VTE score to retrospectively evaluate the risk of thrombosis in these 53 patients. All of these patients were at high risk (>8 scores), even with prophylactic LMWH (-3 score), but if these patients received the therapeutic dose of LMWH, their risk of thrombosis would be reduced to intermediate-risk (score of 4–7). It has been shown that the IMPEDE VTE score may be better than the others for predicting thrombus risk.

In addition, three of the 53 patients suffered a pulmonary embolism and venous thromboembolism of the lower limb. The three patients were men and were accompanied by diabetes, coronary heart disease, and a pelvis fracture; they should be evaluated as high-risk (12 scores) for VTE according to the IMPEDE VTE score. At the very early stage of the newly acquired VTE, thrombosis is often unstable and easy to fall off and enter the pulmonary artery, leading to a pulmonary embolism. Doctors generally might adjust the preventive dose of LMWH to the therapeutic dose of LMWH when the VTE occurs in the lower limb. Nurses should take an active role by educating the patient to reduce lower limb movement to avoid thrombus shedding and closely monitoring the patient's breathing. Nurses should always be concerned about preventing the patient from getting out of bed to prevent compression of blood vessels, properly elevating the limbs to promote reflex, using pressure circulation to drive the pump, or asking the patient to wear lower limb socks to improve edema symptoms to avoid pulmonary embolism, which can be accompanied by chest tightness, shortness of breath, difficulty breathing, and even respiratory arrest leading to death. Once the pulmonary embolism symptoms occur, it is important to notify the doctor immediately and provide appropriate treatment.

In patients with IMiDs, glucocorticoid-based therapy is recommended for the concurrent prevention of VTE. The risk of VTE is highest during the first six cycles of induction therapy due to the greater tumor burden and the release of procoagulant factors by tumor cell apoptosis. At this time, nurses must be aware of potential VTE complications, including pulmonary embolism, evaluate patients according to RAM, and prevent thrombosis. After 6 months, the risk of VTE is relatively low, and prophylactic regimens can be adjusted according to the treatment response of MM patients. It is important to note that, although the incidence of VTE in China is relatively lower than in the United States and Europe, each NDMM should receive RAM scoring to prevent the risk of thrombosis. In summary, as the main caregivers of hospitalized patients, nurses' knowledge of VTE is the key to preventing VTE. Nurses' long-term and repeated use of risk assessment forms can enhance their mastery of VTE-related knowledge. In-depth and standardized VTE training should be carried out according to the characteristics of clinical nurses and school students and the weak areas of VTE knowledge so as to improve the VTE prevention ability of nurses (18–20).

5. Conclusion

Nursing is important in preventing VTE in patients with NDMM, the risk of thrombosis should be assessed for each patient,

and appropriate measures must be implemented. Although a small number of patients still develop VTE, especially pulmonary embolism, this suggests that existing risk stratification algorithms are limited in their ability to stratify the risk of VTE and that more effective risk assessment models are needed. In addition, the IMPEDE VTE score is a VTE scoring method developed in recent years that may have better results for predicting the risk of thrombosis.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

BY and CL collected data and completed data analysis. CG and ZL completed the patient condition analysis. ZZ wrote the article. All authors have reviewed the article. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1153694/full#supplementary-material

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A practical guide to managing cardiopulmonary toxicities of tyrosine kinase inhibitors in chronic myeloid leukemia

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Tyrosine kinase inhibitors (TKIs) have revolutionized the treatment of chronic myeloid leukemia (CML) but their use was associated with a range of serious cardiopulmonary toxicities including vascular adverse events, QT prolongation, heart failure, pleural effusion, and pulmonary arterial hypertension. Dedicated clinical management guidelines for TKI-induced toxicities are not available. This review aims to discuss TKI-associated cardiopulmonary toxicities and proposes a practical guide for their management.

KEYWORDS

chronic myeloid leukemia, tyrosine kinase, heart failure, pulmonary arterial hypertension, QT prolongation, dasatinib, ponatinib, pleural effusion

Introduction

Protein kinases are enzymes involved in different signaling pathways and regulate various cellular functions such as proliferation, differentiation, and death (1, 2). Thus, carcinogenesis can be triggered by disturbances in such processes. Kinase inhibitors have brought about a paradigm shift in the treatment of many diverse malignancies (1). Chronic myeloid leukemia (CML), a rare myeloproliferative disease, is associated with chromosomal translocation (i.e., Philadelphia chromosome), which encodes BCR::ABL1 oncoprotein, through the fusion of BCR and ABL1 genes, with active tyrosine kinase activity (3–6). Thus, tyrosine kinase inhibitors (TKIs) are currently the cornerstone treatment of CML (6). TKIs are categorized into small-molecule TKIs and monoclonal antibody drugs. The latter group inhibits proliferation, angiogenesis, and invasion of tumor cells. The former group is less selective than the other one and may cause more adverse events. Different TKIs, namely imatinib, dasatinib, nilotinib, bosutinib, and ponatinib, are approved in Europe and the United States for the treatment of CML. Although the five TKIs inhibit BCR::ABL1, they differ in their targeted site, mechanisms, efficacy, and safety. Cardiovascular toxicities of TKIs have several manifestations such as heart failure, arrhythmias, and fluid retention (1, 2). However, it is hard to distinguish between the cardiovascular events caused by TKIs from the events that occur due to the patient's risk factors (7). Vascular adverse events and pulmonary toxicities are also associated with TKI therapy (Figure 1) (3, 8). Herein, this review discusses the TKI-associated cardiopulmonary toxicities, including their mechanisms of toxicity, and proposes a practical guide to their recognition, management, and monitoring.

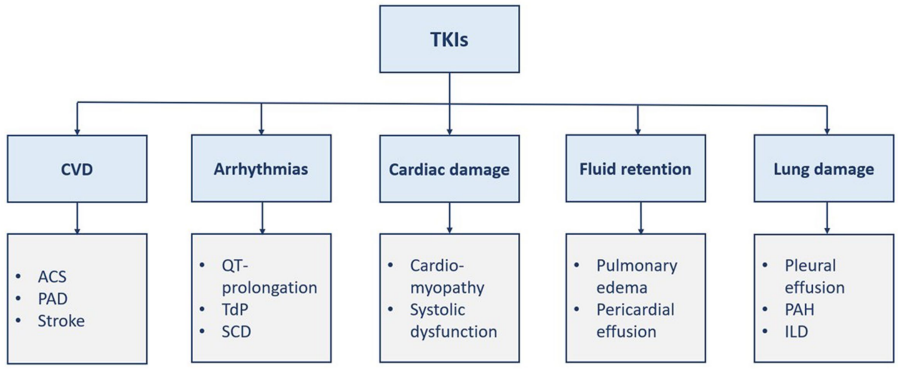

FIGURE 1
Manifestations of TKI-induced cardiopulmonary toxicities. ACS, acute coronary syndrome; CVD, cardiovascular disease; ILD, interstitial lung disease; PAD, Peripheral arterial disease; PAH, pulmonary arterial hypertension; SCD, sudden cardiac death; TdP, torsade de pointes; TKI, tyrosine kinase inhibitor(s).

Tyrosine kinase inhibitors

Tyrosine kinase inhibitors have revolutionized the treatment of CML with the first-generation agent, imatinib (4, 9). The subsequent more effective second-generation agents (bosutinib, dasatinib, and nilotinib) were developed to overcome imatinib resistance (4, 8, 9). However, both generations are highly resistant in the presence of BCR::ABL1T315I mutation, which leads to rapid disease progression and limited mortality improvement. Consequently, the third-generation ponatinib was developed and approved in patients with BCR::ABL1T315I mutation (6, 9). Table 1 summarizes the general characteristics of TKIs (1–3, 8, 10). The three additional third-generation agents have been investigated in TKI-resistant CML and included olverembatinib, asciminib, and vodobatinib. Olverembatinib (HQP1351) was well-tolerated and effective in TKI-resistant chronic and accelerated phase CML, particularly in the presence of BCR::ABL1T315I mutation (9). It has been granted a fast-track designation for CML in the United States (May 2020) and a breakthrough therapy designation for CML in the European Union and China (November 2021) (11). Although the first approval of asciminib was received in October 2021 (United States) for Philadelphia chromosome-positive CML, there are several ongoing trials investigating new indications as monotherapy in chronic phase CML patients with or without T315I mutation previously treated with two or more TKIs or as add-on therapy to other TKIs (12). In a dose-escalating Phase I study, vodobatinib showed encouraging efficacy in both ponatinib-treated and ponatinib-naïve patients with chronic phase CML (13). An innovative fourth-generation agent, PF-114, that structurally resembles ponatinib can inhibit wild-type and mutated BCR::ABL (14, 15). PF-114, unlike Ponatinib, does not inhibit the vascular endothelial growth factor receptor (VEGFR), hence having the benefit of reducing cardiovascular adverse effects (15). Table 2 presents the general characteristics of the novel TKIs (9, 11, 12, 15).

Abbreviations: BNP, B-type natriuretic peptide; CXR, chest x-ray; ECHO, echocardiography; hs-CT, high resolution computed tomography; mPAP, mean pulmonary arterial pressure; PAH, pulmonary arterial hypertension; PCWP, pulmonary artery wedge pressure; PFTs, pulmonary function tests; PH, pulmonary hypertension; PVR, pulmonary vascular resistance; RHC, right heart catheterization; TE, thromboembolism; TKI, tyrosine kinase inhibitor; TRV, tricuspid regurgitation velocity; VQ, ventilation perfusion; WU, wood units.

Cardiovascular toxicity

Classification and severity of cardiovascular toxicity

The types of cardiotoxicities that have been described earlier are acute, sub-chronic, early-onset chronic, and late-onset chronic cardiotoxicities. A newer classification of chemotherapy-related cardiac dysfunction (CRCD) included Type I (irreversible) and Type II (reversible) CRCD (Table 3) (1). The National Cancer Institute (NCI) developed the Common Terminology Criteria for Adverse Events (CTCAE) to ensure the consistent description and grading of cardiovascular adverse events of therapeutic agents. The severity of TKI-induced cardiovascular adverse events was also graded using NCI CTCAE (Table 4) (16, 17). Cardiovascular and pulmonary toxicities of TKIs are demonstrated in Table 5 (1–3, 5, 8, 18–21). The recent 2022 European cardio-oncology guidelines described consensus definitions for several cancer therapy-related cardiac dysfunction (CTRCD) such as heart failure and cardiomyopathy, myocarditis, cardiac arrhythmias, corrected QT interval (QTc) prolongation, vascular toxicities, and hypertension (22).

Extent and consequences of TKI-induced cardiovascular toxicity

A case/non-case study using the Food and Drug Administration (FDA) adverse event reporting system (FEARS) database examined the cardiovascular adverse events reports of TKIs for the treatment of CML. Cases referred to reports with one or more of prespecified cardiovascular events such as cardiomyopathy, QT prolongation/torsade de pointes, pulmonary hypertension, or embolic events, whereas non-cases referred to any report for other not specified serious adverse events. FEARS allows the analysis of an enormous number of reports that helps in detecting safety signals. More than

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TABLE 1 The general characteristics of tyrosine kinase inhibitors in chronic phase chronic myeloid leukemia.

Generation	First generation	Second generation			Third generation
Agents	Imatinib	Dasatinib	Nilotinib	Bosutinib	Ponatinib
Approval year (EMA/FDA)	2001/2003	2006/2006	2007/2007	2013/2012	2013/2012
Potency versus imatinib	–	300 times	30 times	45 times	500 times
Molecular targets	BCR-ABL, c-KIT, CSF-1R, PDGFR-α, −β, DDR-1, −2	BCR-ABL, c-KIT, SFKS, PDGFR- α, −β	BCR-ABL, CSF-1R, c-kit, DDR, PDGFR- α, −β	BCR-ABL, c-KIT, Hck, PDGF, Src	BCR-ABL, c-KIT, EGFR, PDGFR, VEGFR
First line TKIs	Yes	Yes	Yes	No	No
Second line TKIs					
Intolerance to 1st line	Yes	Yes	Yes	Yes	No
Failure of 1st line imatinib	–	Yes	Yes	Yes	Yes
Failure of 1st line dasatinib or nilotinib	No	Yes	Yes	Yes	Yes
Third line TKIs*	Any not tried TKI				
Daily dose (chronic phase)	400–800 mg	100 mg	300–400 mg twice	400–600 mg	45 mg
Metabolism (CYP enzyme)	CYP3A4 Minor metabolism: CYP1A2, CYP2D6, CYP2C9, CYP2C19	CYP3A4	CYP3A4	CYP3A4	CYP3A4, CYP3A5, CYP2C6, CYP2C8
Cardiopulmonary adverse events	Pleural effusion	VAE, QT prolongation, PH, pleural/ pericardial effusion, HTN, HF	VAE, QT prolongation, AF, HTN, hyperlipidemia, hyperglycemia, IHD, CVA	VAE, pleural/pericardial effusion, HTN	VAE, HTN, IHD, CVA, AF, HF, venous thrombosis, PH
Other non-hematological adverse events	Diarrhea, Hepatobiliary and skin disorders	Hepatobiliary disorders, hemorrhage	Hepatobiliary, skin, metabolism, and nutrition disorders	Diarrhea, Hepatobiliary and skin disorders	Diarrhea, hepatobiliary, and skin disorders
Preferred in	CVD, DM, PAD, PAH	DM, (PAD?)	PAH	CVD, DM, PAD, PAH	–
Less preferred in	–	CVD, PAH	CVD, DM, PAD	–	PAH

*Intolerance and/or failure 2nd line TKI.
CML, chronic myeloid leukemia; CSF1R, colony stimulating factor 1 receptor; CSK, C-terminal src kinase; CVA, cerebrovascular accident (ischaemic); CYP, cytochrome P450; DDR1, discoidin domain receptor 1; EGFR, epidermal growth factor receptor; EMA, European Marketing Authorization; FDA, Food and Drug Administration; IHD, ischaemic heart disease; PAH, pulmonary arterial hypertension; HCK, haemopoeitic cell kinase; PDGFR, platelet-derived growth factor receptor; TKI(s), tyrosine kinase inhibitor(s); VAE, vascular adverse events; VEGFR, vascular endothelial growth factor receptor.

1,300,000 adverse event reports, out of more than 20 million reports, were related to anticancer agents. Finally, 717,163 reports were analyzed after excluding non-serious events, duplicates, and aberrant cases. Cardiovascular events accounted for 64,232 cases, with 3,930 (6.1%) cases related to TKIs. In addition, TKIs were the suspected drugs in 83.2% of the cardiovascular event occurrence. Hospitalization and having fatal issues accounted for 35 and 10.1% of the cases. Of the 3,930 reports related to TKIs, 59, 21.2, 14.4, 4.4, and 1% were related to nilotinib, dasatinib, ponatinib, imatinib, and bosutinib, respectively, with 68.1% of the cases treated for CML. In comparison with other anticancer agents, cardiovascular events were more frequent with TKIs. The adjusted reporting odds ratio was 6.6 (95% confidence interval (95% CI): 5.6–7.8) for QT prolongation/torsade de pointes, 1.7 (95% CI: 1.4–2.1) for cardiac arrhythmias, 2.4 (95% CI: 2.2–2.6) for heart failure, and 3.9 (95% CI: 3.2–4.7) for pulmonary hypertension (6).

Wang et al. investigated the long-term follow-up of TKI use in CML patients ($n = 7,119$) and its association with cardiovascular toxicity using SEER (surveillance, epidemiology, and end results) program between 1992 and 2011. The program includes 18 cancer registries in the United States and covers up to approximately 28% of the American population. The authors compared patients' outcomes in the TKI era with that of those in the pre-TKI era. CML diagnosis rate was higher in the TKI era (72.4% versus 27.6%) and was associated with significantly higher overall survival [hazard ratio (HR) 0.22; 95% CI: 0.21–0.24] than in the pre-TKI era. The probability of mortality due to cardiovascular causes was reduced in the TKI era (HR 0.72; 95% CI: 0.59–0.98) (23). Caocci et al. reported survival data of Italian patients with chronic phase CML who were on second- or third-generation TKIs ($n = 656$) and 15% of them had a history of cardiovascular disease. Following TKI therapy, 7.3 and 1.4% of patients experienced arterial occlusive events and ischemic heart disease, respectively. Peripheral arterial disease rate was higher with nilotinib and ponatinib (7.3 and 5.9%) than dasatinib and bosutinib (1.7 and 1.6%; $p = 0.02$), whereas stroke rate was higher with bosutinib and ponatinib (5 and 3%) than nilotinib and dasatinib (0.7 and 0%; $p = 0.01$), respectively. Death was reported in 37 patients of them 12 cases were related to cardiovascular complications. The 15-year overall and cardiovascular mortality free-survival rates were 83.3 and 93%, respectively (24). Interestingly, imatinib, but not dasatinib or nilotinib, caused a significant reduction in the estimated glomerular filtration rate on a long-term follow-up which may have contributed to the occurrence of ischemic events (25).

TABLE 2 The general characteristics of novel tyrosine kinase inhibitors.

Generation	Third generation			Fourth generation
Agents	Olverembatinib	Asciminib	Vodobatinib	PF-114
Alternative names	• APG 1351; D 824; GZD 824; HQP 1351	• ABL 001; ABL-001; STAMP inhibitor	• K0706	–
Approval	• US: fast-track designation for CML • EU and China: breakthrough therapy designation for CML	• US: accelerated approval for Ph + CML • US: full approval for Ph + CML-CP with the T315I mutation	• EU and US: granted orphan drug designation for treatment of CML	–
Molecular targets	• BCR-ABL (including T315I), c-KIT, FLT3, PDGFR- α, FGFR1	• BCR-ABL1 Kinase	• BCR-ABL1 point mutants • No activity against BCR-ABL1T315I	• Native BCR-ABL • BCR-ABL T315I
Daily dose	• 40 mg every other day	• 80 mg • 400 mg (T315I mutation)	• 204 mg (12–240 mg)	• 200–600 mg
Metabolism (CYP enzyme)	–	• CYP3A4	–	–
Indications in CML	• Resistance or failure to TKI	• Adults with Ph + CML-CP, previously treated with ≥2 TKIs • Adults with Ph + CML-CP with the T315I mutation • Resistance or failure to TKI	• Resistance or failure to ≥3TKIs, except for patients carrying BCR-ABL T315I mutation	• Resistance or failure to ≥2 s generation TKIs
Adverse events	• Thrombocytopaenia, anemia, leukopenia, neutropenia • Skin pigmentation, hypocalcaemia, proteinuria, hypertriglyceridemia, arthralgia, fatigue	• Skin hyperpigmentation, hypertriglyceridemia, proteinuria, thrombocytopenia • Hypertension, pericardial effusion, arrythmias, retinal-vein occlusion or palpitations	• Anemia, pneumonia, neutropenia, gout, thrombocytopenia, dementia, amnesia	• Reversible skin toxicity; psoriasis-like skin lesions
DDI	–	• Not fully characterized • CYP3A4 inducers and inhibitors (e.g., imatinib) may affect asciminib level • Asciminib inhibits CYP3A4, CYP2C9 and P-gp	–	–

CML-CP, chronic phase chronic myeloid leukemia; EU, European Union; FGFR1, fibroblast growth factor receptor 1; PDGFRα, platelet-derived growth factor receptor-α; P-gp, P-glycoprotein; Ph+, Philadelphia chromosome- positive; US, United States.

TABLE 3 Classification of cardiotoxicity.

Classic types			
Non-chronic toxicity		Chronic toxicity	
Acute cardiotoxicity	Sub-chronic cardiotoxicity	Early-onset chronic cardiotoxicity	Late-onset chronic cardiotoxicity
• Rare • Manifests immediately after the first drug administration, and is dose-independent • Symptoms: hypotony, arrhythmias, and myocardial ischemia • Usually reversible after drug discontinuation	• Rare • Its onset is observed in the first weeks of treatment with high doses of drugs • Manifests with myocarditis or pericarditis • Example: after anthracyclines administration	• Develops within a few weeks after discontinuation of treatment • Manifests as progressive heart failure	• Develops many years after the end of the treatment and leads to heart failure
New classification			
Type I CRCD		Type II CRCD	
• Irreversible • Example: after anthracycline administration		• Potentially reversible • Induced by new-generation HER2-targeted agents and kinase inhibitors	

CRCD, chemotherapy-related cardiac dysfunction; HER2, human epidermal growth factor receptor 2.

Mechanisms of TKI-induced cardiovascular toxicity

The mechanisms of cardiovascular toxicity are generally divided into on- and off-target mechanisms. On-target mechanisms refer to the anticancer effect of TKIs through the inhibition of a molecule in the tumor cells but can also adversely affect the function of normal cells. While off-target mechanisms imply the non-selective inhibition of other kinases in normal cells, along with the kinase of the tumor cells that cause cardiotoxicity (1, 2, 7). However, the molecular mechanism of TKI-induced cardiotoxicity is not fully elucidated (1, 2, 19). Examples of target receptors that regulate signaling pathways (e.g., PI3K, MEK, and AKT) include BCR::ABL, VEGFR, epidermal growth factor receptor (EGFR),

TABLE 4 National Cancer Institute CTCAE (CTCAE 4.03) grading severity of cardiac events.

CTCAE v4.0 term	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Heart failure	Asymptomatic with laboratory (e.g., BNP) or cardiac imaging abnormalities	Symptoms with mild to moderate activity or exertion	Severe with symptoms at rest or with minimal activity or exertion; intervention indicated	Life-threatening consequences; urgent intervention indicated (e.g., continuous IV therapy or mechanical hemodynamic support)	Death
Left ventricular systolic dysfunction	–	–	Symptomatic due to drop in ejection fraction responsive to intervention	Refractory or poorly controlled heart failure due to drop in ejection fraction; intervention such as ventricular assist device, intravenous vasopressor support, or heart transplant indicated	Death
Pulmonary edema	Radiologic findings only; minimal dyspnea on exertion	Moderate dyspnea on exertion; medical intervention indicated; limiting instrumental ADL	Severe dyspnea or dyspnea at rest; oxygen indicated; limiting self-care ADL	Life-threatening respiratory compromise; urgent intervention or intubation with ventilatory support indicated	Death
Pulmonary hypertension	Minimal dyspnea; findings on physical exam or other evaluation	Moderate dyspnea, cough; requiring evaluation by cardiac catheterization and medical intervention	Severe symptoms, associated with hypoxemia, right heart failure; oxygen indicated	Life-threatening airway consequences; urgent intervention indicated (e.g., tracheotomy or intubation)	Death
Electrocardiogram QT corrected interval prolonged*	QTc 450–480 ms	QTc 481–500 ms	QTc ≥501 ms on at least two separate ECGs	QTc ≥ 501 or > 60 ms change from baseline and Torsade de pointes or polymorphic ventricular tachycardia or signs/symptoms of serious arrhythmia	–
Pleural effusion	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Symptomatic; intervention indicated (e.g., diuretics or limited therapeutic thoracentesis)	Symptomatic with respiratory distress and hypoxia; surgical intervention including chest tube or pleurodesis indicated	Life-threatening respiratory or hemodynamic compromise; intubation or urgent intervention indicated	Death
Peripheral ischemia	–	Brief (<24 h) episode of ischemia managed non-surgically and without permanent deficit	Recurring or prolonged (≥24 h) and/or invasive intervention indicated	Life-threatening consequences; evidence of end organ damage; urgent operative intervention indicated	Death

*QT is the duration of ventricular depolarization and repolarization.
ADL, activities of daily living; BNP, B-Natriuretic Peptide; CTCAE, Common Terminology Criteria for Adverse Events; ECG, electrocardiogram; IV, intravenous; NCI, National Cancer Institute; QTc, corrected QT interval.

TABLE 5 Frequencies of cardiopulmonary toxicities of tyrosine kinase inhibitors in chronic myeloid leukemia.

Generation	First	Second			Third
Agents	Imatinib	Dasatinib	Nilotinib	Bosutinib	Ponatinib
QT prolongation	1–5%	5–10%	1–10%	>10%	1–5%
QT prolongation (weighted average)	3.1%	8%	2.7%	11.5%	2.5%
QTc >500 ms	0.02%	1%	0.2%	0	2.7%
Cardiomyopathy	1–2%	1.6%	<5%	–	–
Fluid retention	1–2%	1–5%	3.9%	1%	–
VAE	–	–	1.2–29.4%	–	4.9–17.1%
Pleural effusion	0–0.8%	6–35%	2%	≤5%	–
PAH	0.4%	0.45–5%	–	–	–

CML, chronic myeloid leukemia; PAD, peripheral arterial disease; PAH, pulmonary arterial hypertension; QTc, corrected QT interval; TKIs, tyrosine kinase inhibitors; VAE, vascular adverse events.

platelet-derived growth factor receptor (PDGFR), and c-KIT. TKIs are usually multi-target agents and only a few of them have one or two targets such as bosutinib (2, 3). The off-target inhibition spectrum can range from six (nilotinib) to 28 (dasatinib) target receptors. Furthermore, the effects of this inhibition depend on the specific agent and its dosage (3). There is also between-patient variability in cardiotoxicity even when treated with the same TKI, which reflects an interaction between different factors such as TKI targets, genetic predisposition, and cardiovascular risk factors (7). A new study reported a novel mechanism for the cardiovascular toxicity caused by the second- and third-generation BCR::ABL1 TKIs through their ability to activate the Rho-associated coiled-coil-containing kinase (ROCK). ROCK activity may be a prognostic biomarker for cardiovascular adverse events and,

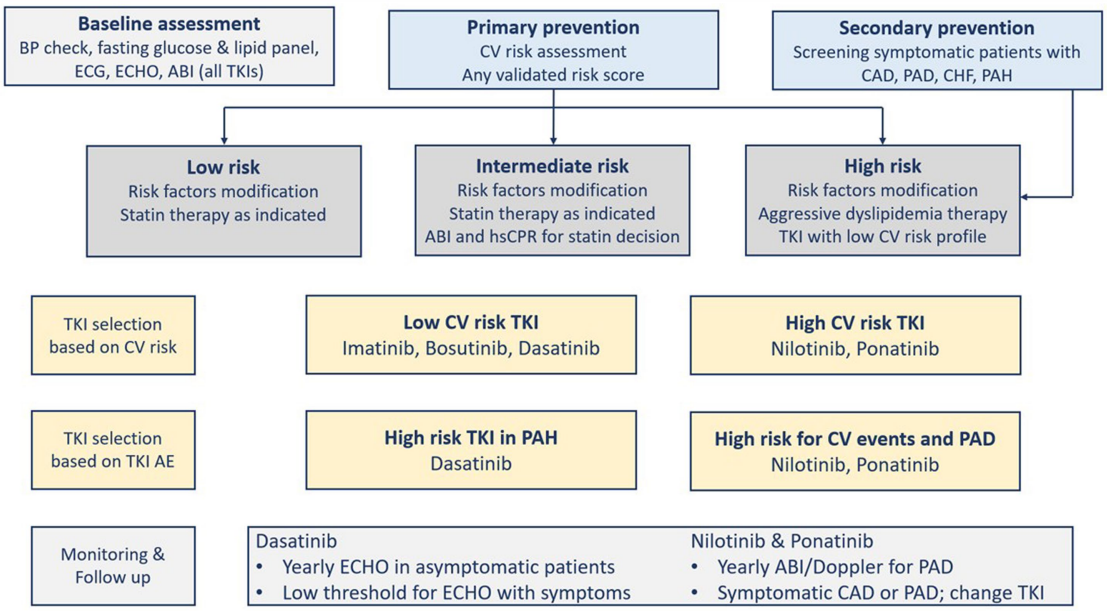

FIGURE 2
Cardiovascular risk assessment and tyrosine kinase inhibitors selection and monitoring. ABI, Ankle-brachial index; AE, Adverse Events; BP, blood pressure; CAD, coronary artery disease; CHF, congestive heart failure; CV, cardiovascular; ECG, electrocardiogram; ECHO, echocardiography; PAD, Peripheral arterial disease; PAH, pulmonary arterial hypertension; TKI, tyrosine kinase inhibitor(s).

therefore, its inhibition may be promising in preventing and/or treating cardiovascular adverse events due to the implicated TKIs (26, 27).

General approach to cardiovascular toxicity

The factors that determine the selection of a TKI comprise the goal of therapy, comorbidities, patient's age, and the TKI's toxicity profile (5, 28). Before deciding on the TKI therapy, cardiovascular risk factors should be evaluated. The presence of comorbidities such as coronary artery disease, hypertension, or diabetes should be closely monitored and followed up (2, 18). In addition, except for imatinib, TKIs are associated with serious cardiovascular complications (Table 1). These concerns should prompt cardiovascular risk assessment before starting TKI therapy (1, 18). Figure 2 shows the cardiovascular considerations when selecting a TKI agent (18). Given the absence of biomarkers that predict the cardiovascular risk associated with BCR::ABL1 TKI therapy, traditional risk scores derived from the general population are usually used in CML patients as well. However, such scores may underestimate the risk (27).

The prevention of cardiovascular diseases starts with the aggressive management of modifiable cardiovascular risk factors in patients with or without CML (7). The "ABCDE" approach lists the classic steps to reduce cardiovascular risk as follows: Assessment of cardiovascular disease signs and symptoms with aspirin use in select patients; Blood pressure control; Cigarette/tobacco cessation and Cholesterol level monitoring; Diabetes control and Diet/weight management; and Exercise (18, 28). International guidelines recommend calculating a risk score (e.g., Framingham, SCORE, QRISK, and Reynolds risk scores), and if the decision to consider statin is uncertain, then other factors can be considered in decision-making, for example, coronary artery calcium score, high-sensitivity C-reactive protein, or ankle-brachial index (ABI). Although such recommendations are not validated in CML patients, they can direct proper aggressive management of the modifiable risk factors. Statin therapy is effective for primary prevention in patients at high cardiovascular risk who can tolerate the therapy, particularly those on ponatinib (7). A systematic review and meta-analysis that used molecular docking to analyze the impact of aspirin and rosuvastatin on hub genes associated with nilotinib cardiotoxicity found that rosuvastatin can be effective in this case (29). Aspirin, in primary prevention, reduces the composite of serious cardiovascular events but is associated with increased gastrointestinal and intracranial bleeding risk. Thus, it needs to balance ischemic and bleeding risks. Aspirin use is more challenging in CML patients using certain TKIs (i.e., dasatinib) because it can inhibit platelet aggregation leading to increased bleeding risk (Table 6) (7). After the baseline assessment (i.e., cardiovascular risk, physical examination, vitals, laboratory tests), there should be follow-up visits at 1, 3, and 6 months (Figure 2) (18, 28). Another approach to reducing adverse events is the use of lower TKI doses and shorter durations (8). The analysis of the German CML-Study IV (Chronic Myeloid Leukemia-Study IV) has concluded that de-escalating a higher dose of imatinib (i.e., 800 mg daily) to 400 mg daily sustained deep molecular remission in 90% of patients (30).

Vascular adverse events

Occurrence and consequences

TKI-associated vascular events usually involve arterial beds (i.e., cerebral, coronary, and peripheral), which generally arise from various etiologies, for example, atherosclerosis, vasospasm, thrombosis, vasospasm, or vasculitis. Unfortunately, the definition of TKI-associated vascular events has not been consistent among the TKI studies and the vascular events have not been adjudicated in CML studies. A study, for example, defined vascular adverse events as myocardial infarction, ischemic stroke, venous thromboembolism, and arterial occlusive disease (31). The most frequently used oncology-related terminology was peripheral artery (or arterial) occlusive disease (PAOD) (18). PAOD refers to the obstruction or occlusion of large arteries (but not coronary arteries), aortic arch, or the arteries supplying the central nervous system. PAOD shares risk factors similar to those contributing to atherosclerosis such as age, obesity, smoking, male gender, dyslipidemia, diabetes, and hypertension. Kim et al. in their study defined a clinically manifest PAOD as experiencing an acute PAOD event or having typical peripheral ulcerations or imaging-identified lesions. PAOD diagnosis can be established by abnormal ABI measurement (i.e., value <0.9) and the use of imaging modalities such as angiography or ultrasonography can aid in identifying the affected arteries and localizing the related lesions (32). Measuring the ABI in CML patients verified the associated proatherogenic effects, as abnormal ABI is sensitive and specific in detecting peripheral artery disease (8). As such, the mechanism that explains the effect of TKI therapy may be the accumulation of atherosclerosis in numerous arterial beds (18). In a prospective screening for PAOD in chronic phase CML patients on TKIs, ABI was available for 81% of patients and 18.6% of them had pathological or abnormal ABI. The rates of pathological ABI were significantly higher with nilotinib use, either as first (26%; $p = 0.0297$) or second-line (35.7%; $p = 0.0029$) therapy than with imatinib first-line therapy (6.3%), accounting for PAOD relative risk of 10.3 with nilotinib first-line therapy. Clinical manifestations of PAOD were only seen in patients on nilotinib. In the first-line nilotinib group, pathological ABI developed after 21–56 months of therapy (32).

Vascular events are clinically important adverse events that were observed with second and third generations TKIs. Earlier, there was little data about the incidence of vascular adverse events with long-term TKI use and the predisposing factors because initial Phase I and II trials did not capture these events (8, 33). However, their frequencies considerably varied in the subsequent reports (e.g., 1–29% over 2 years) (8). Dahlén et al. retrospectively detected vascular events incidence in Swedish CML patients ($n = 896$) using first- and second-generation TKI therapy with a median follow-up of 4.2 years. The relative risks for arterial and venous events were 1.5 (95% CI: 1.1–2.1) and 2.0 (95% CI: 1.2–3.3), respectively. Myocardial infarction rates were higher with nilotinib or dasatinib than with imatinib use. However, the authors stated that patients may have been prescribed multiple TKI agents during the study (34). The retrospective evidence showed that the vascular events rate (1%) was significantly lower with imatinib than that with nilotinib or ponatinib use. Similarly, the events rate was low with both dasatinib and bosutinib (8). Chen et al. tested the incidence of vascular adverse events in Taiwanese CML patients ($n = 1{,}111$) treated with dasatinib, imatinib, or nilotinib and showed that the latter TKI significantly increased the incidence rate compared with imatinib (HR 3.13; 95% CI: 1.30–7.51), while dasatinib was associated with a non-significant numerical rise in events rate (HR 1.71; 95% CI: 0.71–4.26). The identified risk factors included old age, nilotinib use, and previous history of cerebrovascular disease (31). In a meta-analysis of randomized trials, the use of dasatinib [odds ratio (OR) 3.86, 95% CI: 1.33–11.18], nilotinib (OR 3.42, 95% CI: 2.07–5.63), and ponatinib (OR 3.47, 95% CI: 1.23–9.78) was associated with higher vascular occlusive events rate than with imatinib. Only a trend of an increased risk was found with bosutinib use (OR 2.77, 95% CI: 0.39–19.77) (33).

The reported rate of PAOD related to nilotinib ranged from 1.2 to 12.5% (32). Cardiovascular events associated with nilotinib seem to appear at a long-term follow-up (18). For example, at the 12- and 24-month follow-up of the ENESTnd (Evaluating Nilotinib Efficacy and Safety in Clinical Trials–Newly Diagnosed Patients) trial, there was not a safety signal of vascular adverse events (35, 36). Subsequently, PAOD started to be apparent after a minimum of three-year follow-up and did not necessitate drug discontinuation. Most of the patients with PAOD events had baseline risk factors for PAOD and more patients treated with nilotinib experienced ischemic heart adverse events (37). Similar pattern was seen at the 5-year follow-up (38). At a 10-year follow-up, the cumulative cardiovascular events rates continued to increase with nilotinib use for up to 16.5% (300 mg twice daily) and 23.5% (400 mg twice daily) compared with 3.6% for imatinib, even in patients with low Framingham risk score (39). In the ENESTnd extension study, dose escalation from 300 mg to 400 mg twice daily resulted in only a few new adverse episodes (40). The mechanism of vascular adverse events of nilotinib is multifactorial. The direct effect of nilotinib on the vascular cells by exerting proatherogenic and antiangiogenic effects leads to arterial stenosis and inhibition of repair mechanisms, respectively. Other nilotinib-related factors comprise vasospasms and metabolic effects (i.e., elevated cholesterol and glucose levels), all of which can provoke atherosclerosis (8, 18, 33). Ponatinib caused vascular adverse events even in patients without prior treatment with nilotinib, which led to ponatinib transient withdrawal from the market by the FDA in 2013 (8, 18). There is no sufficient data available on the vascular effects of ponatinib, but it may hinder the growth and survival of endothelial cells. Risk factors for TKI-related adverse events comprise those implicated in the process of atherosclerosis such as age, smoking, obesity, diabetes, hypertension, and hypercholesterolemia (8, 18, 33). In a prospective study, the rate of adverse events with ponatinib use was 8.9 and 17.1% after 11 and 24 months, respectively (8).

Management

Preventing vascular adverse events is of paramount importance in the management of CML patients because their prognosis is considered good when treated with TKIs, but vascular adverse events can affect their morbidity and transplant eligibility. Risk factors for PAOD are common in the general population which necessitates individual risk determination using a validated risk score along with aggressive treatment of the underlying comorbidities and modifiable risk factors as discussed above (Figure 2). Individual risk scores can also help in selecting a safe and optimal TKI in CML patients, which is an important initial step in the prevention of adverse events. In patients with multiple risk factors or high-risk scores, nilotinib or ponatinib are not the preferred TKIs if other agents can be considered (8, 33). It was suggested to reserve ponatinib for advanced disease in

the presence of a T315I mutation (33). Moreover, the presence of multiple risk factors for PAOD subjects the patient to pleural effusion while on dasatinib. Thus, the aforementioned agents (dasatinib, nilotinib, and ponatinib) are not the optimal choice, particularly in the elderly (8). The claim that bosutinib is a better option should be confirmed by clinical trials. However, the management of vascular adverse events that develop during nilotinib or ponatinib therapy should be individualized. Patients with low-grade PAOD, i.e., grades I and II, should be treated with optimal PAOD therapy and aggressive management of all cardiovascular risk factors, whereas the management of patients with high-grade PAOD, i.e., grades III and IV is more difficult, and some patients need to continue nilotinib or ponatinib based on the mutation status of the CML. Otherwise, the TKI should be stopped or replaced by another agent. Higher doses of TKIs are associated with more clinically relevant vascular adverse events. As a result, lower TKI doses and shorter durations of therapy may reduce the adverse event rates (8). Dorer et al. pooled data from three studies ($n = 671$) and found a significant correlation between the intensity of ponatinib dose and arterial occlusive events risk (OR 1.71). Ponatinib dose reduction by 15 mg daily decreased arterial occlusive events risk by 33% (41). The OPTIC (Optimizing Ponatinib Treatment in CP-CML) Phase II study explored a dose-reduction approach to examine three ponatinib starting doses (45, 30, and 15 mg daily) on the safety and efficacy in highly resistant CML patients. For 45-mg and 30-mg groups, the dose was reduced to 15 mg daily when treatment response was seen (i.e., BCR::ABL1IS transcript level is 1% or less). The response at 12 months (i.e., primary endpoint) was attained in 44.1, 29, and 23.1%, respectively. The arterial occlusive events of severity grade 3 or above in response to treatment occurred in three patients in the 15-mg group and five patients in each of the other groups. The investigators concluded that optimal efficacy and safety outcome was attained when starting treatment with 45 mg daily and then reducing it to 15 mg daily upon achieving a response (42). Patients with PAOD who require interventional revascularization due to cerebral ischemia or myocardial infarction should consider switching to another TKI therapy, along with the guidelines-recommended antiplatelet and anticoagulation therapies (8).

QT prolongation

Occurrence and consequences

QT prolongation and torsade de pointes are considered acute events. Their incidence was higher with TKIs (6.8%) than that with other anticancer agents (1.2%). The occurrence of QT prolongation was associated with increased polymorphic ventricular arrhythmia and the consequent fatal arrythmias and sudden cardiac death (2). Although the absolute risk is minimal, the risk of torsade de pointes has been associated with prolonged QT intervals, especially when QTc is above 500 milliseconds (ms) (16). There is a 2–3-fold increased risk for torsade de pointes when QTc is 500 ms or above compared with a value of less than 500 ms (22). When quantifying the risk, studies found that each 10-ms increase in QTc interval causes an increase of approximately 5–7% in cardiac events risks such as syncope, cardiac arrest, or death (19). Third-generation TKIs have been associated with QT prolongation (1). QT prolongation warranted a black box warning for nilotinib (18, 19). One of the on-target proposed mechanisms for QT prolongation is the inhibition of the PI3K signaling pathway that led to changes in sodium and potassium current, accounting for more than 70% of the overall prolongation (1, 2, 16). An off-target mechanism occurs through the interference of TKIs with cardiomyocytes potassium channel protein called human Ether-a-go-go (hERG) (2, 19). hERG facilitates ventricular repolarization of potassium current potential during phases 2 and 3 of the action potential. TKIs have variable effects on QT prolongation, with dasatinib being the most implicated agent (2). Potential risk factors that can potentiate the risk of QT prolongation include drug–drug interaction with inhibitors of cytochrome P450 (CYP) 3A4 (CYP3A4) and CYP2D6 enzymes (Table 6), genetic predisposition, electrolyte disturbances, and conditions (e.g., renal or hepatic failure) that reduce the elimination of TKIs (16, 19). Overall, the rate of drug-induced QT prolongation is considered low and patients with malignancies, in comparison with healthy individuals, may be at higher risk for torsade de pointes due to their underlying disease and its treatment. In addition, those with advanced disease may accept the risk of cardiotoxicities of their therapy that prolongs their survival unlike patients with less-advanced cancers who may not (16).

Management

The QT interval is usually measured based on leads II and V5 because they show the earliest onset of the QRS complex and the T-wave offset. There are different formulae to calculate the QT interval which correct the QT interval for the heart rate. The most common ones are the Bazett, Fridericia, and Hodges formulas (19). Although none of them is superior to another (1), the Fridericia formula is the one recommended in cancer patients and compared with other formulas, it has shown fewer errors at high and low heart rates (43). In the general population, the upper 99% limits of normal QTc values are 450 ms for men and 460 ms for women. QTc prolongation to 480 ms or above during cancer therapy is relatively frequent and requires closer monitoring. The change in QT interval of more than 60 ms from baseline does not necessitate an alteration in treatment decision as long as QTc remains below 500 ms (22). There is no risk score to help identify the patient's risk factors for QTc prolongation while on TKI therapy (19). However, Tisdale et al. developed and validated a risk score for the prediction of QT prolongation in hospitalized patients which may guide monitoring and therapeutic decisions. The risk score classifies patients into three risk categories as low (score < 7), moderate (score 7–10), and high (score ≥ 11), according to a calculated score based on the presence of nine risk factors (age ≥ 68 years, female sex, loop diuretic use, serum potassium ≤ 3.5 mEq/L, admission QTc ≥ 450 ms, QTc-prolonging drugs, acute myocardial infarction, sepsis, and heart failure) (44).

Before receiving a QT prolonging-TKI, a complete past medical and medication history should be collected (2, 16, 19). There are different reported congenital (e.g., Brugada and congenital long QT syndromes) and acquired reasons for QT prolongation. Acquired causes can be cardiac (e.g., cardiomyopathy and ischemia), metabolic (e.g., hypothyroidism and electrolytes), drug-induced (e.g., antiarrhythmics, psychotropics, and antimicrobials), or others (e.g., hypothermia) (16). Baseline and regular electrocardiogram (ECG) with QTc monitoring are advised during TKI therapy. Other monitoring measurements include blood pressure, electrolytes, thyroid hormones, B-type natriuretic peptide (BNP), and cardiac biomarkers (1, 2, 16). Immediate and careful patient evaluation

TABLE 6 Drug–drug interactions between tyrosine kinase inhibitors and potentially interacting drugs.

	TKIs CYP inhibitor	TKIs CYP substrate	TKIs Other effects
QT prolongation (drugs with known risk and their alternatives)			
Antiarrhythmic drugs	• Amiodarone, disopyramide, dofetilide, dronedarone, quinidine with imatinib DDI: moderate; monitor QT and toxicities Effect: TKI increase serum level of antiarrhythmics Mechanism: CYP3A4 inhibition	• Dronedarone with bosutinib and ponatinib DDI (ponatinib): moderate; monitor QT and its risk factors DDI (bosutinib): moderate; avoid combination Effect: increase serum level of TKI Mechanism: CYP3A4 inhibition	• Flecainide DDI (bosutinib): minor; no action needed DDI (dasatinib, nilotinib): moderate; monitor QT and its risk factors • Amiodarone, disopyramide, dofetilide, dronedarone (only with bosutinib), procainamide, quinidine, sotalol with bosutinib, dasatinib and nilotinib DDI (bosutinib): moderate; monitor QT and its risk factors DDI (dasatinib): major; consider alternatives DDI (nilotinib): major; avoid combination Effect: enhance QTc-prolonging effect of TKI Mechanism: additive effect
Alternatives	–		
Common antimicrobial drugs	–	• Clarithromycin (major), fluconazole (moderate) with bosutinib DDI: moderate; avoid combination DDI: major; avoid combination • Clarithromycin (moderate), fluconazole (minor) with imatinib • Clarithromycin (major), fluconazole (moderate) with ponatinib • Fluconazole (moderate) with dasatinib DDI: minor; no action needed DDI: moderate; monitor TKI toxicities DDI: major; consider alternatives Effect: increase serum level of TKI Mechanism: CYP3A4 inhibition	• Clarithromycin (major), azithromycin (dasatinib), fluconazole (nilotinib), levofloxacin, moxifloxacin, pentamidine (IV) (moderate), ciprofloxacin (minor) with nilotinib and dasatinib • Azithromycin, levofloxacin, moxifloxacin, Pentamidine with bosutinib (minor) DDI: minor; no action needed DDI: moderate; monitor QT and its risk factors DDI: major; consider alternatives Effect: enhance QTc-prolonging effect Mechanism: additive effect
Alternatives	Penicillin, cephalosporins, doxycycline, anidulafungin		
Prokinetic and antiemetic drugs	• Droperidol (moderate) with nilotinib DDI: moderate; monitor for QT and its risk factors • Domperidone (major) with imatinib and nilotinib DDI: major; avoid combination Effect: increase serum level of antiemetics Mechanism: CYP3A4 inhibition	–	• Ondansetron (moderate) with nilotinib • Chlorpromazine (moderate), domperidone, droperidol (minor) with bosutinib DDI: minor; no action needed DDI: moderate; monitor QT and its risk factors • Chlorpromazine with dasatinib and nilotinib DDI (dasatinib): major; consider alternatives DDI (nilotinib): major; avoid combination Effect: enhance QTc-prolonging effect Mechanism: additive effect • Domperidone, droperidol with dasatinib DDI (droperidol): moderate; monitor QT and its risk factors DDI (domperidone): moderate; consider alternatives Effect: enhance QTc-prolonging effect Mechanism: additive effect
Metoclopramide (conditional risk)	–	–	• Metoclopramide (minor) with dasatinib and nilotinib DDI: minor; no action needed Effect: enhance QTc-prolonging effect Mechanism: additive effect
Alternatives: Aprepitant, fosaprepitant, palonosetron	• Aprepitant (not with ponatinib), fosaprepitant with imatinib, nilotinib, and ponatinib DDI: moderate; avoid combination Effect: increase serum level of antiemetics Mechanism: CYP3A4 inhibition	• Aprepitant with bosutinib and dasatinib DDI (dasatinib): moderate; monitor TKI toxicities DDI (bosutinib): moderate; avoid combination Effect: increase serum level of TKI Mechanism: CYP3A4 inhibition	–

(Continued)

TABLE 6 (Continued)

	TKIs CYP inhibitor	TKIs CYP substrate	TKIs Other effects
Antipsychotic drugs	• Haloperidol, thioridazine with imatinib • Pimozide with ponatinib DDI (haloperidol): minor; no action needed DDI (thioridazine): moderate; monitor for thioridazine toxicities DDI (pimozide): moderate; avoid combination Effect: increase serum level of antipsychotics Mechanism: CYP3A4 (haloperidol/pimozide) or CYP2D6 (thioridazine) inhibition	–	• Pimozide, thioridazine with bosutinib (minor) • Pimozide with nilotinib • Pimozide, thioridazine with dasatinib DDI: minor; no action needed DDI (thioridazine): moderate; monitor QT and its risk factors DDI (pimozide): moderate; avoid combination Effect: enhance QTc-prolonging effect Mechanism: additive effect • Haloperidol with dasatinib, bosutinib, and nilotinib • Thioridazine with nilotinib DDI: moderate; monitor QT and its risk factors Effect: enhance QTc-prolonging effect Mechanism: additive effect
Alternatives	• Brexpiprazole (moderate) with ponatinib and nilotinib DDI: moderate; monitor Effect: increase serum level of brexpiprazole Mechanism: CYP3A4 inhibition		
Antidepressants	–	–	• Citalopram and escitalopram with bosutinib, dasatinib and nilotinib Effect: enhance QTc-prolonging effect of TKI DDI (bosutinib): minor; no action needed DDI (dasatinib/nilotinib): moderate; monitor QT and its risk factors Mechanism: additive effect
Alternatives: Desvenlafaxine, bupropion, vortioxetine	–	–	• Desvenlafaxine, vortioxetine with dasatinib DDI: major; monitor bleeding Effect: enhance antiplatelet properties Mechanism: additive effect
Pulmonary arterial hypertension			
Endothelin receptor antagonists	• Macitentan with imatinib, nilotinib DDI: moderate; monitor for macitentan toxicities Effect: increase serum level of macitentan Mechanism: CYP3A4 inhibition	• Bosentan with imatinib, dasatinib, nilotinib, ponatinib, and bosutinib DDI: moderate; monitor TKI efficacy DDI (Bosutinib): major; avoid combination Effect: decrease serum level of TKI Mechanism: CYP3A4 induction	–
Phosphodiesterase type-5 inhibitors	• Sildenafil/Tadalafil with imatinib, and nilotinib DDI: moderate; monitor for adverse effects (e.g., hypotension, headache) Effect: increase serum level of sildenafil Mechanism: CYP3A4 inhibition	–	–
Other classes	Soluble cyclic cGMP stimulators, prostacyclin receptor agonists, and prostacyclin derivatives do not interact with TKIs		
Primary and secondary cardiovascular disease prevention			
Antiplatelets	• Ticagrelor with imatinib and nilotinib DDI: moderate; monitor for adverse effects (e.g., bleeding) Effect: increase serum level of ticagrelor Mechanism: CYP3A4 inhibition	–	• Aspirin, clopidogrel, prasugrel, ticagrelor with dasatinib DDI: major; monitor for bleeding Effect: enhance antiplatelet properties Mechanism: additive effect; dasatinib carries risk of thrombocytopenia

(Continued)

TABLE 6 (Continued)

	TKIs CYP inhibitor	TKIs CYP substrate	TKIs Other effects
Statin*	• Atorvastatin, lovastatin, simvastatin with imatinib and nilotinib DDI: moderate; monitor for increased statin adverse effects (e.g., myopathy) Effect: increase serum level of statin Mechanism: CYP3A4 inhibition	–	–
Heart failure			
RAAS[§]	• Eplerenone with imatinib and nilotinib DDI: moderate; use maximum of 25 mg daily for heart failure and 25 mg twice for HTN Effect: increase serum level of eplerenone Mechanism: CYP3A4 inhibition	–	–
Other classes	Beta-blockers except for sotalol (see antiarrhythmics), thiazide or loop diuretics, and SGLT2i do not interact with TKIs		
Other relevant drug classes			
Fibrates	–	–	• Gemfibrozil with imatinib DDI: moderate; monitor therapy Effect: decrease serum level of imatinib Mechanism: possible inhibition of imatinib intestinal absorption and conversion to active metabolite (CYP1C8)
DHP-CCB	• Amlodipine (minor), felodipine (moderate), nifedipine (moderate) DDI: monitor for adverse effects (e.g., edema, hypotension) Effect: increase serum level of DHP-CCB Mechanism: CYP3A4 inhibition	–	–
Non-DHP-CCB (as CYP3A4 inhibitors)	–	• Diltiazem/Verapamil with bosutinib/ponatinib (moderate) and nilotinib (minor) DDI (bosutinib): moderate; avoid combination DDI (ponatinib/nilotinib): monitor TKI toxicities Effect: increase serum level of TKI Mechanism: CYP3A4 inhibition	–
Non-DHP-CCB (as substrates)	• Diltiazem (minor)/Verapamil (moderate) with imatinib and nilotinib DDI: monitor for side effects (e.g., hypotension, bradycardia) Effect: increase serum level of non-DHP CCB Mechanism: CYP3A4 inhibition	–	–

ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; ARNI, angiotensin receptor and neprilysin inhibitors; CCB, calcium channel blockers; cGMP, cyclic guanosine monophosphate; DHP, dihydropyridine; DDI, drug–drug interaction; HTN, hypertension; IV, intravenous; PCSK9i, proprotein convertase subtilisin/kexin type 9; QTc, corrected QT; RAAS, renin-angiotensin-aldosterone system; SGLT2i, sodium-glucose cotransporter-2 inhibitors; TKI(s), tyrosine kinase inhibitor(s).
*No DDI with ezetimibe, PCSK9i, or other unmentioned statins.
[§]No DDI with ACEI/ARB/ARNI, or spironolactone.
Reference: Lexicomp® available from: http://online.lexi.com/lco/action/interact; accessed on December 8, 2022.

should be performed upon detecting a prolonged QTc interval as defined (19). General measures comprise correcting and monitoring identifiable causes such as electrolytes imbalance (i.e., hypokalemia, hypomagnesemia, and hypocalcemia) (2, 16), and the presence of QT-prolonging drugs that interact with TKIs. The drug regimen should be modified or discontinued (Table 6). The presence of palpitation, syncope or presyncope, or QT prolongation with new-onset bradycardia of a rate less than 60 beats per minute and high-degree heart block should prompt immediate evaluation with continuous monitoring. The ECG must be repeated on a daily basis until QT prolongation is resolved. Correcting the QT prolongation by using drugs should be considered if there are concerning ECG signs for torsade de pointes. Treatment includes intravenous magnesium sulfate, beta-adrenergic agent (e.g., isoproterenol), lidocaine infusion and temporary pacing for refractory cases, and changing rate setting in patients with implantable cardiac devices (19). A proposed management algorithm for QT interval prolongation surveillance before and during TKI therapy is summarized in Figure 3 (2, 16, 18, 19).

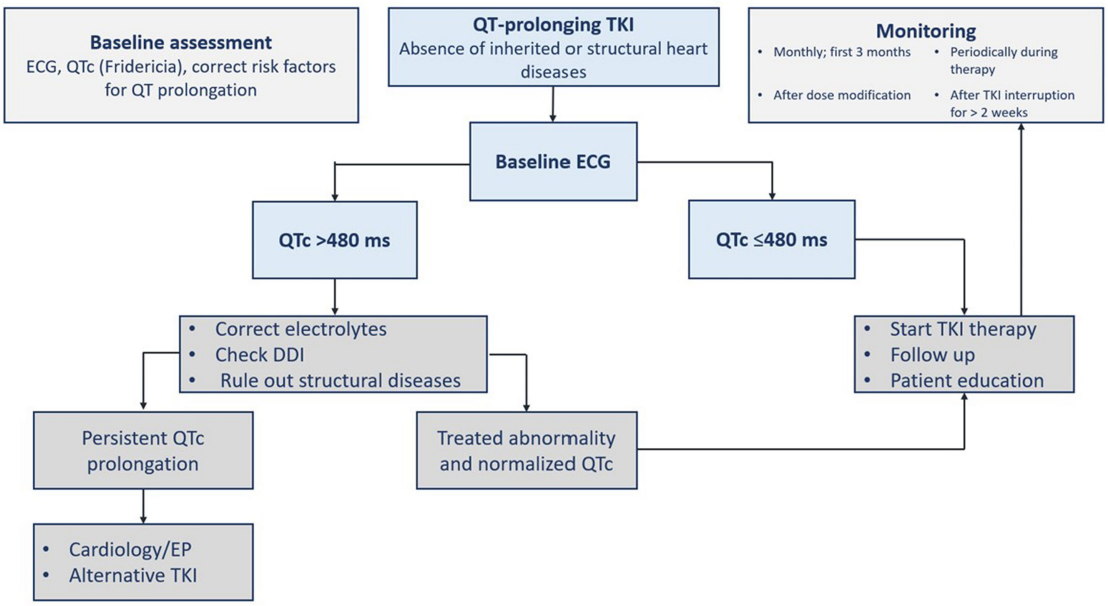

FIGURE 3
Assessment of QTc prolongation before and during tyrosine kinase inhibitor therapy. ECG, electrocardiogram; DDI, drug-drug interaction; EP, electrophysiology; TKI, tyrosine kinase inhibitor(s); QTc, corrected QT interval.

Heart failure

Occurrence and consequences

Cardiomyopathy or cardiac dysfunction induced by chemotherapy may range from asymptomatic myocardial injury [i.e., evident by a rise in cardiac troponin or decrease in global longitudinal strain (GLS)] to severe heart failure with reduced left ventricular ejection fraction (LVEF). Chemotherapy-induced cardiomyopathy is usually defined as a reduction in LVEF by more than 10% points and reaching a value below the lower limit of normal. Among the other cardiomyopathy types, it has the worse prognosis, adding to that the need for chemotherapy interruption or switching to a less effective alternative, hence leading to reduced survival in cancer patients (31, 45). Heart failure due to cardiomyopathy is a serious adverse event of TKI treatment. Dasatinib caused serious cardiotoxicity including heart failure and cardiomyopathy in 1.6% of patients receiving it. Mitochondrial damage, cardiac energy balance alterations, and contractile protein dysfunction can lead to left ventricular systolic dysfunction and consequent heart failure. It was hypothesized that inhibiting PDGFR and other tyrosine kinase receptors affects the myocytes' normal response to hypertensive stress. Cardiac damage may result from interfering with the ribosomal S6 kinase family as it inhibits the phosphorylation of apoptosis-activating factors and thus determines the survival of the cardiomyocytes (1).

Management

The risk of systolic dysfunction and heart failure is probably underestimated in the relevant studies (1). TKI-induced cardiotoxicity occurs independently of the dose and at any time throughout therapy (i.e., a few days to months). Early recognition of cardiomyopathy and initiation of heart failure therapy increases the chances of left ventricular function improvement (45). Patients on potentially cardiotoxic agents should be closely monitored for any cardiac dysfunction. Monitoring of patients on a TKI with unclear risk such as imatinib is only recommended in the presence of predisposing comorbidities, or signs and symptoms of cardiac disease. Patients with cancer therapy-induced heart failure should be managed according to the international heart failure guidelines regardless of their cancer status. Before the initiation of TKI therapy, LVEF should be assessed at baseline (1, 45). For patients on TKI, the surveillance strategy is not supported by adequate evidence. The gold standard in chemotherapy-induced cardiomyopathy monitoring is the assessment of LVEF. The utilization of cardiac troponin and BNP has been proposed and supported by evidence for the early detection of subclinical cardiotoxicity (45). For asymptomatic heart failure, the European cardio-oncology guidelines defined the respective CTRCD based on reductions in LVEF and/or changes in GLS (22). Thavendiranathan et al. in their study support the utilization of GLS to guide cardioprotective therapy to prevent CTRCD and reductions in LVEF (46). Earlier, Negishi et al. found that GLS was an early predictor of reductions in LVEF as a consequence of cancer therapy (47). Since chemotherapy-induced heart failure usually improves with guidelines-directed medical therapy, implantable cardiac devices should be reserved for patients with persistent systolic dysfunction or when there is a prognostic mortality benefit. In the absence of dedicated randomized trials, evidence from clinical practice showed a potential long-term efficacy of CML treatment. Patient education about the potential risk, the possibility of prevention, and the management approaches can help benefit from long-term therapy with TKIs (Figure 4) (16).

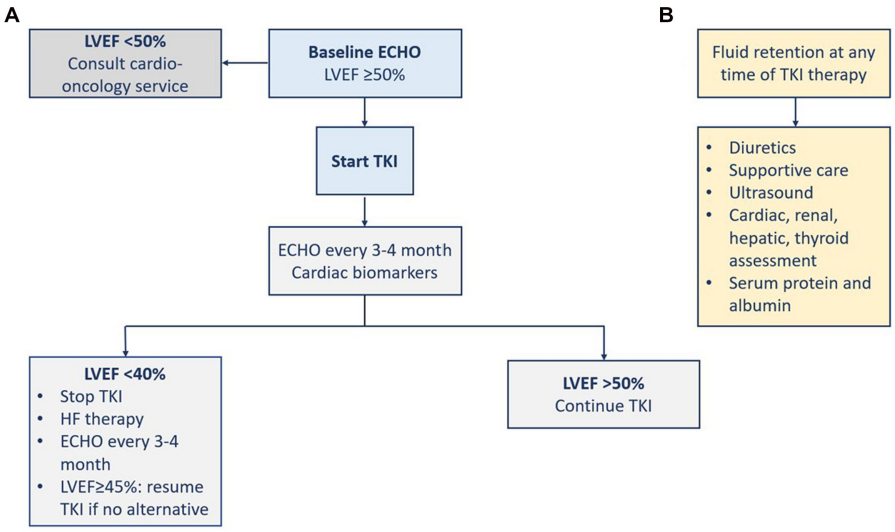

FIGURE 4
Management of heart failure **(A)** and fluid retention **(B)**. ECHO, echocardiography; LVEF, left ventricular ejection fraction; TKI, tyrosine kinase inhibitor.

Fluid retention

Occurrence and management

Fluid retention induced by some TKIs can result in pericardial effusion or pulmonary edema (1). At the 5-year follow-up of the ENESTnd trial, severe fluid retention was more frequent with imatinib (23.2%) than with the two doses of nilotinib (11.1 and 14.4%). Severe fluid retention included peripheral edema, pleural effusion, pulmonary edema, pericardial effusion, or cardiac tamponade (38). However, at the 10-year follow-up, severe fluid retention was infrequent (39). Although it has been described with the use of imatinib or bosutinib, heart failure was rarely observed (~1%). Pulmonary edema due to fluid retention was observed with dasatinib use. The inhibition of PDGFR signaling was proposed as a possible mechanism since it regulates the homeostasis of interstitial fluid. The standard management of fluid retention is diuretics and supportive care. The dose of the TKI can be reduced or the TKI can be stopped if needed. There should be an assessment for the cardiac, renal, hepatic, and thyroid functions, in addition to the serum levels of protein and albumin. Ultrasound modality is indicated to diagnose and monitor patients with any sign of pulmonary edema, pericardial or pleural effusion, and ascites (Figure 4) (1).

Pulmonary toxicity

The incidence of TKI-induced pulmonary toxicities is less than 1%. The frequent toxicities included pleural effusion, pulmonary arterial hypertension (PAH), pulmonary edema, interstitial lung disease, pneumonitis, chylothorax, and upper respiratory tract infection (5). The available data about the mechanisms of pulmonary toxicity involves that of dasatinib. The long-term toxicity data for both bosutinib and ponatinib is scarcely given their relatively recent approval. Luckily, the adverse events are usually reversible after drug discontinuation. Although there are various possible overlapping mechanisms for TKI-induced pulmonary toxicities, the strongly implicated ones are, direct cell injury, endothelial cell dysfunction, excess reactive oxygen species at cellular levels, or indirect inflammatory-mediated events. The management of TKI-induced pulmonary toxicity should be individualized considering the severity of respiratory status and hematology service involvement (3).

Pleural effusions and interstitial lung diseases

Occurrence and consequences

The incidence of pleural effusions differs between the TKIs, with dasatinib being the most implicated TKI. Interstitial lung diseases are rarer in occurrence but both conditions should be suspected in TKI-treated patients when experiencing non-specific respiratory symptoms. None of the conditions was reported with ponatinib. An incidence of pleural effusion of less than 5% occurred with bosutinib over 5 years, with more frequent rates in older patients (3). Chylothorax, a subgroup of pleural effusion, is a rare pulmonary toxicity that was reported with dasatinib (5). A retrospective study ($n = 212$) found that 55% of chronic phase CML patients on dasatinib had adverse events. Pleural effusions were the most frequent ones (25%) and the predominant reason for permanent drug discontinuation. The authors suggested the use of the lowest effective dose particularly in older patients to decrease the risk of pleura effusion occurrence (34, 48). The FDA has announced a warning about the cardiopulmonary risk of dasatinib therapy (18). However, the response to dasatinib therapy and the survival (i.e., both progression-free and overall) did not differ between patients who developed pleural effusion and who did not (3). The significant risk factors for the development of pleural effusion associated with dasatinib included twice daily dasatinib schedule, duration of CML, skin rash, duration of dasatinib therapy, and comorbidities such as cardiac history, hypertension, hypercholesterolemia, and autoimmune disease (3, 5, 8).

Management

A detailed investigation for pleural effusion is warranted given the various possible causes of it. If there is a sufficient effusion volume, diagnostic thoracentesis is indicated to exclude pleural infection and to eliminate other differential diagnoses. The use of diuretics and corticosteroids has been described in the literature but has not been tested in trials. Their use may be dependent on the causative element of the pleural effusion. The size and consequences of the effusion usually dictate specific management. Close clinical and diagnostic monitoring can be sufficient with minimal effusion volume, whereas effusions with moderate or large volumes may necessitate reducing the TKI dose, withdrawing, or changing the used TKI. Since the occurrence of interstitial lung diseases with TKI therapy is rare, other possible causes should be ruled out. TKI-induced pneumonitis resolves spontaneously with TKI discontinuation or corticosteroid use. It has been reported that interstitial lung disease due to imatinib did not occur when changing to nilotinib. However, re-challenging with the use of the offending TKI can be considered based on the risk–benefit assessment for an individual patient (3).

Dasatinib is associated with high occurrence rates of pleural effusion, with the patient's age being the most significant factor for its incidence. However, it is usually a reversible adverse event in most cases (5). Dose reduction (e.g., 100 mg once daily), dose interruption, TKI discontinuation, and drug therapy have been suggested for the management of dasatinib-induced pleural effusion (3, 5, 7). Naqvi et al. updated the results of their initial study and continued to demonstrate the efficacy and tolerability of dasatinib 50 gm daily for newly diagnosed CML patients in the chronic phase (49, 50), with 6% of patients experiencing pleural effusion and 80% of them needed further dose reduction (50). In the multicenter single-arm DAVLEC (DAsatinib, Very Low-dose, for Elderly CML-CP patients) Phase II Japanese study ($n = 52$), the standard dasatinib starting dose was reduced by 20% and 20 mg daily was administered in new diagnosed elderly patients above 70 years of age. Pleural effusion, not pulmonary hypertension, was observed in 7.7% of patients. The investigators encouraged dose reduction consideration and conducting more studies on larger and more diverse cohorts (51). However, dasatinib dose reduction did not prevent the recurrence of pleural effusion in all the cases. Drug withdrawal was reported in 22% of patients who developed dasatinib-induced pleural effusion without the need for a therapeutic thoracentesis. Switching from dasatinib to bosutinib resulted in the recurrence of pleural effusion in 30% of the cases. Although not widely used, therapeutic drug monitoring of TKIs may minimize pleural effusion rates (3). There are some reports on the use of diuretics and corticosteroids as mentioned above (3, 7). The use of vasopressin V_2-antagonist (tolvaptan) with diuretics, in some reports, improved pleural effusion and allowed continuing or reintroducing dasatinib (3).

Patients with Class 1 pleural effusion do not require intervention. In asymptomatic patients with Class 2 or more, TKI therapy should be interrupted, and diuretics can relieve fluid retention if present. TKI should be resumed after effusion resolution with dose reduction in case of further occurrences. If patients with Class 2 or more are symptomatic or with Class 3 or more but asymptomatic, dasatinib therapy should be interrupted and then resumed after the effusion is resolved. However, it should be discontinued if the pleural effusion recurs. In addition, corticosteroids such as 40 mg of prednisone per day, should be given for 4 days, and the pleural fluid should be examined for other potential causes. Another classification approach defines small effusion as a volume of less than 500 ml with blunting costophrenic angle; medium effusion as with opacity above the costophrenic angle; and large effusion when having more than 30–50% of hemithorax. The respective management and monitoring of each severity are presented in Figure 5 (5).

Pulmonary arterial hypertension

Occurrence and consequences

Pulmonary arterial hypertension has been reported with dasatinib therapy, rarely with bosutinib, nilotinib, or ponatinib, whereas no reports with imatinib (3). In PAH, there is usually an increase in pulmonary vascular resistance (PVR) and pulmonary artery pressure (PAP) leading to right ventricular failure (7); mean PAP is above 20 mmHg and PVR is 3 Wood units (WU) or above with normal left cardiac filling pressures (i.e., pulmonary artery wedge pressure (PCWP) $\leq$15 mmHg) (3, 52). Dasatinib is implicated in increasing the pressure in the pulmonary arterial system, hence causes a pre-capillary PAH (i.e., Clinical Group 1) (7). Earlier, a study using data from the French pulmonary hypertension registry identified nine dasatinib-treated patients with pulmonary hypertension, from dasatinib approval from November 2006 to September 2010. The estimated incidence of dasatinib-related pulmonary hypertension in France was 0.45%. There were not any incidental pulmonary hypertension cases related to other TKIs at the time of diagnosis. Four months after the discontinuation of dasatinib, there were hemodynamic, clinical, and functional improvements. However, there was not a complete recovery in most of the patients after 9 months of follow-up (20). Another study from the same French registry investigated the long-term outcomes of 21 patients with dasatinib-induced PAH which was confirmed by right-heart catheterization. Nineteen patients with CML received dasatinib for a median of 42 months before PAH diagnosis. Dasatinib was discontinued in all patients, and half of them ($n = 11$) received therapy for PAH such as bosentan, sildenafil, and calcium channel blockers. Despite improvement after drug discontinuation, PAH continued in 37% of patients. Concomitant pleural and pericardial effusions were detected in 62 and 29% of patients, respectively (53). A multicenter study from Australia retrospectively studied 212 patients with chronic phase CML. The study estimated an occurrence of 5% for dasatinib-induced PAH which was frequently associated with pleural effusion. PAH was reversible in most of the cases after dasatinib discontinuation. Permanent discontinuation of dasatinib in immunologically competent patients was not necessary (48).

Cases with reversible PAH may have an intense pulmonary arterial vasoconstriction (3), while the frequently reported cases with the persistence of PAH may suggest an irreversible pulmonary arterial remodeling (3, 7). The latter proved valid with histological examination of explanted lungs from a patient who required lung transplantation. The survival of patients with dasatinib-induced PAH (i.e., 90.5 and 85.7% at 1- and 5-year follow-up, respectively) was similar to that reported in dasatinib randomized trials in CML (3). PAH, however, is considered a life-threatening consequence of dasatinib long-term therapy, particularly when accompanied by pleural effusion. If left untreated, it may cause right ventricular failure (5). Finally, although ponatinib was associated with a 6% increase in venous thromboembolic events, there were no cases reported for thromboembolic PAH due to TKIs use (3).

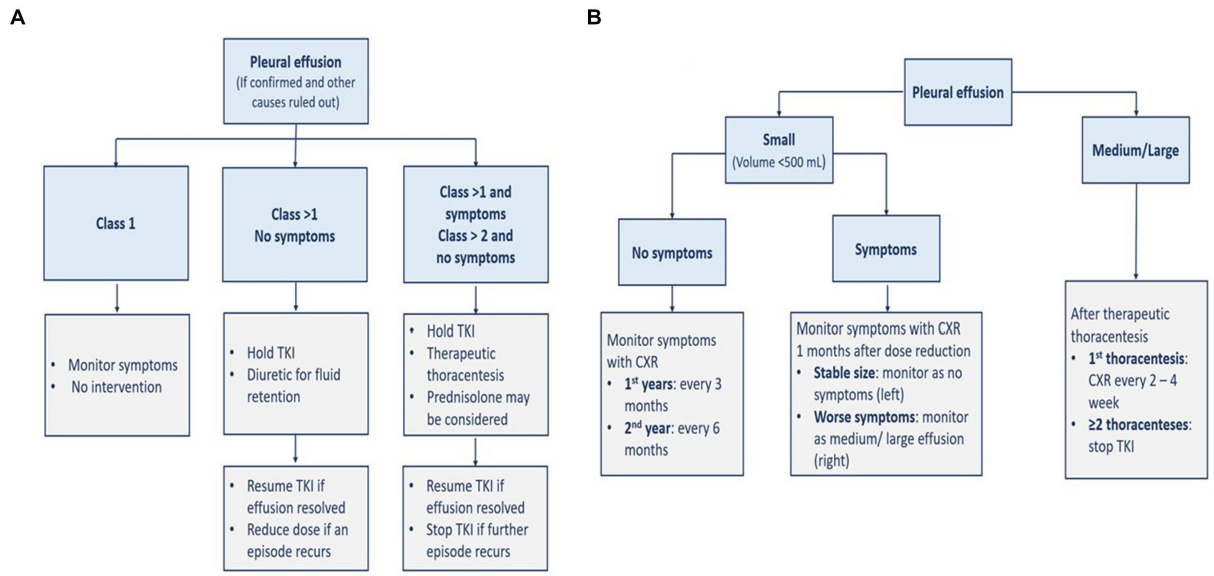

FIGURE 5
Management **(A)** and monitoring **(B)** of pleural effusion. CXR, chest x-ray; TKI, tyrosine kinase inhibitor.

Management

The incidence of symptomatic PAH is relatively low and does not warrant PAH screening in asymptomatic patients (3). Dasatinib is the TKI with the potential to cause PAH. Early PAH diagnosis and TKI therapy discontinuation are recommended (5). The symptoms of PAH are usually not specific such as dyspnea, fatigue, atypical chest pain, or unexplained syncope. The presence of non-specific symptoms should raise suspicion about PAH diagnosis and prompt investigation using chest X-ray and echocardiogram with Doppler flow, as well as considering referral to cardiology service if warranted. A baseline echocardiogram before dasatinib initiation may be performed, if feasible, to identify a pre-existing PAH (3). If PAH is suspected during TKI therapy, a chest X-ray is performed to rule out pleural effusion, and an echocardiogram is performed to assess PAP and then identify the cause of the elevated PAP (≥25 mmHg). A right-heart catheterization is indicated to confirm PAH diagnosis (5, 7, 18).

When there is a high probability of a new pulmonary hypertension occurrence in a CML patient (i.e., peak tricuspid regurgitation velocity (TRV) is more than 3.4 m/s which is equivalent to an estimated PAP of 50 mmHg or more), TKI therapy should be discontinued until ruled out or confirmed with right-heart catheterization (22). More specifically, if dasatinib-induced PAH is diagnosed, dasatinib therapy should be discontinued (5, 7, 22), and alternative TKI is recommended (22). If dasatinib-treated patients have new asymptomatic peak TRV between 2.9 and 3.4 m/s, the dasatinib dose can be reduced and peak TRV should be monitored every 4 weeks with echocardiography. If peak TRV keeps rising and PAH is confirmed by right-heart catheterization, dasatinib should be stopped (22). Symptoms and PAP usually improve after dasatinib discontinuation (5, 7) but usually without complete recovery of the hemodynamic parameters (5). The overall treatment of pulmonary hypertension should be according to the respective societal guidelines (22). The management of PAH is directed toward the FDA-approved drug classes for PAH treatment and collaboration with the PAH team (5, 7). FDA-approved drug classes include phosphodiesterase type-5 inhibitors (e.g., sildenafil and tadalafil), endothelin receptor-1 antagonists (e.g., bosentan, ambrisentan, and macitentan), prostacyclin derivatives (e.g., systemic epoprostenol, inhaled iloprost, and oral and systemic treprostinil), soluble guanylate cyclase stimulator (e.g., oral riociguat), and prostacyclin receptor agonist (oral selexipag) (5). The steps for the diagnosis and management of TKI-induced PAH are demonstrated in Figure 6 (3, 5, 7, 22). There is not any evidence suggesting the effectiveness of calcium channel blockers in dasatinib-induced PAH (5).

Drug–drug interactions

Most of the TKIs are metabolized by CYP enzymes and transported by P-glycoprotein (P-gp) (2, 7). Strong CYP enzyme inhibitors can increase TKIs plasma concentrations (1, 16), and vice versa as TKIs can inhibit CYP enzymes to varying degrees (7). Dasatinib can also influence P-gp, the drug transporter (2). Table 6 lists the drug–drug interactions between TKI agents and the most used drugs in cardiopulmonary conditions (54).

Summary

The introduction of TKIs is considered a milestone in the treatment of CML but their use was associated with a range of serious cardiopulmonary toxicities including vascular adverse events, QT prolongation, heart failure, pleural effusion, and PAH. The current fully approved TKIs (imatinib, bosutinib, dasatinib, nilotinib, and ponatinib) have different efficacy and safety profiles. The exact underlying mechanisms for cardiopulmonary toxicities are not fully known but on- and off-target effects may be partly responsible. Imatinib has the most favorable safety profile among TKIs. Nilotinib and ponatinib were associated with vascular occlusive adverse events, whereas dasatinib was with pleural effusion and PAH. QT prolongation was mostly linked to third-generation agents. TKI-induced adverse events can lead to serious morbidities if left untreated.

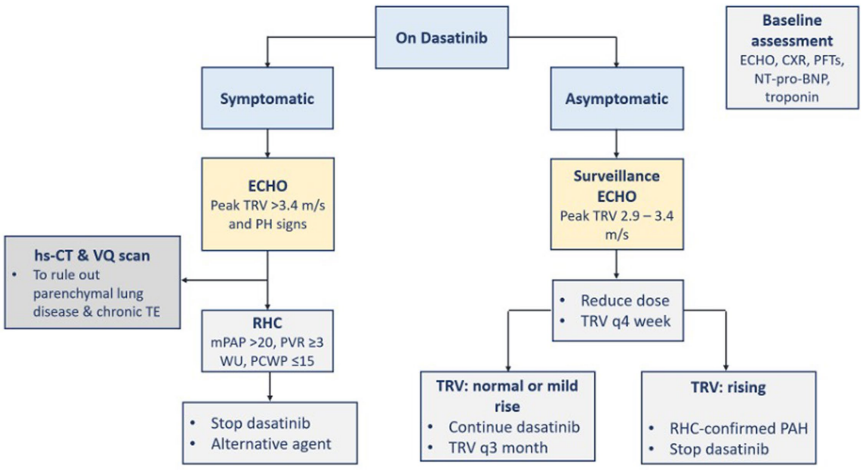

FIGURE 6
Diagnosis and management of TKI-induced PAH. BNP, B-type natriuretic peptide; CXR, chest x-ray; ECHO, echocardiography; hs-CT, high resolution computed tomography; mPAP, mean pulmonary arterial pressure; PAH, pulmonary arterial hypertension; PCWP, pulmonary artery wedge pressure; PFTs, pulmonary function tests; PH, pulmonary hypertension; PVR, pulmonary vascular resistance; RHC, right heart catheterization; TE, thromboembolism; TKI, tyrosine kinase inhibitor; TRV, tricuspid regurgitation velocity; VQ, ventilation perfusion; WU, Wood units.

The assessment of cardiovascular risk is crucial before starting TKI therapy. The choice of TKI agent should be dictated by its safety profile, disease state, patient comorbidities, and other concurrent therapies considering their interacting tendency. The management of cardiopulmonary adverse events should be integrated with a multidisciplinary collaboration between the respective specialties. Early recognition, frequent monitoring, optimal intervention, and adequate follow-up are essential to managing adverse events while maintaining the long-term TKI therapy benefit. Awareness of the potential TKIs toxicities and balancing the risks and benefits of such therapy should be considered. Future research should fill in the gaps in knowledge about molecular mechanisms, frequencies, definitions, classifications, predictors, and management guidelines of TKIs toxicities.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication. All authors contributed equally to the manuscript.

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Conflict of interest

RK, WD, KA, and MY were employed by the Hamad Medical Corporation.

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Glossary

Acronym	Explanation
ABI	Ankle-brachial index
CML	Chronic myeloid leukemia
CRCD	Chemotherapy-related cardiac dysfunction
CTCAE	Common Terminology Criteria for Adverse Events
CTRCD	Cancer therapy-related cardiac dysfunction
CYP	Cytochrome P450
DAVLEC	DAsatinib, very low-dose, for elderly CML-CP patients
ECG	Electrocardiogram
EGFR	Epidermal growth factor receptor
ENESTnd	Evaluating Nilotinib Efficacy and Safety in Clinical Trials–Newly Diagnosed Patients
FDA	Food and Drug Administration
FEARS	Food and Drug Administration adverse event reporting system
GLS	Global longitudinal strain
hERG	Human Ether-a-go-go
LVEF	Left ventricular ejection fraction
NCI	National Cancer Institute
OPTIC	Optimizing Ponatinib Treatment in CP-CML
OR	Odds ratio
PAH	Pulmonary arterial hypertension
PAOD	Peripheral arterial occlusive disease
PAP	Pulmonary artery pressure
PDGFR	Platelet-derived growth factor receptor
QTc	Corrected QT interval
ROCK	Rho-associated coiled-coil containing kinase
TKI(s)	Tyrosine kinase inhibitor(s)
VEGFR	Vascular endothelial growth factor receptor

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Splenectomy is significantly associated with thrombosis but not with pulmonary hypertension in patients with transfusion-dependent thalassemia: a meta-analysis of observational studies

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Introduction: Thromboembolism (TE) and pulmonary hypertension (PH) constitute frequently occurring complications in patients with transfusion-dependent thalassemia and have been associated with splenectomy in different studies. Nevertheless, the size of the possible association varies greatly in literature. Herein, we sought to provide pooled effect estimates regarding the impact of splenectomy on TE and PH in transfusion dependent thalassemia (TDT) by retrieving relevant, available studies.

Methods: We systematically searched articles published in PubMed, Cochrane library, Scopus and gray literature from inception until the 30th of May, 2023. Pooled estimates in terms of odds ratios (OR) and 95% confidence intervals (CI) were calculated according to outcome measures. Risk of bias and quality of studies were evaluated.

Results: Regarding TE, 4 studies were selected for meta-analysis and the pooled data demonstrated that splenectomy was significantly associated with this outcome in TDT patients [OR = 4.08, 95% CI (1.03, 16.11), $p = 0.04$]. On the other hand, we pooled data from seven investigating PH, and, interestingly, the quantitative analysis revealed no association between splenectomy and PH [OR = 1.76, 95% CI (0.91, 3.41), $p = 0.1$].

Conclusion: Splenectomy is associated with higher risks of TE, but not with PH in patients with TDT.

KEYWORDS

thalassemia, splenectomy, thrombosis, pulmonary hypertension, meta-analysis, observational

1. Introduction

Thalassemia syndromes are a diverse collection of congenital autosomal recessive hemoglobinopathies caused by globin gene abnormalities, most of which are minor nucleotide substitutions. The two primary types of the disease, alpha-thalassemia and beta-thalassemia, are distinguished by decreased or absent synthesis of either the alpha-or beta-globin chains of the hemoglobin molecule (1). Cooley and Lee described thalassemias for the first time almost a century ago, when they reported cases of severe anemia with splenomegaly and distinctive bone changes (2). At least one variant globin allele is carried by an estimated 5% of the global population, with a higher prevalence observed in the Mediterranean region, the Middle East, Africa, Southeast Asia, and the Indian subcontinent (1).

Patients with beta-thalassemia have a variety of clinical manifestations. Historically, this illness was classified as thalassemia major, intermedia, and minor based on the globin chain ratio (alpha/beta), degree of anemia, and clinical history. Asymptomatic microcytic anemia is common in patients with beta-thalassemia minor (carrier or trait). Thalassemia syndromes are nowadays classified into two groups depending on clinical severity and transfusion requirement: TDT and Non-Transfusion-Dependent Thalassemia (NTDT). TDT patients are unable to produce enough hemoglobin to survive without transfusions. Transfusions are rarely required in NTDT patients (3). TDT necessitates maintaining a pre-transfusion hemoglobin level of 95–105 g/L, which suppresses erythropoiesis and allows for a reduction in blood consumption. The higher standards for pretransfusional hemoglobin levels that current transfusion guidelines in TDT are setting are usually attained by more frequent transfusions (4).

Despite advances in therapeutic care in the era of novel drugs such as luspaterecpt, splenectomy remains an essential treatment option in TDT patients, particularly in low-income countries, and is considered in the following cases: higher transfusion needs (200–220 mL of red blood cells/kg/year), symptomatic splenomegaly, signs of hypersplenism resulting in clinical problems (4). The therapeutic rationale for splenectomy, particularly in patients suffering from poor health due to thalassemia-induced medical conditions, is to protect against the establishment of extramedullary hematopoiesis by increasing hemoglobin levels, decreasing the need for transfusions, and, as an ultimate result, minimizing iron overload (5). Both open and laparoscopic techniques are employed for total splenectomy, with the latter requiring shorter hospitalization and appearing to offer lower morbidity and short-term mortality. In a limited number of centers, partial splenectomy, which preserves some immune functions of the spleen, as well as embolization of splenic tissue are evaluated, although not widely accepted. Adverse events following splenectomy include bleeding, atelectasis, subphrenic abscess, extreme thrombocytosis and overwhelming post-splenectomy sepsis. Morbidities are common in people with TDT. The procedure of splenectomy has been related to major long-term complications including thromboembolic and PH consequences, as well as infections, which increase morbidity and death risk in these patients (4). The spleen normally removes damaged red cells. In the absence of spleen, high levels of negatively charged RBCs, as well as high levels of platelets which present with hyperactivity, seem to contribute to the hypercoagulable state of thalassemia, as these cell elements stay in the blood circulation and activate thrombin production mechanisms (6).

PH is most frequently identified in NTDT, although it is also becoming more common in TDT lately. This complication is diagnosed and monitored by echocardiography (tricuspid valve jet velocity), while cardiac catheterization is often used for validation. Pulmonary vasodilator therapies are used for the management of PH. Additionally, thalassemia patients tend to present with an increased risk for arterial and venous thrombosis. Risk factor education with regard to the avoidance of other risk factors, aspirin prophylaxis for at-risk individuals and routine anticoagulation are included in the therapeutic management of TE in TDT patients. Nevertheless, TDT is a lifelong high-burden disease both for patients and for healthcare systems (3). The scientific community must invest in better understanding the etiology of the disease complications and the elements that influence its natural history, since this will lead the development of new therapeutics, as well as appropriate and timely use of the already available agents.

Therefore, we sought to determine the impact of splenectomy on two major complications, namely TE and PH, by performing the first in the literature systematic review and meta-analysis of relevant studies.

2. Methods

This systematic review and meta-analysis is conducted in accordance with the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines (7) and the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines (8).

2.1. Eligibility criteria

We searched the articles published in PubMed, Cochrane library, Scopus and gray literature from inception until the 30th of May, 2023. We searched for studies enrolling adult patients diagnosed with TDT who were splenectomized compared to non-splenectomized TDT patients, reporting on the complications TE and PH. We did not impose any restriction regarding language of publication, study design (retrospective, prospective), setting and sample size. We excluded case reports, case series, former meta-analyses (if any), editorial and opinion papers, narrative reviews.

2.2. Search strategy

We searched articles published in PubMed, Cochrane library, Scopus, and gray literature, namely conference proceedings, including full-text articles in English. We did not impose any filter regarding sample size, study setting, or publication language. MeSH terms were used for both intervention and outcomes, along with free-text words. We also used the Boolean operators "OR" and "AND." The searching strategy applied in PubMed is shown in Supplementary Appendix Table 1.

2.3. Data extraction

Following deduplication, two independent reviewers (T-VK, KD) screened all records at title and abstract level and then assessed the full

text of eligible records. Any disagreements were resolved by consultation of a third reviewer (EV).

Two independent reviewers (T-VK, KD) extracted the data from the eligible reports. Relevant information was extracted and recorded on a data collection form developed in Microsoft Excel©. Extracted information included the following: first author, year of study conduction, country of origin, study sample size, key clinical outcomes (TEE, PH), measurement method of PH, type of thrombosis.

The Newcastle-Ottawa Scale (NOS) (9) was used by two independent reviewers (T-VK, KD) to assess the quality of the included observational studies. The included studies were evaluated based on three general criteria: study participants, group comparability, and determination of either the exposure or outcomes of interest. Any individual study can receive up to four stars for selection, two stars for comparability, and three stars for outcome, with a maximum score of nine stars. Divergent views among reviewers were settled through debate, consensus, or arbitration by a third senior reviewer (EV).

2.4. Data synthesis and analysis

We planned to assess major clinical endpoints (TE, PH) representing dichotomous variables, thus the OR with 95% CI were estimated. To generate the pooled estimates of the outcomes, the Mantel–Haenszel (M-H) random effects formula was implemented. To assess the extent to which statistical heterogeneity in meta-analysis is due to differences between studies rather than accidental, we used I^2 statistic. Heterogeneity was considered to be low if I^2 was between 0 and 25%, moderate if I^2 was between 25 and 50%, or high if I^2 was greater than 75% (10). The forest plots were used for a visual representation of the presence and nature of statistical heterogeneity. A value of $p < 0.05$ was considered statistically significant. All statistical analyses were performed using the RevMan 5.3. software (11, 12).

3. Results

3.1. Data sources and selection process

As shown in the corresponding PRISMA flow diagram (Figure 1), our search strategy retrieved 445 results in total. After deduplication, we initially screened 173 records at title and abstract level. Finally, we assessed 21 records in full text. Nine (13–21) of them were evaluated as eligible for inclusion in our qualitative synthesis. Of these, four studies were included in the quantitative synthesis regarding the outcome TE and seven studies were used in the final PH meta-analysis. Twelve observational studies were excluded for various reasons (Figure 1). Five studies (22–26) were excluded from the analysis due to different population (pediatric population). Moreover, three studies (27–29) were not included due to the lack of control group. The results presented by another study were also excluded, due to the fact that a different outcome was assessed (quality of life) (30). The study of Alieva et al. (31) was not included because the full study was only available in Russian. Additionally, the study by Derchi et al. (32) was not taken into account due to study design differences. Finally, the study by Taher and colleagues (33) could not be included, as original data were not available after contact with the author.

3.2. Characteristics of the included studies

A detailed description of participants' baseline characteristics is provided in Table 1. Regarding the primary outcome TE, we pooled data from four studies in a total of 690 enrolled subjects. Overall, 15 cases of thrombosis were detected, of which 14 (93%) were observed on splenectomized patients. Thromboembolic events included portal vein thrombosis (PVT), deep vein thrombosis (DVT), pulmonary embolism (PE), transient ischemic attack (TIA) and cerebrovascular disease. The commonest adverse event was PVT (47%), followed by DVT (27%). Most events were recorded between 1 and 5 years after splenectomy, while the patients were receiving low dose aspirin (80-100 mg). As far as the outcome PH is concerned, data were collected from 7 studies, with a total of 395 participants. Sixty-three patients were referred with PH, 45 (71%) of whom were splenectomized.

3.3. Meta-analysis

As shown in Figure 2, we demonstrated that splenectomy is associated with a statistically significant higher prevalence of TE in TDT patients [OR = 4.08, 95% CI (1.03, 16.11)], with the test for overall effect conforming statistical significance ($p = 0.04$). Notably, no association between splenectomy and PH was proved [OR = 1.76, 95% CI (0.91, 3.41), $p = 0.10$] according to Figure 3. Heterogeneity of the included studies was low in both analyses ($I^2 = 0$).

3.4. Assessment on quality of studies

The risk of bias appraised among the included studies is shown in Table 2. Study quality scores ranged from 6 to 9, and all of them were of good quality.

4. Discussion

In this study we attempted to synthesize and assess the already documented evidence, as derived by the existing observational studies, regarding the risk of splenectomized thalassemic patients in developing TE or PH. In order for this to be achieved, a systematic review of the already existing bibliography was conducted according to the PRISMA guidelines (7). Thus, we utilized meta-analysis as a robust tool and followed an organized approach, in order to assimilate data and combine results from multiple independent studies answering our research question.

According to our results, statistical significance ($p = 0.04 < 0.05$) was observed with regard to the outcome TE, but no evidence of significant association was found regarding PH ($p = 0.10 > 0.05$). Our conclusions may be less definite, as some of the comparisons in the included studies arise from retrospective data and historical controls, while, additionally, a retrospective design often lacks full availability of risk factors data and comorbidities. Specifically, both thrombosis and PH are rather complicated and multifactorial phenomena, so a direct causative relation between these events and splenectomy in TDT patients is difficult to be unraveled. Nevertheless, our results should be highlighted, although they must be confirmed in large-scale prospective, well-designed trials in the future.

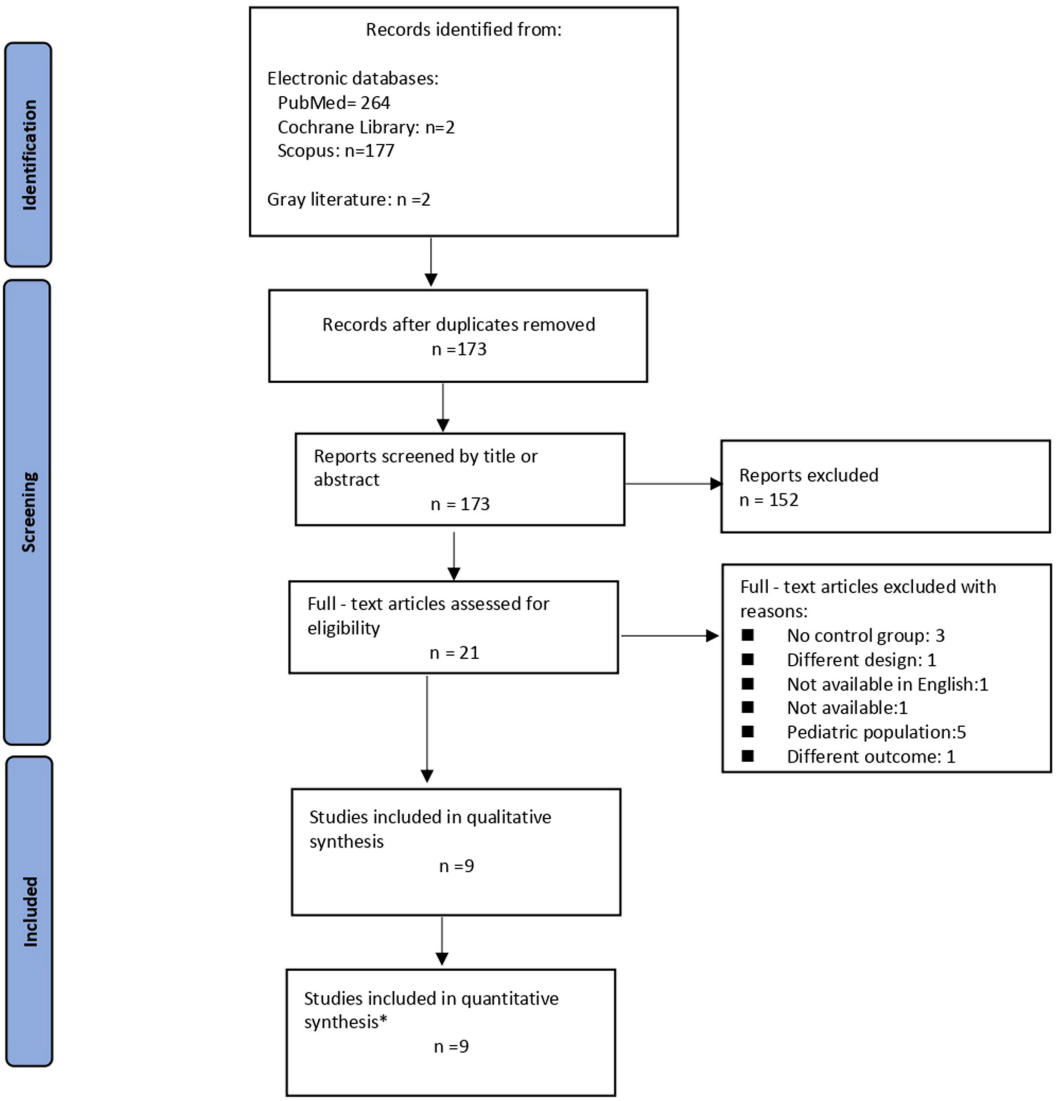

FIGURE 1
Flow diagram illustrating the study selection process. *Two studies were common fo the two outcomes (TE, PH) and were used in both analyses.

Certain studies suggest that there is still a lot of evidence that splenectomy has negative effects on both healthy people and people with hematological diseases, such as TDT. The most frequently documented and concerning complications are increased susceptibility to infections and TE. However, a significant portion of thalassemic patients will continue to undergo splenectomy until a substitute is advised by evidence-based guidelines. These patients, along with those who have already undergone splenectomy, constitute a substantial group of patients who are at a possible risk for splenectomy-related complications.

TDT patients may benefit from splenectomy in cases that, despite advantageous chelation therapy, iron overload in not sufficiently reduced, leading to life-threatening excess iron deposits mainly in the liver, heart, and endocrine organs. For these patients, splenectomy, in the context of a comprehensive management of iron overload, is efficient in reducing the rate of transfusional iron loading. Moreover, this procedure is valuable in erasing the symptoms of early satiety and left upper quadrant pain, as well as the risk of a possible splenic rupture, in patients experiencing massive splenomegaly, thus, improving quality of life and reducing the morbidity and mortality risk. Last but not least, thalassemia patients who experience clinical conditions such as bleeding or recurrent infections, as a result of hypersplenism causing thrombocytopenia and leucopenia respectively, may overcome these adverse events through splenectomy.

However, splenectomy has been related to many disadvantages and unpleasant conditions. Except for PH and hypercoagulation, which are more thoroughly discussed in the present work, infections constitute a sizable long-term risk. Overwhelming sepsis in splenectomized TDT patients is commonly related to encapsulated pathogens such as *Streptococcus pneumoniae* (75% of cases), *Haemophilus influenzae* and *Neisseria meningitidis*, gram-negative organisms and protozoa, with a greater risk being documented after 1–4 years following the procedure. Prevention of overwhelming sepsis is the most important measure to avoid this complication. Proper education should be offered to patients, so that they have the ability to recognize febrile illnesses and report them to their physician. Moreover, pneumococcal, *Haemophilus influenzae* and meningococcal

TABLE 1 Baseline characteristics of the included studies.

Study	Splenectomized/ non-splenectomized	Country	Outcome	Outcome assessment	Follow-up	Results
Cappellini (13)	48/17	Italy	TE	U/S, color doppler/ venography, V/Q scan, angiography, CT, MRI	10 years	S group: 1 transient ischemic attack NS group: 0
Esfahani (14)	31/29	Iran	PH	echocardiography	–	S group: 4 NS group: 0
Hagar (15)	17/11	USA	PH	echocardiography	–	S group: 10 NS group: 6
Hassan (16)	160/160	Iran	TE	color Doppler ultrasound	11 years	S group: 5 PVT(1 month to 3 years after splenectomy) NS group: 0
Kalamara (17)	73/68	Greece	TEE PH	U/S, color doppler/ venography, v/q scan, angiography, CT echocardiography	25.75 ± 11.7 years	■ TEE S group: 4 (DVTx3, PVT x1), NS group: 1 DVT ■ PH S group: 7 NS group: 3
Meera (18)	6/9	India	PH	echocardiography	–	S group: 2 NS group: 1
Meloni (19)	24/36	China, Southeast Asia, Indian Subcontinent, Italy, Greece, Cyprus, Middle East	PH	echocardiography	21 months	S group: 1 NS group: 0
Morsy (20)	36/15	Saudi Arabia	PH	echocardiography	–	S group: 14 NS group: 6
Osataphan (21)	TE: 44/20 PH: 27/12	Thailand	TEE PH	echocardiography	5 years	■ TEE S group: 4 (cerebrovascular disease x2, pulmonary embolism x1, PVT x1) NS group: 0 ■ PH S group: 7 NS group: 2

polysaccharide vaccine should be properly and timely administered to thalassemic patients undergoing splenectomy for the achievement of immunoprophylaxis. Finally, children under 5 years of age should be treated with prophylactic antibiotics, such as chemoprophylaxis with oral penicillin (4, 6).

Many studies have shown that increased red blood cells and platelet count were associated not only with higher risk of TE events, but also with shorter time between splenectomy and TE. Red-cell senescence antigens, such phosphatidylserine and membrane proteins undergo iron-dependent oxidation in hemolytic anemia, which in turn causes thalassemic RBCs to be stiff, distorted, and aggregate, leading to premature cell removal (28, 33–41). Phospholipids with a negative charge may be present in thalassemic RBCs, which may eventually lead to an increase in the production of thrombin (42, 43). Splenectomized patients had considerably more circulating RBCs with negatively charged phospholipids (44). Additionally, such patients had, also, considerably higher amounts of circulating RBC microparticles (submicrometric membrane fragments with procoagulant potential) compared to controls (45). After a blood transfusion the quantity of circulating damaged RBCs is reduced (46). These results may help to partially explain why patients with high nuclated RBC counts or transfusion-naive patients experienced more TE episodes. A few observational studies have also shown that thalassemic patients who receive blood transfusions present less often with TEE, PH, and silent brain infarcts than transfusion-naive patients (47, 48). Correction of the underlying inefficient erythropoiesis and the consequent damaged RBCs with thrombogenic potential may be responsible for this. As a result, early transfusion therapy that aims to prevent the effects of chronic hemolytic anemia may assist patients by preventing such issues rather than only treating them after they have already occurred and are irreversible. It is, also, of note, that in the recent years, several studies have linked transfusions of red blood

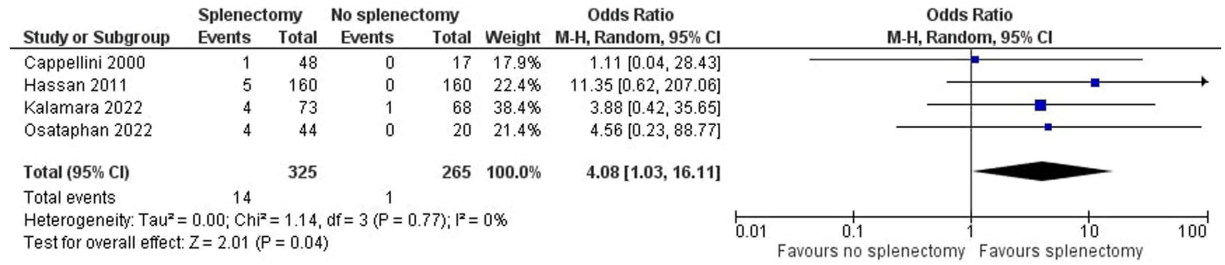

FIGURE 2
Odds ratio for thromboembolism.

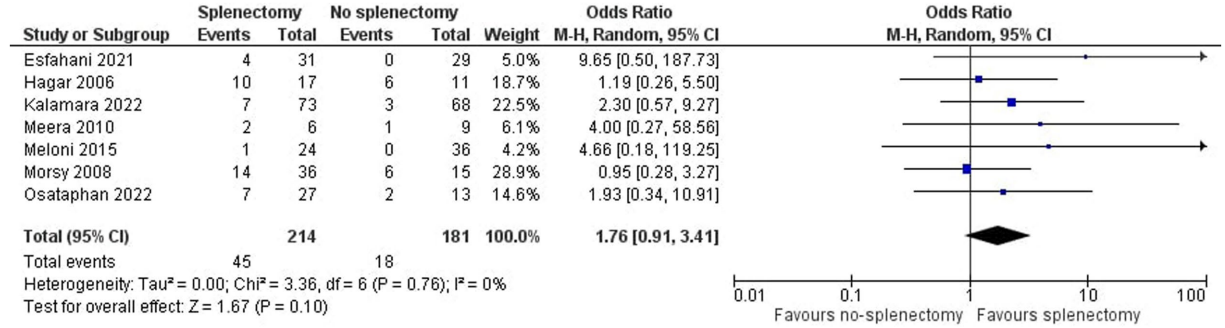

FIGURE 3
Odds ratio for pulmonary hypertension.

cells to thromboembolism (49), in the context of a variety of illnesses affecting both inpatients and outpatients. Lin and colleagues in an observational retrospective study with over 41,000 participants found that blood transfusions were significantly associated with venous thromboembolism, but, also, that with the administration of warfarin this risk was decreased (50). So, anticoagulation issues, as well as a possible thrombophilic predisposition should be always taken into consideration by physicians.

Patients with thalassemia and hereditary stomatocytosis were the first to suggest a connection between splenectomy and PH. According to estimates, it takes a significant amount of time (between two and 35 years) after splenectomy for PH to manifest (13, 51–53). In 58 thalassemia patients who had undergone splenectomy, 54% had pulmonary vascular alterations suggestive of microthromboemboli, as opposed to 16% of the remaining patients (13, 42, 44–59). The PH in asplenic patients with thalassemia is typically categorized as chronic thromboembolic pulmonary hypertension, which typically affects the distal pulmonary arteries and has a distinctive histopathology (42, 55). It is also likely that the process is actually "*in situ*" thrombosis, which is characterized by medial hypertrophy, intimal fibrosis, and plexiform lesions and is associated with idiopathic PH (55). Increased cardiac output as a result of chronic anemia, decreased plasma concentration of antithrombotic agents such as protein C, S and Antithrombin III could lead to platelet activation and microthrombotic episodes leading to RBC membrane malformations (57). The asymmetric RBC membrane phospholipids (56, 57), nitric oxide scavenging by free hemoglobin, and subsequent endothelial dysfunction brought on by nitric oxide depletion are some putative pathophysiologic mechanisms of PH in patients with hemolytic anemia. Those highly thrombogenic red blood cells would normally be eliminated by splenic macrophages, but, in the absence of this organ, they tend to be present in blood circulation for much longer and can increase hypercoagulability (58, 59).

Vasoconstriction expressed by abnormal narrowing of the pulmonary arteries is a primary mechanism in pulmonary hypertension. This constriction can be due to imbalances in the production of vasoconstrictors such as endothelin-1 or deficiencies in vasodilators like nitric oxide (NO) (60). In turn, increased vasoconstriction leads to elevated resistance and pressure in the pulmonary arteries. That, in addition to dysfunction of the endothelial cells, lining the pulmonary arteries, is commonly observed in pulmonary hypertension (60, 61). Endothelial cells play a crucial role in maintaining vascular tone and regulating blood flow. The remodeling of pulmonary arteries is, also, a hallmark of pulmonary hypertension. It involves structural changes in the arterial walls, including smooth muscle cell proliferation, fibrosis, and the formation of plexiform lesions (62). These changes lead to the narrowing of the vessel lumen, further increasing pulmonary vascular resistance. Another pathophysiologic mechanism involves Inflammation and immune dysregulation. Immune cells, such as macrophages and lymphocytes, infiltrate the pulmonary arteries, releasing pro-inflammatory cytokines and growth factors that promote vascular remodeling and vasoconstriction (60, 61). As far as genetic and hereditary factors are concerned, they can also be related with certain forms of pulmonary hypertension. Mutations in genes involved in signaling pathways, such as bone morphogenetic protein receptor type 2, can disrupt normal vascular homeostasis and contribute to the

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TABLE 2 Newcastle-Ottawa quality assessment form regarding included studies.

Studies	Representative exposed cohort	Selection of non-exposed cohort	Ascertainment of exposure	Precision of Exposure Dose Ascertainment	Outcome of interest not present at baseline	Adjustment for confounding	Outcome assessment	Was follow-up long enough for outcomes to occur?	Adequacy of follow-up	Quality
Cappellini (13)	⋆	⋆	⋆	⋆	⋆	–	⋆	⋆	⋆	good
Esfahani (14)	⋆	⋆	⋆	⋆	⋆	–	⋆	–	⋆	good
Hagar (15)	⋆	⋆	⋆	⋆	⋆	–	⋆	–	–	good
Hassan (16)	⋆	⋆	⋆	⋆	⋆	–	⋆	⋆	⋆	good
Kalamara (17)	⋆	⋆	⋆	⋆	⋆	–	⋆	⋆	⋆	good
Meera (18)	⋆	⋆	⋆	⋆	⋆	–	⋆	–	–	good
Meloni (19)	⋆	⋆	⋆	⋆	–	–	⋆	⋆	–	good
Morsy (20)	⋆	⋆	⋆	⋆	⋆	–	⋆	–	–	good
Osataphan (21)	⋆	⋆	⋆	⋆	⋆	–	⋆	⋆	⋆	good

development of pulmonary hypertension (60). Lastly, pulmonary vascular thrombosis can contribute to the development or exacerbation of PH (62). Someone can safely state that the pathophysiology of PH in splenectomized thalassemic patients is a rather complicated phenomenon with a large number of contributing factors. The extent of the effect of each factor cannot be measured according to the already existing literature, so further studies should be conducted in the direction of further explaining this rather difficult circumstance.

As far as NTDT is concerned, there is a decline in the use of splenectomy over the last years, as this procedure has been linked to several important negative outcomes. Similarly to TDT, hypercoagulability issues arise from the removal of the spleen, as procoagulant RBCs, erythroblasts and platelets, and probably iron free fractions, cannot be scavenged. NTDT patients present with an increased (reaching 5-fold) risk of venous TE, PH, silent cerebral infarction and leg ulcers, compared to unsplenectomized patients. These results arise from a number of previous studies, although, to our knowledge, no synthesis of the available data in the form of a meta-analysis has been conducted and published. Pulmonary hypertension in NTDT patients is characterized by increased pressure in pre-capillary pulmonary vessels. Probable causes have been identified and it seems that the exact pathophysiology of the phenomenon is rather complex. Chronic thromboembolic disease, chronic anemia/hypoxia and the subsequent hyperdynamic circulation as well as disruptions in the synthetic pathways of vasodilators such as nitrous oxide derivatives can be blamed as probable causes of this clinical syndrome (63). Thus, a probable explanation of the fact that PH was not associated with splenectomy in TDT patients according to our results, but has been observed in NTDT population according to numerous studies, could be the chronic hemolytic anemia and the subsequent hyperdynamic circulation derived by chronic hypoxia, which in TDT may be alleviated by recurrent transfusions.

In accordance with our study results, that showed an association between splenectomy and the serious adverse event of TE, we would not recommend splenectomy as a standard-of care measure. Patients should be advised to undergo splenectomy only in cases of extreme transfusion requirements and clinical conditions that make this procedure inevitable. We should emphasize on availability of optimal transfusion regimens and strict transfusion protocols and chelation treatment, which minimize the incidence of splenomegaly and reduce iron overload. In cases that patients are already splenectomized, careful monitoring is of great significance. Awareness of the possible risks is required. TE events and infections should be immediately addressed and patients should be well informed and trained to recognize these conditions. In cases that splenectomy must be conducted, laparoscopic procedure seems to be a safer option, and precise immunization and chemoprophylaxis protocols should be followed. Low dose aspirin should be prescribed to all post-splenectomy patients, unless this is contraindicated. In cases that other risk factors for thrombosis are present, low molecular weight heparin prophylaxis should also be considered.

To our knowledge, this is the first meta-analysis and systematic evaluation of the relationship between splenectomy and TEE and PH in TDT. Our study has certain advantages. We established the link between splenectomy and thrombosis through evidence synthesis, and the finding may have important clinical implications for in the clinical setting. Moreover, we showed that splenectomy

had a neutral effect on PH in these patients, despite the traditional views on this subject. Additionally, we created appropriate inclusion and exclusion criteria, resulting in a data collection that is rather homogeneous, according to the heterogeneity testing. The inclusion of quality assessment was also a great tool, allowing readers to appraise the level of evidence. Finally, two independent reviewers completed the research and data extraction, which enables us to confirm the review's comprehensiveness and accuracy. Nevertheless, an important limitation to be mentioned is that, despite the high link between splenectomy and thrombosis, causality could not be fully established, as some of the included studies were retrospective, cross-sectional or case–control studies. Thus, more prospective well-designed cohort studies are needed for this result to be confirmed in the future.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

T-VK: Writing – original draft, Writing – review & editing. KD: Writing – original draft, Writing – review & editing. EV: Writing – review & editing.

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Caplacizumab for immune thrombotic thrombocytopenic purpura: real-world multicenter data

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Given the limited real-world data of caplacizumab, our multicenter real-world study was designed to assess the safety and efficacy of caplacizumab in immune thrombotic thrombocytopenic pupura (iTTP), compared to historic controls. We have studied 70 patients: 23 in the caplacizumab and 47 in the historic control group. Plasma exchange was applied in all episodes except for two patients that denied plasma exchange. Rituximab as first-line treatment was more common in the caplacizumab group compared to historic control. Caplacizumab (10 mg daily) was given at a median on day 7 (1–43) from initial diagnosis for 32 (6–47) dosages. In the caplacizumab group, a median of 12 (8–23) patients required plasma exchange sessions versus 14 (6–32) in the control group. Caplacizumab administration did not produce any grade 3 complications or major hemorrhagic events. After a median of 19.0 (2.6–320) months since the iTTP diagnosis, 5 deaths occurred (4 in the control group and 1 in the caplacizumab group, $p = 0.310$). Caplacizumab patients achieved early platelet normalization and ADAMTS13 activity normalization at the end of treatment. Relapse was observed only in 2/23 (9%) caplacizumab patients, compared to 29/47 (62%) historic controls ($p < 0.001$). Overall, caplacizumab is safe and effective in treating iTTP, including cases refractory to plasma exchange, re-administration, and cases without previous plasma exchange treatment. No major hemorrhagic events were observed. Cessation of dosing guided by ADAMTS13 has ensured a low relapse rate.

KEYWORDS

caplacizumab, thrombotic thrombocytopenic purpura, plasma exchange, ADAMTS13, multicenter real-world study

1. Introduction

Almost one century ago, in 1924, Thrombotic Thrombocytopenic Purpura (TTP) was clinically described for first time by Eli Moschcowitz. In particular, a 16-year-old girl was diagnosed with a fatal thrombotic microangiopathy (TMA) syndrome, characterized by fever, transient focal neurologic symptoms, severe thrombocytopenia, and microangiopathic hemolytic anemia. These findings were linked to the presence of autopsy-defined systemic visceral microthrombosis of the terminal arterioles and capillaries (1). During the past 20 years, acquired or immune TTP (aTTP or iTTP) has been transformed from a clinical diagnosis of exclusion into a fully-described pathophysiologic diagnosis, based on specific clinical and laboratory features (2). It is now a well-established medical emergency requiring a rapid diagnosis and management. Death may occur usually during the acute phase, of the disease, resulting from uncontrolled formation of microvascular thrombi (3). Severe ADAMTS13 deficiency (<10%) is both, sensitive and specific for the diagnosis of TTP (4). Despite the advances in treatment options for TTP, there are still limited high quality data to inform clinicians regarding the recently introduced targeted type of treatment.

Established treatment approaches—plasma exchange and immunosuppression—replenish functional ADAMTS13 enzyme, but do not adequately address microvascular thrombosis (5). Caplacizumab represents the first drug to receive a regulatory approval for the treatment of iTTP. Caplacizumab, an anti–von Willebrand factor humanized, bivalent variable-domain-only immunoglobulin fragment, inhibits interaction between von Willebrand factor multimers and platelets (6). The drug demonstrated efficacy and safety in the placebo-controlled phase- 2 TITAN and phase-3 HERCULES studies. Both studies concluded that caplacizumab treatment is generally well tolerated, hastens platelet recovery, and reduces the recurrence rates. Relapses, however, were more common among caplacizumab- treated patients in both studies (7, 8). Despite the safety and efficacy of caplacizumab, several questions remained unanswered by these randomized clinical trials and the subsequent analyses (9). Caplacizumab has also been used in pregnancy (10) and for treatment of pediatric patients (11). Data from clinical trials also suggest that the benefit of caplacizumab is greatest when it is given earlier in the course of disease (8), although this is not always feasible in clinical practice.

Given the limited real-world data of caplacizumab, our multicenter real-world study was designed to assess the safety and efficacy of caplacizumab, compared to historic controls.

2. Materials and methods

2.1. Patients

We recorded clinicobiological data from consecutive adult patients (≥18 years of age), diagnosed with iTTP in the last 10 years (2011–2022). Diagnosis was based on clinical presentation (anemia, thrombocytopenia, and microangiopathic hemolytic anemia) and was confirmed with measurement of plasma ADAMTS13 activity by commercially available ELISA kit (Technozym), indicating severe ADAMTS13 deficiency (<10%). Patients were treated according to current guidelines, as implemented by their treating physicians. International guidelines were implemented in all centers since 2020 (6). In the era of caplacizumab, ADAMTS13 activity levels were available within 48 hours from diagnosis, and they were also used to guide caplacizumab dosing, which was administered until ADAMTS13 activity was raised above 10% (defined as ADAMTS13 normalization). Both, caplacizumab and historic control patients have been continuously monitored until data cut-off (October 2022). TTP event occurring more than 30 days after the end of daily plasma exchanges, was referred to as relapses. Exacerbations were defined as recurrent thrombocytopenia within 30 days after the end of daily plasma exchanges that required reinitiation of daily exchanges. Major or minor bleeding events were determined by treating physicians. Patients provided written informed consent to participate in the study which was conducted according to the Helsinki Declaration.

2.2. Study design

We conducted this comparative real-world multicenter study at 11 Hematology Departments (10 based in Greece and one in the United Kingdom). Patient records were documented retrospectively in a predefined CRF format. Standard of Care (SOC) treatment was implemented according to each center's protocol. The majority of the participating centers administered steroids, as methylprednisolone 1mg/kg, along with daily plasma exchange treatment with subsequent tapering of steroids and frequency of plasma exchange. Most centers also administered rituximab weekly x 4 doses. The historical control group received rituximab based on physician's decisions, mostly in refractory/relapsed patients or patients with severe presentation (i.e., neurological manifestations). Membrane filtration was used in six centers, while centrifugal plasma exchange in five centers. Relapse was defined as in deterioration after 30 days in remission.

2.3. Statistical analysis

Analysis was performed using the Statistical Package for Social Sciences (SPSS) v22.0 for Windows (SPSS Inc., Chicago, IL, United States). Results are presented for continuous variables as mean ± standard deviation or as median ± interquartile range for non-normal variables and for qualitative variables as frequencies. Differences between the two groups were evaluated by the *t*-test for parametric and the Mann-Whitney U test for non-parametric variables. Pearson's or Spearman's rank tests were performed for univariate comparisons of continuous variables and the Chi-Square

Test for qualitative variables. Logarithmic transformation was used when indicated for non-normally distributed data. When logarithmic transformation did not result in normal distributions, non-parametric tests were performed. The Kaplan-Meier method was used for survival analysis, and survival curves of the two groups were compared, using a log-rank test. Cox regression analysis was performed for univariate and multivariate predictors of survival. Considering multicollinearity issues, factors with a significant univariate association were inserted into the multivariate analysis.

3. Results

3.1. Baseline characteristics

We have studied 70 consecutive white patients in total (median age 45 years, range 19–85), of whom 23 were enrolled in the caplacizumab group and the remaining 47 in the historic control group (Figure 1A). Baseline characteristics are presented in Table 1. No significant differences were observed in patient or disease characteristics between the two groups. Comorbidities refer only to clinically significant underlying diseases before TTP diagnosis, such as diabetes, connective tissue disorders. The difference was not meaningfully significant and therefore, no safe conclusions can be made due to the limited sample size.

3.2. Treatment modalities

Plasma exchange was applied in all episodes, except for two patients that denied plasma exchange, one in the caplacizumab and one in the historic control group; both with favorable outcomes. All patients received corticosteroids, and the majority of them also received rituximab in both groups, as shown in Table 1. Rituximab as first-line treatment (day 1) was more common in the caplacizumab group compared to historic control (68% versus 32%, $p < 0.001$), possibly reflecting current international guidelines recommendations. Low-dose aspirin (100 mg) was given in all patients and low-dose molecular heparin was started after the increase of platelets at levels above 50,000/μL.

Caplacizumab (10 mg daily) was given at a median on day 7 (1–43) following initial diagnosis for 32 (6–47) dosages. The drug was provided through regular market access. The majority of patients received caplacizumab at the first iTTP episode (15/23; 65%). All patients intended to receive caplacizumab as first-line treatment, except four patients, who received caplacizumab after being unresponsive to plasma exchange (median day 18, range 8-43 from diagnosis). Administration of caplacizumab within 72 hours from diagnosis was achieved in most cases (16/23; 70%), while 3/23 were delayed due to access issues (median day 6, range 4-8 from diagnosis). In two cases, iTTP occurred early after COVID-19 infection. Median plasma exchange sessions were 12 (8–23) in the caplacizumab group versus 14 (6–32) in the control group.

3.3. Safety

Caplacizumab administration did not produce any grade 3 complications or major bleeding events. Minor bleeding events were reported in 21% patients that received caplacizumab. and 15% of historic control group patients.

3.4. Outcomes

All patients achieved early platelet normalization 5 (3–7) days from caplacizumab administration. In all caplacizumab-treated patients, ADAMTS13 levels became detectable (25–52%) by end of treatment (6–47 doses). No significant difference was found in platelet recovery or plasma exchange duration based upon timing of caplacizumab initiation: earlier initiation (within <72 h of diagnosis; $n = 16$) vs later initiation (>72 hours; $n = 7$).

No exacerbation was observed in the caplacizumab setting. 2/23 patients treated in the caplacizumab group (that also had higher rates of first-line rituximab administration) relapsed within 13 and 18 months of their first caplacizumab dose, with low ADAMTS13 activity

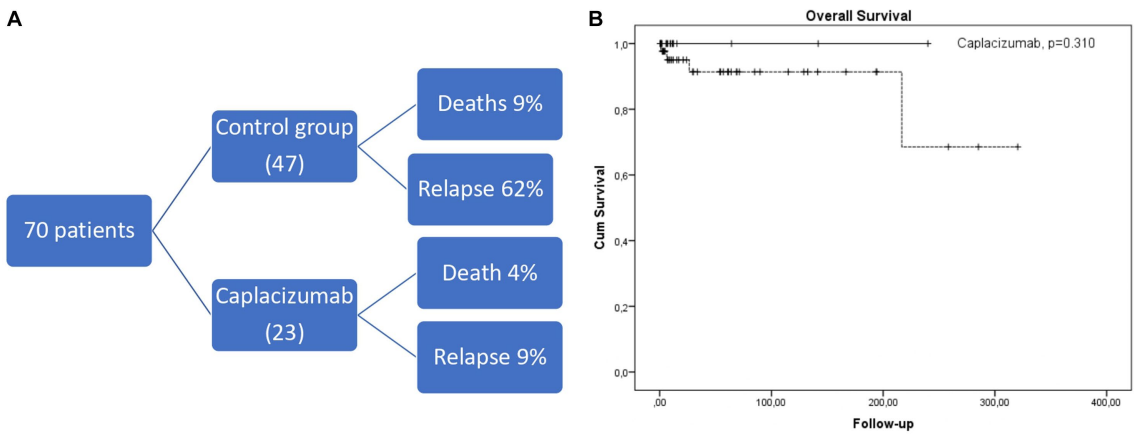

FIGURE 1
(A) Short diagram of patients included and outcomes. **(B)** Kaplan-Meier curve depicting overall survival in caplacizumab (black line) compared to historical control group (dotted line).

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TABLE 1 Baseline patient characteristics in both groups.

Characteristics	Caplacizumab ($n = 23$)	Historical group ($n = 47$)	All patients ($n = 70$)	p-value
Age (year)				
Median	47	45	46	0.231
Range, IQR	24–74, 20	19–85, 19	19–85, 17.1	
Female gender (%)	65%	71%	66%	0.485
BMI (kg/m^2)				
Median	27	28	27	0.563
Range, IQR	22–33, 4.1	23–31, 3.5	22–33, 4.2	
Hb at diagnosis (gr/dL)				
Median	8.7	9.5	9.0	0.342
Range, IQR	5.3–14.3, 2.8	5.6–13.4, 3.2	5.4–14.3, 3.2	
Plt at diagnosis (plt/μL)				
Median	23,000	21,000	22,000	0.763
Range, IQR	3,000–115,000, 15,719	5,000–100,000, 2,300	3,000–115,000, 18,500	
MCV at diagnosis				
Median	94.7	88.9	93.2	0.231
Range, IQR	62.6–105, 20.1	80–109; 12.3	62.6–109, 14.2	
Schistocytes (%)				
Median	12.3	7.3	11.2	0.654
Range, IQR	3–22, 3.2	1–18, 3.8	1–22, 3.4	
Creatinine (mg/dL)				
Median	1.02	1.16	1.05	0.112
Range, IQR	0.7–1.99; 0.4	0.6–3, 0.5	0.6–3, 0.5	
Fibrinogen (gr/L)				
Median	1.3	2.7	1.8	0.423
Range, IQR	1.7–5.31, 1.9	1.2–7.6, 2.1	1.2–7.6, 2.0	
LDH (mg/dL)				
Median	831	753	794	0.312
Range, IQR	549–1,109, 235	621–1,243, 543	549–1,243, 425	
Glasgow coma scale				
Median	13	12	13	0.129
Range, IQR	12–15, 1.2	8–15, 2.1	8–15, 2.1	
Elevated troponin, % *	31%	43%	37%	0.182
Comorbidities, %	30%	6%	23%	0.102
CNS involvement, %	50%	53%	50%	0.763
Corticosteroids, %	100%	100%	100%	NA
Median TPE, range, IQR (n)	12, 8–23, 8.6	14, 6–32, 10	12, 6–32, 10	0.231
Refractory to TPE, %	19%	44%	24%	0.184
Rituximab doses, %	4–9, 79%	2–8, 64%	2–9, 72%	0.287

No significant differences observed between caplacizumab treated and historical control group patients. IQR, interquartile range; BMI, body mass index; Hb, hemoglobin; Plt, platelets; MCV, mean corpuscular volume; LDH, lactate dehydrogenase (upper limit of normal reference range 245 mg/dL); CNS, central nervous system; TPE, therapeutic plasma exchange; NA, not applicable. *Elevated troponin was defined as above the local upper limit of normal reference range.

(<1%), compared to 29/47 (62%) patients from the historic control group ($p < 0.001$). The first patient re-received caplacizumab at regular doses from day 1 of relapse with adjunctive steroids but without plasma exchange. The second patient received caplacizumab 10 days after the initial relapse because of poor response to plasma exchange and steroids. Both patients' platelet count normalized rapidly within 10 and 4 days, respectively, from the start of caplacizumab. After a median of 19.0 (2.6–320) months since the iTTP diagnosis, 5 deaths

TABLE 2 Summary of real-world data comparing outcomes in caplacizumab-treated versus historical control patients.

Country (year)	Centers	Control group	Treatment	Patient number	% presenting with recurrent TTP	% with ADAMTS13 <10%
France (2021) (11)	Multicenter	Historical	Caplacizumab + SOC	90	13	100
			SOC	180	12	100
UK (2021) (15)	Multicenter	Historical	Caplacizumab + SOC	85	NA	99
			SOC	39	NA	NA
Spain (2022) (16)	Multicenter	Concurrent	Caplacizumab + SOC	77	4.5	100
			SOC	78	20.5	100
Germany/Austria (2023)	Multicenter	Historical	Caplacizumab + SOC	113	2	97
			SOC	119	4	92

UK, United Kingdom; SOC, standard of care; No, number; TTP, thrombotic thrombocytopenic purpura; NA, not available; ADAMTS13, a disintegrin and metalloprotease with thrombospondin type 1 repeats, member 13.

occurred (4 in the control group and 1 in the caplacizumab group, $p = 0.310$, Figure 1B). All deaths were associated with an acute episode, except for one attributed to acute ischemic stroke during remission in the control cohort.

4. Discussion

Our real-world multicenter comparative study yields updated information, and it also shows that caplacizumab is safe and effective in treating iTTP, including cases refractory to plasma exchange, re-administration, and cases without previous plasma exchange treatment. Patients treated with caplacizumab in this study had no major hemorrhagic events or other complications. In addition, cessation of dosing guided by ADAMTS13 has ensured a low relapse rate. Despite early diagnosis and the drug's wide availability, our real-world data confirm that treatment initiation is only sometimes feasible from the day of initial diagnosis.

The latter might account for the lack of statistically significant differences between the control group, and the rather small patient population. This is implied by a recent real-world patient experience, confirming that early initiation confers better outcomes superior to those reported by historic controls (12–14). Therefore, we have summarized available evidence of real-world studies comparing caplacizumab to a control group in Table 2. However, early initiation is not always feasible in the real-world setting, not only due to drug availability issues, but also because ADAMTS13 testing cannot be readily available before the first plasma exchange. Trying to overcome this issue, ISTH guidelines have introduced risk scores, to predict ADAMTS13 activity, which have high accuracy in all patients, except for those with secondary causes. It should be also noted that secondary causes cannot be always recognized immediately, before the first plasma exchange. An additional important component that is neglected by many studies is the importance of ADAMTS13 level measurement in caplacizumab dosing. Furthermore, as the patient population that has received caplacizumab continues to grow, additional questions arise, regarding re-administration and plasma exchange-free treatment approach. Beyond caplacizumab, differences in studies with historical controls need to take into account the increasing use of rituximab over the last years, in accordance with ISTH guidelines. Lastly, high cost remains a challenge to its widespread use until clear evidence is provided for plasma exchange-free treatment (15).

Real-world evidence is of utmost importance in rare diseases, like iTTP (13, 17). This study is a multicenter collection of real-world data, presenting the use of caplacizumab outside of clinical trials. Further patient recruitment is necessary to provide additional data. Given that this is indeed a rare but life-threatening disease, we want to emphasize on the safety and efficacy that caplacizumab has brought not only to the acute setting but also to the relapsed setting. A unique characteristic of our study was that cessation of caplacizumab treatment was based on ADAMTS13 activity, leading to low relapse rates with daily dosing, as noted by Tse et al. (18). Alternate dosing of caplacizumab has been also suggested by the German group, with an individualized algorithm (19). In context with TITAN and HERCULES (7, 8), an important aspect of our trial is also the low number of deaths (only 1) in the caplacizumab group. Deaths in the control group were 4 in our study, possibly due to the long-term follow-up of historical controls.

In conclusion, our study confirms the safety of caplacizumab in treating iTTP. While overall TPE durations did not differ between groups, the addition of caplacizumab was associated with rapid platelet recovery and low relapse rates. Given the limited international clinical experience with caplacizumab, dosing modifications, compared to the clinical trial setting, have non-inferior outcomes in the real-world setting (16, 19). Since ADAMTS13 reduction has emerged as an essential indicator of long-term results, further studies in large real-world populations with longer follow-ups are needed to delineate the iTTP treatment algorithm in the era of personalized medicine.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by General Hospital of Thessaloniki "G. Papanikolaou." The studies were conducted in

accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

EG: Conceptualization, Data curation, Methodology, Project administration, Supervision, Visualization, Writing – original draft. EN: Conceptualization, Methodology, Project administration, Validation, Writing – original draft. EE-K: Methodology. SD-B: Methodology. ChaK: Methodology. AP: Methodology. AM: Methodology. ChaP: Methodology. ChrK: Methodology. AB: Methodology. IT: Methodology. TT: Methodology. TC: Methodology. MP: Methodology. AntS: Methodology. EZ: Methodology. GK: Methodology. IS: Visualization, Writing – review & editing. EM: Writing – review & editing. ZM: Writing – review & editing. DK: Writing – review & editing. AK: Writing – review & editing. ArgS: Writing – review & editing. EK: Writing – review & editing. HP: Writing – review & editing. CL: Writing – review & editing.

Conflict of interest

AntS and ArgS have received grants, honoraria or travel support from Abbvie, Amgen, Bei-Gene, BMS, Gilead, Glaxo, Janssen, MSD, Pfizer, Roche, Sanofi, Servier, SOBI, Takeda. ChaK and ChrK have received honoraria from Pfizer, BMS, Bayer, Takeda. EG and CP have received honoraria from Sanofi.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Case report: Paroxysmal nocturnal hemoglobinuria presenting with hemorrhagic esophageal varices

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We report the case of a female who was cured of hemorrhagic esophageal varices caused by paroxysmal nocturnal hemoglobinuria (PNH) through transjugular intrahepatic portosystemic shunt (TIPS) treatment. PNH complicated by portal vein and visceral veins thrombosis without hepatic veins is extremely rare, and as such, it is easy to incorrectly treat due to lack of awareness. Hemorrhagic esophageal varices due to PNH with PVT have been reported in one case in 1974, and here, we report the second.

KEYWORDS

paroxysmal nocturnal hemoglobinuria, portal vein thrombosis, esophageal varices, hemorrhagic esophageal varices, TIPS

1. Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare type of thrombophilia and hematopoietic stem cell disorder (1). Thrombophilia of PNH has been defined as "the most vicious acquired thrombophilic state known to medicine," representing the leading cause of death in PNH patients (2). PNH with portal vein thrombosis (PVT) without hepatic vein thrombosis has been sparsely reported, with several cases of esophageal varices and only one case of esophageal variceal bleeding (3). The pathophysiology of how PNH causes PVT specifically is still unclear. PVT was once considered a contraindication for transjugular intrahepatic portosystemic shunts (TIPS). In recent years, there has been an increasing amount of literature discussing how patients with PVT may benefit from TIPS to reduce portal venous pressure and complications such as hemorrhagic esophageal varices (4). Long-term portal vein thrombosis can cause cavernous transformation of the portal vein (CTPV). Whether TIPS is used in non-cirrhotic portal vein cavernous transformation is controversial (5). Here, the case of a patient with CTPV and PVT caused by PNH is presented.

2. Case presentation

A 49 years-old female presented to the vascular surgery department of our hospital with melena that had lasted for 18 months. While 18 months prior to this the patient had a little intermittent melena, which resolved with conservative treatment, the symptoms suddenly worsened 6 months before presenting to our hospital after a CT examination at her local hospital revealed esophageal and gastric varices and splenomegaly; she underwent selective splenic artery embolization (SSAE) to relieve hypersplenism and portal vein pressure. After SSAE, her hemoglobin changed from 6.6 to 5.7 g/dL, and her platelet changed from 28 to 19 × 103/

μL. Then, she was discharged from the hospital. However, 5 months after the SSAE, melena reappeared more severely than before. No other gastrointestinal symptoms, such as nausea, vomiting, or abdominal pain, were present during the onset of the disease.

The patient reported symptoms of anemia, leukopenia, and thrombocytopenia 9 years ago and also reported a 9 years history of PNH confirmed by flow cytometry to identify GPI-AP-deficient peripheral blood cells. During routine physical exams 5 years before, contrast-enhanced CT reported PVT, but splenomegaly and varicose veins were not reported. The patient did not show any PVT-related symptoms. She had taken prednisolone and warfarin and had no history of surgical intervention. She had no family history of thrombosis.

The patient's temperature was 36.5°C, heart rate was 92 beats per minute, respiratory rate was 18 breaths per minute, and blood pressure (measured with an electronic cuff) was 86/50 mmHg. The physical examination showed an anemic appearance, splenomegaly, and tenderness on the left upper quadrant.

A complete blood count revealed a leukocyte count of 4.2×10^9/L, a hemoglobin count of 4.7 g/dL, and a platelet count of 29×10^9/L. Her coagulation test showed a D-dimer of 7.63 U/L and fibrinogen degradation products of 33.87 mg/L. She presented with a lactate dehydrogenase (LDH) level of 3,657 IU/L (reference range, 120–250) as a result of hemolysis. A bone marrow smear revealed significant hyperactivity of hematopoiesis, especially in the erythroid lineage.

Computed tomography (CT) scan results from the local hospital 5 months before showed revealing esophageal and gastric varices (EGV) before SSAE (Figure 1A), splenomegaly (18.8 cm), and wider portal vein diameter. A contrast-enhanced CT scan at our hospital revealed portal vein, splenic vein, and superior mesenteric vein thrombosis with extensive collaterals, including the esophageal and gastric varices and CTPV (Figure 1B). Moreover, the size of the spleen was 13.7 cm at this time.

The patient was diagnosed with PNH complicated by varices portal vein thrombosis and superior mesenteric vein thrombosis causing esophageal gastric vein bleeding.

Esophageal–gastric varices are visible through the imaging (Figure 2A). The patient underwent TIPS and superior mesenteric vein and splenic vein stent placement procedures (Figures 2B,C). She was asked to continue using prednisone. Intravenous heparin was administered, and she was transitioned to warfarin with a goal an international normalized ratio of 2.0 to 2.5.

The esophageal and gastric varices disappeared, and hemoglobin rose (Table 1). The patient no longer had blood in her stool, and her CT scans revealed smooth blood flow in the stents at her 1 month, 3 months, 6 months, and 1 year follow-up after the surgery (Figure 1C). There were no complications, such as hepatic encephalopathy, at the follow-ups.

3. Discussion

PNH is a rare, acquired, potentially life-threatening hematologic disorder characterized by chronic intravascular hemolysis caused by uncontrolled activation of the terminal complement pathway (1). The mechanisms of thrombosis in PNH are still poorly understood; possible causes include the percentage of GPI protein-free granulocytes (PNH granulocytes >50%), endothelial cell damage, platelet activation because of the absence of CD59, and nitric oxide due to intravascular hemolysis, among others (7). Rother et al. (8) pointed out that thromboembolism is mainly due to hemolysis; although the mechanism is not fully understood, hemolysis has been implicated in the initiation of platelet activation and aggregation. Thrombotic events (TEs) account for up to 67% of deaths with a known cause in patients with PNH. Thrombosis can appear anywhere in the body (9). Hepatic veins are the most commonly involved site in PNH, and the isolated involvement of portal veins with visceral veins without the involvement of the hepatic veins is rare (10). PVT involving superior mesenteric veins and mesenteric venous arches may lead to intestinal ischemia, obstruction, and fatal intestinal infarction (11). PNH with PVT, on the other hand, is very rare and is easy to mistreat due to complex complications. A literature search of relevant articles on the PubMed database, published from January 1977 to May 2023, was conducted using the keywords "paroxysmal nocturnal hemoglobinuria" and "portal vein thrombosis." One article presenting hemorrhagic esophageal varices due to PNH with PVT was identified (6).

The two cases are very similar, with both subjects being female and having experienced melena and splenomegaly (Table 2). In their case, hematemesis indicated that the patient may have bled faster and more than ours. In our case, hemoglobin was lower and LDH higher, possibly because of the more severe hemolysis caused by PNH. In addition, our patient presented with CTPV. In terms of treatment, their patient underwent portal vein dissection, splenectomy, and

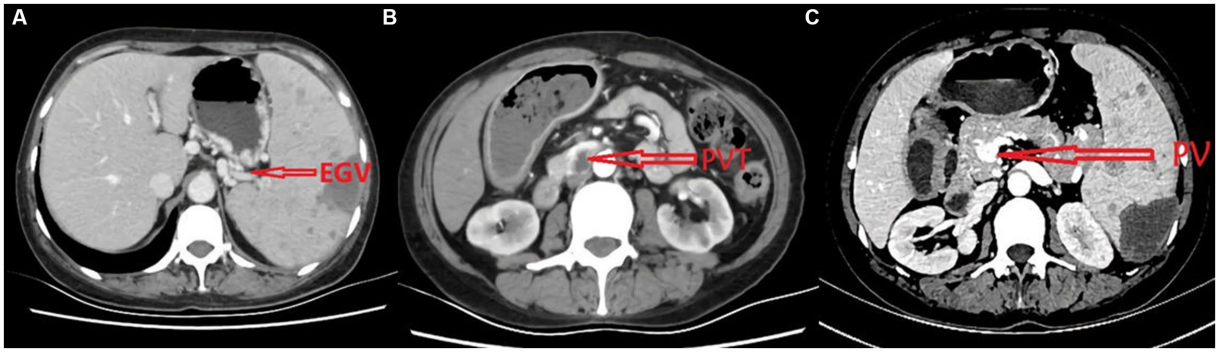

FIGURE 1
The CT scan before SSAE **(A)**, before TIPS **(B)**, and after TIPS **(C)**.

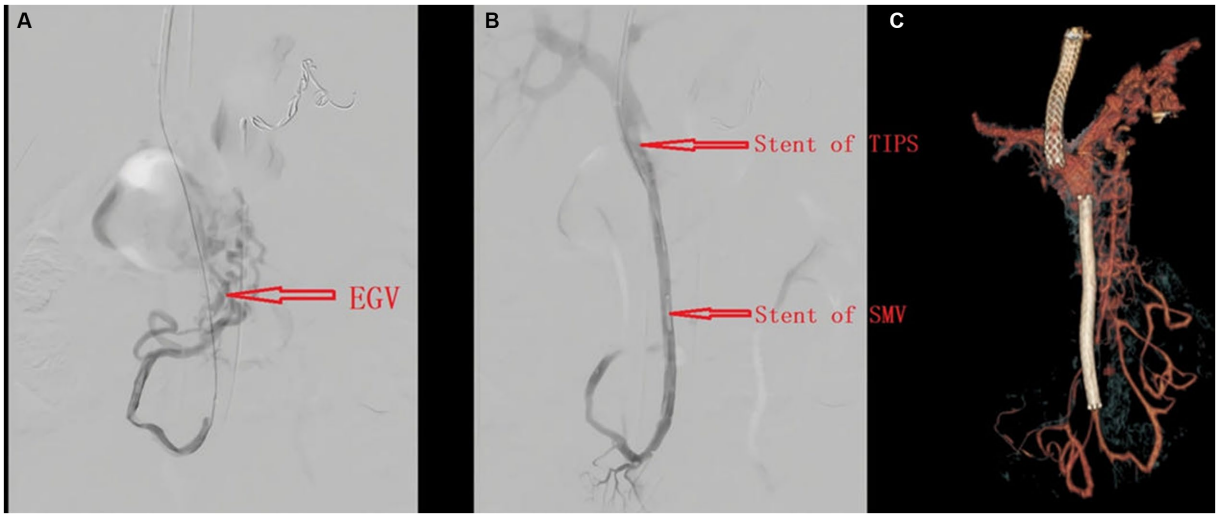

FIGURE 2
Angiography before **(A)** and after **(B)** TIPS placement; CT angiography after TIPS **(C)**.

TABLE 1 **Changes in blood routine before and after TIPS.**

Representative blood counts	Preoperative	POD 0	POD 1	POD 2	POD 3	POD 7	1-year follow-up
HGB (g/dL)	4.7	5.7	6	6.3	6.1	6.3	5.9
PLTs ($\times 10^3$/μL)	29	46	25	27	23	17	34
Leukocyte (10^9/L)	4.2	10.6	4.6	5.3	5	2.2	3.4
RBCs (10^{12}/L)	1.35	1.7	1.82	1.86	1.82	1.79	1.8

TABLE 2 **Previous similar case compared to ours.**

Case	Age/sex	Chief complaint	Physical examination	HGB g/dL	LDH IU/L	Techniques	Thrombus site	Treatment	Ref.
1	37/female	Hematemesis and melena	Hepatosplenomegaly, anemic appearance	9	1,605	Endoscopy and angiogram	Splenic and portal vein	Portal-azygos disconnection, splenectomy, and pyloroplasty	(6)
Our case	49/female	Melena	Splenomegaly, anemic appearance and tenderness on left upper quadrant	4.7	3,657	CT and angiogram	Splenic, portal, and superior mesenteric vein	TIPS and superior mesenteric vein stent	

pyloroplasty. Our treatment modalities were more minimally invasive than their surgery and less invasive to the patient, gastric bleeding did not recur during follow-up, and hemoglobin recovered steadily.

There have also been several reports of PNH complicated by PVT and abdominal thrombosis (10) but with no bleeding of the esophageal varices. It is possible that our patient had not received effective treatment for a long time, and worsening portal hypertension led to hemorrhagic esophageal varices.

We conducted a thorough evaluation before treating the patient. The patient had completely blocked the portal vein and had previously undergone SSAE surgery for the treatment of regional portal hypertension. At this time, the patient developed refractory esophageal variceal bleeding and severe anemia, and we tried to save their life by using TIPS surgery to relieve portal hypertension. The right internal jugular vein was punctured for TIPS. The femoral vein was used as a puncture route for infarction of the superior mesenteric vein and portal vein, followed by a stent. Note that the superior mesenteric venous stent should completely cover the thrombus site while avoiding covering the main portal vein, and warfarin should be given to the patient after surgery.

The use of TIPS in PVT has been studied with the possibility of achieving recanalization by disrupting the thrombus and mechanical thrombectomy. The feasibility rate of performing TIPS in PVT ranges between 75% and 100% (12). CTPV was once considered a relative contraindication to TIPS, and with their attempts, some scholars believe that TIPS can be used for refractory CTPV. Currently, whether TIPS can be used in patients with CTPV, especially those without cirrhosis, is controversial (5). Our case is a deliberate and bold attempt.

We consider trilineage cytopenia in our patient primarily because of PNH instead of hypersplenism. Alleviating hypersplenism does not cure trilineage cytopenia, and hemorrhagic esophagus further aggravates anemia. After we performed TIPS on our patient and gave her warfarin and prednisolone, her bleeding was controlled, and the hemoglobin returned, but the levels were not very high because of PNH.

Our patient had spleen shrinkage after SSAE, but their thrombocytopenia did not improve. This outcome is different from that found by Araten et al. (13). We believe that SSAE not

only has no value but may also have increased PVT. A CT scan before SSAE showed portal vein widening, suggesting decreased portal hypertension and reduced portal vein flow velocity and that SSAE results in splenic vein congestion that further reduces portal blood flow velocity, which may lead to PVT (14). In our case, portal vein recanalization and TIPS relieved portal vein pressure and cured hemorrhagic esophageal varices, and there were no complications, such as hepatic encephalopathy, at follow-up. TIPS and superior mesenteric vein stent have been shown to be successful in the treatment of hemorrhagic esophageal varices caused by PVT and superior mesenteric vein thrombosis in our PNH patient. Such treatments are effective in avoiding the worsening of the condition, including increased bleeding and intestinal necrosis.

Eculizumab has been found to be highly efficient in reducing intravascular hemolysis and may provide protective antithrombotic action (15). However, high costs and access difficulties have limited the utilization of eculizumab in China (16). Taking warfarin with a goal international normalized ratio of 2.0 to 3.0 is crucial for the unobstructed stent.

In our case, the patient's initial visit was not at our institution, so we lacked some information, including upper endoscopy, etc., but by contrast-enhanced CT and the patient's melena symptoms, we could also confirm the presence of esophageal and gastric variceal bleeding. Our brief report focuses on highlighting the improvement of melena and a steady recovery in hemoglobin after the patient received TIPS surgery. TIPS may become a new treatment option for PNH combined with PVT.

In conclusion, portal vein thrombosis in patients with paroxysmal nocturnal hemoglobinuria is rare and refractory to treatment. The long course of the disease facilitates portal cavernous transformation, and it is difficult to achieve a curative effect via simple spleen embolism. Our patient underwent transjugular intrahepatic portosystemic shunt, which worked well at 1 month, 3 months, 6 months, and 1 year follow-up and could inform clinical decision-making.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the Ethics Committee of the First Hospital of Hebei Medical University. The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from a by-product of routine care or industry. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

RD: Writing – original draft, Writing – review & editing. LiZ: Writing – review & editing, Formal Analysis, Funding acquisition. PL: Data curation. YZ: Investigation. YY: Validation. LeZ: Methodology, Conceptualization. ZZ: Conceptualization, Project administration, Supervision, Funding acquisition.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Endothelial activation and damage as a common pathological substrate in different pathologies and cell therapy complications

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The endothelium is a biologically active interface with multiple functions, some of them common throughout the vascular tree, and others that depend on its anatomical location. Endothelial cells are continually exposed to cellular and humoral factors, and to all those elements (biological, chemical, or hemodynamic) that circulate in blood at a certain time. It can adapt to different stimuli but this capability may be lost if the stimuli are strong enough and/or persistent in time. If the endothelium loses its adaptability it may become dysfunctional, becoming a potential real danger to the host. Endothelial dysfunction is present in multiple clinical conditions, such as chronic kidney disease, obesity, major depression, pregnancy-related complications, septic syndromes, COVID-19, and thrombotic microangiopathies, among other pathologies, but also in association with cell therapies, such as hematopoietic stem cell transplantation and treatment with chimeric antigen receptor T cells. In these diverse conditions, evidence suggests that the presence and severity of endothelial dysfunction correlate with the severity of the associated disease. More importantly, endothelial dysfunction has a strong diagnostic and prognostic value for the development of critical complications that, although may differ according to the underlying disease, have a vascular background in common. Our multidisciplinary team of women has devoted many years to exploring the role of the endothelium in association with the mentioned diseases and conditions. Our

research group has characterized some of the mechanisms and also proposed biomarkers of endothelial damage. A better knowledge would provide therapeutic strategies either to prevent or to treat endothelial dysfunction.

KEYWORDS

endothelial damage, cardiovascular disease, chronic kidney disease, obesity, major depression, sepsis, COVID-19, hematopoietic stem cell transplantation

1. Introduction

At the conference on "Women and Science" organized by the European Parliament and Commission in April 1998 in Brussels, both institutions agreed in a formal statement on the "need to identify efforts to increase the presence of women in research in Europe." Actually, as the scientific career advances, the proportion of women decreases (1). In our case, we are proud to constitute a group of multidisciplinary scientific women who address the same exciting topic: endothelial activation and damage in association with different pathologies and as a consequence of certain therapies.

For years, the vascular endothelium was considered an inert barrier, but, nowadays, it plays a fundamental role in human health and disease. This biologically active interface is constituted by 1 to 6 $\times$ 10^{13} endothelial cells (ECs) in an adult human being and covers a large surface area (between 4,000 and 7,000 m^2) (2–4). The endothelium has a huge range of functions (5), including the maintenance of the vascular homeostatic balance, modulation of the vascular tone, participation in the recruitment of immune cells, and the generation of new blood vessels, among others.

All these functions are differentially regulated in space and time, showing the heterogeneity of the ECs in different organs in terms of morphology, structure, and barrier function (6, 7). The pulmonary endothelium is localized at a crucial interface and it is formed by an heterogenous cell monolayer that acts as a selective barrier between blood, airways, and lung parenchyma (8). Blood vessel endothelium crosses every tissue, exhibiting unique structural and functional properties in each vascular bed. As a result of organ-specific requirements, the vascular system varies in its organization and specifically in cell-to-cell junctions, which are crucial in the integrity of blood vessels, depending on the anatomical site. While tight junctions are well organized in arteries and brain microvessels, they are more unstructured in veins, capillaries, and organs where a higher rate of exchange is needed (9). Due to their location, the endothelium is directly exposed to all physiological and pathological stimuli (10, 11). These cells are able to adapt to a wide range of environmental conditions, however, noxious stimulus induce local or systemic endothelial activation (12).

Endothelial cell activation and damage imply a range of phenotypic changes in the endothelium and differ according to many physiological variables. If the activation stimuli are persistent in time and/or intense enough, the endothelium may become dysfunctional causing abnormal functional and structural changes, being the main consequence of the loss of vascular integrity with the detachment of ECs exposing a more thrombogenic extracellular matrix (ECM) (13). The relevance of endotheliopathy in the progression of several diseases has been increasingly accepted in the scientific community. The present review aims to summarize the complexity of this process in a range of pathological situations that share endothelial damage (ED) as a common feature.

Historically, our team initiated the research on endothelial activation and damage in the context of end-stage chronic kidney disease (CKD). Further collaboration with other teams in the Hospital Clinic and Institut de Recerca August Pi Sunyer helped us to expand the investigation into other pathologies with associated cardiovascular risk, such as obesity and major depression. A joint venture with obstetricians followed to explore the role of the endothelium in pregnancy-related pathologies, such as preeclampsia (PE), in which the dysregulation of the complement system plays a crucial role. Moreover, a tight partnership with the intensive care unit (ICU) prompted us to explore the ED in septic syndromes and how the severity of the disease could have a gradual impact on the endothelium. Then, with the global outbreak of the coronavirus SARS-CoV-2 that caused the COVID-19 pandemic starting in 2020, our efforts were focused on characterizing the associated endotheliopathy. Furthermore, in collaboration with the hematopoietic stem cell transplantation (HCT) unit at the Hospital Clínic, we were one of the first groups to demonstrate the role of the ED as a pathological substrate for the complications appearing in early post-transplantation. We are now progressing in the research of the role of the endothelium in the development of severe complications that may compromise the promising curative potential of new therapies, such as the use of chimeric antigen receptor T (CAR-T) cells. The investigation performed during these years to explore the mechanisms involved has already provided us with diagnostic and prognostic biomarkers and potential therapeutic targets. Future research should still generate additional tools focused on protecting the endothelium (**Figures 1**, **2**).

2. Endothelial activation and damage in end-stage chronic kidney disease

Chronic kidney disease is a major public health issue with an increasing prevalence. It is associated with poorer quality of life, reduced life expectancy, and, therefore, high rates of morbidity and mortality, and increased hospitalization costs (14, 15). An increment in cardiovascular diseases has been highlighted as the main cause of the increased morbidity and mortality in this population; however, it cannot be fully explained by the presence

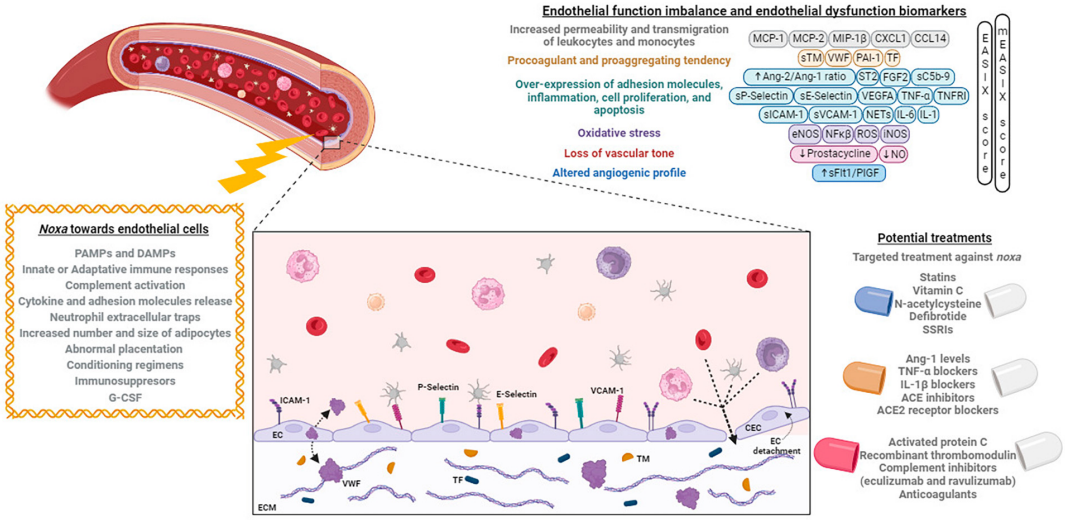

FIGURE 1
Noxa, mechanisms, and biomarkers involved in endothelial dysfunction, and its potential treatment. Different factors alter the endothelial cells phenotype causing an imbalance that can be identified by the expression of different biomarkers. At right, the principal developed treatments targeting the endothelium are exposed. Created with BioRender (available from https://www.biorender.com).

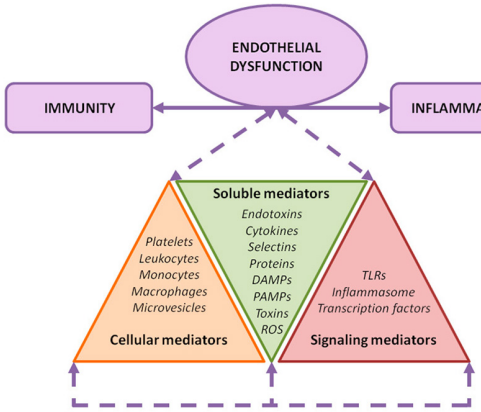

FIGURE 2
Endothelial damage mechanisms. Exposure of the endothelium to different noxa leads to a convergence among inflammation, immunity, and endothelial activation. As depicted, several soluble, cellular, and signaling mediators are involved in this orchestrated response.

of traditional cardiovascular risk factors. Endothelial activation and damage in CKD patients have been described as related to sustained toxic, oxidative stress, and inflammatory conditions. Endothelial dysfunction has been proposed as a pathophysiological substrate for accelerated atherothrombosis, hemostasis alterations, inflammatory activity, and impaired immune response in these patients (16, 17).

Endothelial activation in CKD may be attributed to different factors: the pulsatile blood flow and disturbed shear stress (18), the presence of uremic toxins, such as indoxyl sulfate, and the production and release of oxidative stress and inflammation related products to the circulation (19). All these elements constitute the uremic environment, and could be classified into three major categories of mediators: (i) soluble, (ii) cellular, and (iii) signal transduction mediators (19).

Innate immune system alterations have been also reported in association with end-stage renal disease, aggravated by dialysis procedures. In addition, CKD is related to gut dysbiosis, with a significant loss of the gut microbiota diversity due to the uremic condition, dietary restrictions, administered drugs (antibiotics, phosphate binders, and oral iron supplementation), and hypervolemia, leading to intestinal wall congestion and edema (20, 21). In addition to the impaired renal function, there is also a reduced function of the intestinal barrier with increased permeability to different size molecules. All together enrich the toxic milieu in uremia

There is *in vivo* and *in vitro* evidence demonstrates endothelial activation and damage in association with CKD, causing impaired endothelium-dependent vasodilatation and increased plasma levels of circulating cell adhesion molecules, such as intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin (22–24). Also, other ED-derived proteins, such as monocyte chemoattractant protein-1 (MCP-1) (25), angiopoietin-2 (Ang-2) (26), tissue factor (TF) (27–29), and total von Willebrand factor (VWF) (30, 31) are elevated in circulation. Endothelial activation is considered an early trigger for atherosclerosis and, therefore, in the setting of CKD may explain the increased cardiovascular risk in this population, beyond traditional cardiovascular risk factors (32).

Our research group has carefully studied the ED in uremia by exposing ECs to culture media supplemented with sera from patients on renal replacement therapy. ECs exhibited alterations in their morphology, with accelerated proliferation (33). They also showed signs of inflammation, expressing VCAM-1, ICAM-1 on their surface, with activation of the intracellular protein p38MAPK (34). The ECM produced by these ECs was characterized by a less intricate network of fibrils (27) and an increased thrombogenicity, mainly due to a higher expression of TF (27), VWF (34), and

thrombomodulin (TM). No changes in ADAMTS-13 activity, the VWF metalloprotease, were detected in patients' plasma (35).

The analysis of the proteome of ECs grown under uremic conditions versus control (36) provided information on the differentially expressed proteins. Also, antioxidant enzymes, such as glutathione peroxidase, superoxide dismutase, and peroxiredoxin, were detected to be increased, suggesting an adaptive response to the oxidative stress induced by uremic media. Most of the proteins found to be unregulated in the uremic ECs are related to nuclear factor kappa B (NF-κB) (35). This protein is key in mediating inflammatory and immunological responses and oxidative stress. In addition, elements participating in innate immunity, such as Toll-like receptor 4 and the inflammasome nucleotide-binding oligomerization domain-like receptor prying domain-containing-3 (NLRP3, also known as NALP3) were also upregulated in ECs exposed to the uremic milieu (37). ED can also develop into apoptotic changes (38, 39).

Therefore, endothelial activation is associated with inflammation, oxidative stress, and alterations of immune mechanisms in CKD patients. Therapies focused on protecting the endothelium at different levels could diminish the accelerated cardiovascular risk in this population.

3. The endothelium in obesity

Obesity is a chronic systemic metainflammation. Is associated with oxidative stress, endothelial dysfunction, and vasculopathy, and, therefore, constitutes an important risk factor for atherothrombotic cardiovascular morbidity and mortality (40, 41). In addition, obesity is related to dyslipidemia, hypertension, insulin resistance, and diabetes mellitus, which are conditions that also have a deleterious impact on the endothelium.

The increase in the number and size of adipocytes appears to be the initial event of adipose tissue dysfunction, resulting in hypoxia and defects in the lipids accumulation, together with infiltration of inflammatory macrophages, the switch of adipose tissue-resident macrophages to a proinflammatory phenotype, and conversion of preadipocytes to macrophages. Activation of non-adipocyte stromal cells and secretion of factors from the adipose tissue lead to an increased presence of chemokines and cytokines in plasma, which may participate in the development of chronic inflammation, angiogenesis, and atherothrombotic changes (42–46). There is evidence of the secretion of cytoadipokines from different adipose tissue depots (42, 47–49).

In studies performed by our group, cultured ECs were exposed to the secretome of adipose tissue from visceral and subcutaneous locations of obese and non-obese individuals (50). The cytokines secreted by the adipose tissue of obese subjects caused an adverse effect on the cultured ECs (50), characterized by increased proliferation, morphology alteration, higher expression of VCAM-1, ICAM-1, and VWF, and production of a more thrombogenic ECM. The visceral secretomes induced the strongest expression of these markers, which occurred through NF-κB activation in ECs, together with an increased presence of proinflammatory cytokines (interleukin-6, IL-6), and neutrophil and monocyte chemo-attractants (MCP-1, MCP-2, MIP-1β, CXCL1, and CCL14), in the secretomes from obese adipose tissue (42–47). All these alterations could be involved in vascular and systemic aging (51, 52). Our findings are in agreement with observations by several groups of a better cardiovascular risk profile for those obese individuals with low levels of visceral adipose tissue, known as "healthy obesity."

We believe that excessive fat accumulation causes activation of the stromal cell fraction, altering the adipose tissue secretion pattern. The synergic proinflammatory and prothrombotic profiles of the obese secretome are responsible for the systemic macrovascular endothelial activation observed in obesity.

4. Major depression and cardiovascular risk: role of the endothelium

Major depression and cardiovascular disease are two comorbid conditions highly prevalent that constitute an important health concern in developed countries Signs of ED have been demonstrated in major depression (53, 54). In this regard, inflammation and ED are mechanisms potentially connecting depression to cardiovascular disease (55–57).

Our group was able to demonstrate significant elevation in circulation of different biomarkers of ED, such as circulating endothelial cell (CEC), VWF, and soluble VCAM-1, in patients with the diagnose of major depression (58). Moreover, treatment with the selective serotonin reuptake inhibitor (SSRI) escitalopram exhibited a protective role since biomarker levels decreased substantially in a gradual manner. In addition, when cultured ECs were exposed to the sera from these patients, these findings were reproducible. ECs exhibited signs of inflammation, oxidative stress, and increased thrombogenicity of the ECM generated, which were inhibited significantly by the presence of escitalopram *in vitro* (58).

There is strong evidence on the role of serotonin in the cardiovascular system. Platelets, the main carriers of serotonin (59), play a key role in the development of cardiovascular events. In experimental studies performed by our group, exogenous addition of serotonin to blood samples potentiated platelet functions, increasing their procoagulant behavior, and enhancing thrombus formation on damaged vascular surfaces, effects that were inhibited by the presence of SSRIs (60, 61). In addition, in experiments performed with blood samples from patients with major depression, a pronounced procoagulant profile, with increased platelet thrombus and fibrin formation, was observed at the moment of diagnosis and normalized after 24 weeks of treatment with escitalopram (62). Altogether, our results suggest that both platelets and endothelium are two key hemostatic components, whose responses may be altered and may be acting synergistically in major depression (63).

5. Endothelial damage in pregnancy-associated complications

Preeclampsia is a life-threatening pregnancy-associated disorder that affects 2–8% of pregnancies (64). It is defined as

new-onset hypertension with other signs of endothelial systemic damage, accompanied by signs of end-organ damage, such as proteinuria, acute kidney failure, liver dysfunction, hemolysis or thrombocytopenia, occurring after 20 weeks of gestation (65). PE is a heterogenous disorder, with a large variability in its associated risk factors and clinical presentation (66). The exact pathophysiology remains unclear despite exhaustive investigation (67). However, the most accepted hypothesis is known as the two-stage model claiming that complications originate during abnormal placentation at the beginning of pregnancy, followed by generalized inflammation and progressive maternal ED (68). Resulting placental insufficiency and overt clinical signs of PE do not manifest usually until the last pregnancy trimester (69) with a possibly rapid and unexpected progression from mild to severe PE (70). Unfortunately, there is no effective treatment and delivery if the only available intervention (71).

Endothelial dysfunction has been accepted as one of the key mechanisms in PE development (72). In normal pregnancy, the uterine vasculature undergoes significant adaptations to ensure proper blood supply to the developing fetus (73). These adaptations mainly involve increased vasodilation and decreased vasoconstriction, allowing for higher blood flow to the uterus (73). However, these adaptations are disrupted in PE, leading to vasoconstriction and inadequate blood supply to the placenta and fetus (74).

The dysfunctional endothelium promotes the production and release of pro-inflammatory cytokines (75), such as tumor necrosis factor-alpha (TNF-α), interleukin 6 and 1b (IL-6 and IL-1b), and adhesion molecules (VCAM-1 and ICAM-1) (76). The cytokines activate immune cells, causing excessive inflammatory responses (77), and the adhesion molecules promote the breakdown of EC-cell contacts (78). Prolonged ECs activation results in a cycle of inflammation and oxidative stress. This inflammatory environment contributes to the extensive ED and organ dysfunction seen in PE, including kidney, liver, and brain involvement (79). Furthermore, this ED is associated with a dysregulation of the complement system (80). The maternal innate immune system is crucial throughout pregnancy, providing protection against pathogens while inducing tolerance to semi-allogeneic fetal and placental development (81). Dysregulation of the maternal immune system during PE leads to overstimulation of the complement system as a compensatory mechanism (82), with recruitment of phagocytic cells and neutrophils to the site of stimulation (83). This phenomenon manifests with elevated plasma C5b9 in PE mothers and C5b9 deposits on ECs (76, 83).

In addition, there is imbalance in the coagulation system (80). Damaged endothelium does not produce sufficient anticoagulant factors, such as tissue factor pathway inhibitor (TFPI), and TM (84), in association with increased VWF (85). This prothrombotic state increases the risk of thrombosis and microvascular fibrin deposition, further impairing placental blood flow (86) and contributing to the development of maternal organ dysfunction (87).

Angiogenic factors have emerged as the most specific biomarkers of PE ever described and have recently been incorporated as essential components in the prediction, diagnosis and prognosis of PE (88). A proper angiogenic balance during pregnancy is critical for adequate development of fetus and placenta together with appropriate maternal cardiovascular adaptation to pregnancy (89). Indeed, angiogenic factors are essential not only for new vessel formation but also to keep the maternal endothelium healthy by promoting vasorelaxation, adequate permeability and cell survival (90). In PE, placental inflammation and increased oxidative stress cause release of larger amounts of sFlt1 over PIGF (91), with an antiangiogenic profile reflecting placental malfunctioning and maternal endothelial dysfunction (92).

In conclusion, endothelial dysfunction is key in the pathogenesis of PE. Impaired NO production, increased vasoconstriction, inflammation, innate immunity dysregulation, and coagulation and angiogenic imbalance contribute to the hypertension, poor placental perfusion, and multiple organ damage occurring in this condition. Other pathways, like altered lipid metabolism (93), mitochondrial dysfunction (94), and maternal response to circulating trophoblast-derived extracellular vesicles (95) may be also involved. Furthermore, endothelial dysfunction seems to act as a cardiometabolic stressor that may culminate in long-term cardiovascular complications in women who developed PE during pregnancy (96). Further elucidation of the molecular mechanisms involved is critical for the development of potential therapeutic strategies aim at preventing or reducing the adverse consequences associated with this syndrome.

6. Endothelial alterations in septic syndromes

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection (97). The endothelium orchestrates a beneficial local host response to infection by regulating the vasomotor tone, leukocyte trafficking, vascular permeability and hemostasis. However, when the response is overproduced, a systemic and untargeted dysregulated inflammatory response leads to endothelial hyperactivation, resulting in tissue hypoperfusion and subsequent multi-organ failure and death. In the lung, this translates as a localized injury to the alveolar-capillary membrane, fostering the onset of acute respiratory distress syndrome (ARDS). ARDS is characterized by an acute onset of respiratory failure typically requiring mechanical ventilation and radiographic bilateral pulmonary opacities of non-cardiogenic origin. Direct ARDS occurs after a direct insult to the lung tissue, leading to an increase in the capillary hydrostatic pressure and interstitial and alveolar flooding, impaired gas exchange, and decreased lung compliance. Indirect ARDS is triggered by a systemic insult and the release of inflammatory mediators that eventually damage the pulmonary endothelium. Its most common cause is sepsis (98).

Activation of the endothelium in sepsis occurs directly by recognition of pathogen-associated molecular patterns (PAMPs) through patron recognition receptors, such as Toll-like receptors (TLRs) expressed in ECs, and, indirectly, by released proinflammatory cytokines, such as TNF-α, IL-6, and IL-1, complement components or neutrophil extracellular traps (NETs) (99). Damage-associated molecular patterns (DAMPs) can also be recognized by ECs, contributing to the amplification of the inflammatory cascade. This endothelial activation leads to a proadhesive, proinflammatory, prothrombotic, and proapoptotic phenotype.

The endothelial barrier integrity is altered during sepsis due to the action of inflammatory mechanisms, such as metalloproteinases and heparanase, causing glycocalyx deconstruction and disruption of ECs junctions, and increasing endothelial permeability, albumin extravasation, and capillary leak (100). Glycocalyx degradation may be potentially compounded by fluid resuscitation practices (101). In addition, there is dysregulation of the NO pathway with a decreased activity of the endothelial nitric oxide synthase (eNOS) and an increased NO production by inducible nitric oxide synthase (iNOS), altering the vascular vasomotor tone toward vasodilatation and producing reactive nitrogen species (102). Moreover, infection triggers ECs to produce ROS, leading to EC apoptosis and production of proinflammatory cytokines, acting not only as a victim but also as an active participant and amplifier of the inflammation. This proinflammatory environment increases expression of adhesion molecules (VCAM-1, ICAM-1, and selectins) on the ECs surface, promoting subsequent leukocyte rolling, adhesion and transmigration, contributing to inflammation and progression of endothelial dysfunction (103). The activated endothelium also interacts with platelets, which are activated directly by PAMPs or indirectly by innate immune cells, promoting a prothrombotic phenotype in which an increased production of VWF seems to play a major role. ADAMTS-13 activity has been found to be decreased in patients with sepsis, which may increase platelet-vessel wall interaction by an increased presence of ultra-large VWF multimers (104, 105). Furthermore, activated platelets also contribute to inflammation by releasing proinflammatory proteins that help establish a "cross-talk" with the endothelium (106). Indeed, thrombocytopenia is frequently seen in sepsis, probably due to excessive peripheral consumption, and it is associated with increased disease severity and mortality (107).

Upon sepsis-induced endothelial activation, there is also an increased expression of TF with subsequent extrinsic coagulation pathway activation. This procoagulant state is favored by a dysregulation of the endothelial anticoagulant and fibrinolytic properties, with decreased protein C activation, TM and TFPI and increased PAI-1 release. This procoagulant state favors thrombosis in the microvasculature, potentially causing disseminated intravascular coagulation (DIC), which is associated with poor prognosis in patients with sepsis (108, 109).

Neutrophil extracellular traps have also an important role in sepsis-induced endothelial dysfunction, causing the expression of adhesion molecules, participating in the prothrombotic state by increasing platelet adhesion on the ECs surface, and contributing to thrombin-mediated fibrin generation (110, 111).

This ED significantly alters the microcirculation, with decreased vascular flow and subsequent organ hypoperfusion, with mitochondrial dysfunction leading to organ failure and death. Experimental and clinical studies have demonstrated a correlation between endothelial dysfunction and sepsis severity, highlighting the crucial role of the endothelium in the pathophysiology of sepsis-induced organ dysfunction, and arising as an attractive therapeutic target (112–114). Thus, in the last decades, experimental and clinical studies have strived to find effective treatments targeting sepsis-induced endothelial dysfunction (111), however, none of them have shown survival improvement in large randomized clinical trials. Our group has demonstrated the utility of an *in vitro* model of ED in sepsis, able to show a gradual effect depending on the severity of the disease, which may constitute a useful tool to explore different treatments.

7. The endotheliopathy developed in COVID-19

The COVID-19 pandemic, caused by the emergence and worldwide spread of the SARS-CoV-2 virus, exerted profound and far-reaching impacts on global healthcare and the economy. It is now widely acknowledged that the endothelium plays a pivotal role in its pathogenesis and manifestations (115, 116). Intracellular penetration of SARS-CoV-2 into human cells occurs via union of its spike protein to the angiotensin converting enzyme 2 (ACE2) (117). While ACE2 receptors are ubiquitous, they are particularly overexpressed on the alveoli and the ECs (118), leading to an immediate interaction with the endothelium from the very initial stage of infection. As viral replication progresses, more severe symptoms may appear accompanied by a hyperinflammatory activation of host immunity. Key features at this stage are elevated acute phase reactants, such as C-reactive protein, D-dimer, and ferritin, as well as increased circulating cytokine levels. As discussed below, the endothelium is a primary contributor in sustaining and intensifying this maladaptive immune response (119, 120).

Critically ill COVID-19 patients develop pulmonary infiltrates leading to acute respiratory distress syndrome with an eventual need for respiratory support. The development of ARDS in COVID-19 involves a complex interplay of immune responses and inflammatory processes. Both direct (lung tissue-specific) and indirect (systemically triggered) mechanisms are involved in the pathogenesis of ARDS, but there is no evidence of a specific phenotype related to COVID-19 (121). The clinical diagnosis of ARDS is based on the Berlin definition (122) of sudden refractory hypoxemia and bilateral shadows in the lung fields. The endothelium plays a pivotal role in the spreading of inflammation and damage to the lung and the alveolar-capillary membrane, which culminates in fluid accumulation, compromised gas exchange, and pulmonary vasculature hypertension. The initial virus mediated pneumocyte injury triggers local cytokine production and, via paracrine cell communication, the alveolar capillary ECs sense the signaling distress (123). This activates the endothelium, which responds with the induction of a proinflammatory cell recruitment state, mediated by the expression of cell adhesion molecules (124). As a result, oxidative stress, endotheliitis and EC dysfunction ensue. Multiple mechanisms are involved in the switching of the endothelium into a hypercoagulative state, such as TF coagulation activation, platelet pro-aggregation due to increased release of VWF, NET-mediated thrombin generation and fibrinolytic shutdown. This is supported by the fact that COVID-19 patients present with increased circulating endothelial stress products (proinflammatory cytokine levels, VWF, soluble VCAM-1 and heparan sulfate) in correlation with disease severity (105). Also, biomarkers of complement activation, fibrinolysis inhibition, proangiogenic factors, and NET formation have shown to be significantly higher in the serum of COVID-19 patients (125–127). Several postmortem histological lung studies (128, 129) have widely evidenced the aforementioned mechanisms involved in the cascade of endothelial modifications,

showing increased levels of inflammatory cytokines, such as IL-6 and TNF-α (130), and upregulation of ICAM-1 and VWF (131).

Other organs are often affected in the spectrum of COVID-19 manifestations, notably the cardiovascular system (132, 133). Thrombosis, coronary infarction and stroke are more frequently observed in the setting of critical illness (134). Further evidence pointing toward endotheliopathy as a predictor of COVID-19 morbidity and mortality emerges from the strong association between pre-existing cardiovascular risk factors (i.e., advanced age, hypertension, and obesity) and the likelihood of disease progression (135). In the brain, neurologic symptoms such as anosmia, ageusia or even encephalopathy were widely reported during the pandemics. In the skin, cutaneous lesions have also demonstrated endothelial activation. Histological studies evidenced complement-mediated vascular injury (136), particularly in severe COVID-19.

Current strategies primarily focus on high-efficacy antivirals and immunosuppressants. The combination of therapies targeting inflammation, coagulopathy, and endotheliopathy is a promising strategy to address disease complications. Many publications have explored other approaches to reduce ED, such as defibrotide (137), ACE inhibitors and ACE2 receptor blockers, statins, heparins, and direct oral anticoagulants. These therapies appear to be promising due to their pleiotropic mechanisms of action and their ability to regulate the endothelium (138).

Lastly, evidence of persistent endothelial dysfunction has been found in the subset of patients who develop long-term sequelae after acute infection. Elevated inflammatory markers, cytokine levels, and cytotoxic T cells subsist in convalescent patients (139), and vascular barrier injury is considered to be responsible for the disruption of normal organ physiology (140), leading to severely impairing systemic symptoms. While days of high infection incidence and overwhelming ICU admissions may be behind us, the implications of endothelial dysregulation remain relevant in the current era of persisting COVID-19. Therefore, endothelial protection remains a valid target for preventing both acute critical illness and long-term COVID-19 complications.

Our group of researchers has contributed to improving the knowledge of the endotheliopathy associated with COVID-19. We have provided evidence on biomarkers that may be useful for the stratification of disease severity and also to guide specific therapeutic strategies to prevent endotheliopathy progression. Some of these biomarkers help to differentiate COVID-19 endotheliopathy from the one that occurs in septic syndromes, in which ED is also a pathological substrate (120). Similarly, we have demonstrated that preeclampsia and severe COVID-19, which may be clinically similar, exhibit distinctive biomarker profiles related to ED, coagulopathy, and angiogenic imbalance. Therefore, differential diagnosis of these entities could be done based on these results.

8. The endothelium as a central player in thrombotic microangiopathies

Thrombotic microangiopathies (TMAs) are a group of disorders characterized by microangiopathic hemolytic anemia and ischemic organ dysfunction, resulting in a wide spectrum of symptoms. The most commonly affected organs in TMAs are the brain, kidneys, and gastrointestinal system (141). Although uncommon, these are life-threatening conditions that require urgent management (142). ED is the common underlying mechanism among different forms of TMAs, leading to the microvasculature thrombosis observed histologically (143).

Classically, primary TMAs have been classified according to the identification of the following pathogenic mechanisms: thrombotic thrombocytopenic purpura (TTP), mediated by a deficiency in the activity of ADAMTS-13 enzyme; typical hemolytic uremic syndrome, caused by a Shiga-toxin–producing Escherichia coli (STEC-HUS); and primary atypical HUS (aHUS), due to the dysregulation of the alternative complement pathway (ACP) (142).

Thrombotic thrombocytopenic purpura is characterized by ADAMTS-13 deficiency, resulting in a deficient excision of the ultra-large VWF multimers presented in the VWF molecule on ECs, causing platelet adhesion and aggregation with rapid generation of disseminated microthrombi. However, evidence generated from clinical, *in vitro,* and *in vivo* studies suggests that ADAMTS-13 deficiency may be a necessary but not sufficient condition to induce TTP. Weibel–Palade bodies (WPBs) are endothelial-specific organelles that contain molecules involved in the regulatory functions of the endothelium, such as ultra-large VWF multimers (proaggregating), P-selectin (proinflammatory) or Endothelin-1 (vasoconstricting). The "second hit" model suggests that in TTP, besides ADAMTS-13 deficiency, endogenous (antibodies or cytokines) and/or exogenous (virus or drugs) factors induce endothelial activation leading to an uncontrolled WPBs degranulation and, finally, to endothelial dysfunction (144).

In STEC-HUS, Shiga-toxin (Stx) is thought to be the key element in the pathogenesis of ED through several mechanisms. Stx induces the production of adhesive molecules (E-selectin, ICAM-1, and VCAM-1) and chemokines (MCP-1, IL-8, and fractalkine), leading to the adhesion of leukocytes to cultured human ECs. Moreover, Stx induces rapid release of ultra-large VWF multimers inhibiting its cleavage by ADAMTS-13, therefore enhancing platelet adhesion and clot formation in the microvasculature. In addition, Stx modifies gene expression, with mRNA production, and release of chemokines and cytokines that may aggravate ED (145, 146).

As mentioned before, dysregulation of the ACP occurs as the primary event in aHUS, prompting the activation of the terminal complement phase and the deposit of the lytic complex C5b-9 on the EC surface (146). Eculizumab, a humanized monoclonal antibody against C5, and ravulizumab, a long-acting C5 inhibitor, are first-line treatments for aHUS (147). In this regard, it has been observed that the measurement of C5b-9 deposits on ECs constitutes a reliable tool to explore the complement system dysregulation in aHUS, as well as to monitor the response efficiency to eculizumab treatment in these patients (83).

Although aHUS is the prototype of complement-mediated TMAs, the contribution of dysregulated complement activation to ED has been widely demonstrated in other TMA forms. Increased levels of soluble C5b-9 (sC5b-9) have been detected in patients with acute TTP, probably due to the activation of the classical complement pathway by immunocomplexes of ADAMTS-13 and anti-ADAMTS-13 antibodies (146). In STEC-HUS, the ED is caused by different mechanisms driven by Stx, including ACP activation, resulting in increased levels of C3a, Bb, and sC5b-9

during the active phase of the disease. In this regard, eculizumab has been employed for the treatment of children and adults with STEC-HUS, with no systematic assessment of its efficacy or safety (145).

Secondary forms of TMAs may occur in multiple clinical settings: autoimmune diseases, cancer, pregnancy, solid organ and HCT, certain medications, and infections (141). The underlying pathogenic mechanism has been attributed to a direct ED conferring a more procoagulant and proinflammatory phenotype in ECs, inducing a "second-hit" in which complement activation and its perpetuation occurs, aggravating TMA (148). Among them, TMAs after kidney transplantation should be highlighted due to the well-known involvement of ED in its pathogenesis, which can occur due to the combination of multiple triggers: ischemia-reperfusion injury, immunosuppressive drugs (calcineurin and m-TOR inhibitors), viral infections (mainly cytomegalovirus), and acute or chronic humoral rejection (148).

Thrombotic microangiopathies differential diagnosis is a major challenge due to their variable clinical presentation and the absence of pathognomonic histological findings or specific biomarkers (141). Moreover, differentiation between primary or secondary forms may be difficult in clinical practice because triggering factors, such as infections or drugs, are often identified in patients with primary aHUS (142). Therefore, there is an urgent need for new diagnostic tools, based on functional and genetic studies, to assess the involvement of complement dysregulation in the pathogenesis of the different forms of TMAs (146). In this regard, the use of complement-targeted therapies in patients with secondary refractory TMAs to traditional therapy could be useful. The duration of the treatment should be tailored based on the presence of complement abnormalities and response to therapy (149).

9. Hematopoietic stem cell transplantation and endothelial damage

Hematopoietic stem cell transplantation is the best-known form of cellular therapy, widely used for the treatment of malignant and non-malignant hematologic, metabolic, or autoimmune disorders (150). During the last decades, HCT has experienced significant improvements in terms of donor selection, in allogeneic HCT (allo-HCT), and treatment refinement in both, allo- and autologous HCT (auto-HCT) (151, 152), allowing the expansion of HCT to older adults or patients with comorbidities. More recently, the scope of cellular therapy has expanded through the emergence of immunotherapies based on the cytotoxic effect of autologous cells, as CAR-T cells and tumor-infiltrating lymphocytes (TIL), for refractory/relapsed hematological malignancies and solid tumors, respectively. Despite the curative potential of cell based therapies, there are associated complications that may compromise the success of the treatment. In these complications, the endothelium seems to play a main role.

Sinusoidal obstruction syndrome (SOS), formerly known as veno-occlusive disease, was the first post-HCT complication where endotheliopathy was proven as its pathophysiological substrate and targeted for its treatment (153, 154). Consecutively, growing evidence points to endothelial dysfunction underlying other highly incident HCT-related complications, such as acute graft-versus-host disease (aGVHD) (155, 156), and the main CAR-T cell-related toxicities (157, 158).

Endotheliopathy has not only shown to be involved in the pathogenesis of the complications of cellular therapies but also to be the result of different harms toward ECs before and during the treatment. Mainly due to drugs used during the induction or consolidation chemotherapeutic schemes (159, 160) or the ones used for the conditioning treatment before and after the infusion of the autologous or allogeneic cells (161–165). Consequently, it is essential to understand the involvement of the endothelium and other associated pathways in the pathogenesis of cellular therapies-complications in order to better-stratify risk patients and develop targeted treatments and preventive strategies.

The HCT treatment itself induces endothelial dysregulation, leading to a hypercoagulable state. Studies demonstrate that while procoagulant molecules increase, levels of the main natural-anticoagulant molecules decrease in the context of HCT (166, 167). Furthermore, the innate and adaptative immune reactions, the PAMPs resulting from infections occurring during the HCT process, together with the toxic agents included in the preparative regimens, have been identified as *noxa* toward the endothelium.

Endothelial dysfunction after HCT has multiple origins and varies according to the time after HCT and anatomical location. In most cases, it implies increased leukocyte adhesion and transmigration, molecule extravasations, platelet activation, and cytokine liberation (168). The ED occurring after HCT and derived from the mentioned stressors would consist of the following: (1) increased synthesis of Ang-2, which is involved in endothelial inflammation increasing its permeability (169), (2) overexpression of adhesion molecules (such as ICAM-1, VCAM-1, E-selectin, and P-selectin), responsible for leukocyte recruitment and transmigration through the endothelium (170), (3) dysregulation of the vascular tone, since the endothelial synthesis of NO and prostacyclin is reduced, and (4) elevation of angiogenic molecules, such as vascular endothelial growth factor A (VEGFA), fibroblast growth factor 2 (FGF2), and Ang-2, molecules that have their respective receptors (VEGFR1 and VEGFR2, FGR1, and TIE-2) (171).

The complex link existing between endothelial activation and progression to endothelial dysfunction occurring during the HCT process has been investigated in different *in vitro* and pre-clinical studies. Results obtained indicate that ECs are activated and damaged by different factors, including drugs used in the conditioning regimen, radiotherapy, cytokines released by the injured tissues, endogenous microbial products that translocated through damaged mucosal barriers, immunosuppressants in allo-HCT, the engraftment process itself, and allo-reactivity (163, 168, 172, 173). Studies by our group and others, using an *in vitro* model of ED, allowed the investigation of the specific responses of the endothelium to controlled and well-known stimuli at different stages of the HCT, and also to molecules associated with HCT, such as lipopolysaccharide or TNF-α (174).

In the HCT setting, the ED occurring since the start of the conditioning regimen and during the post-transplant process is involved in a group of early and potentially life-threatening post-HCT endothelial complications (175, 176). These events generally appear during the first 100 days after the stem cell infusion, their diagnosis is mainly based on medical signs and symptoms,

and all of them seem to begin at the capillary level and result from an endothelial dysfunction occasioned by the administration of chemotherapy, calcineurin inhibitors, G-CSF, infections, and allogeneic-derived reactivity (12, 168, 177).

Historically, SOS was the first complication in which the role of ED was proposed. Nevertheless, there is increasing evidence of the implication of the endothelium in the pathophysiology of other post-HCT vascular endothelial complications, such as engraftment syndrome, capillary leak syndrome, transplant-associated thrombotic microangiopathy (TA-TMA), GVHD, and the vascular idiopathic pneumonia syndrome.

Several groups, in which ours is included, have investigated the presence of circulating biomarkers for diagnosis and prognostication of post-HCT vascular endothelial complications (177–180). In general, the majority of the studies show increased levels of ED biomarkers, especially in patients with HCT complications (179, 181–183). The complexity of the diagnosis and clinical management required to treat post-HCT vascular endothelial complications enhances the need to increase the knowledge of predictors and clinical manifestations for the early detection of these syndromes to decrease mortality after HCT (168). Furthermore, the increasing knowledge of the physiopathology of these complications opens up potential pharmacologic interventions to prevent and treat ED and, therefore, to improve the outcome of patients receiving HCT.

10. Endotheliopathy in CAR-T cell immunotherapy

Treatment with CAR-T cells has risen as a viable and safe procedure for the treatment of relapsed/refractory hematological malignancies. CAR-T cell technology is based on the cytotoxic effect of T lymphocytes of the patient modified *in vitro* to target antigens present in tumoral cells. This immunotherapy has proven to be effective for the treatment of acute lymphoblastic leukemia and lymphomas expressing CD19 antigen and for myeloma patients when targeting B-cell maturation antigen. To date, only a few therapeutic schemes and constructs have been approved by Food and Drug Administration and European Medicines Agency (184, 185) whereas many others are still being under assessment in clinical assays (186). Despite the promising remission rates of these novel therapies, cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS), are highly incident toxicities, and potentially life-threatening (187–189). Growing evidence sustains that endotheliopathy underlies and promotes the onset of these toxicities. However, not all endothelial dysfunction can be attributed to the administration of the construct but to different *noxa* before and during immunotherapy.

The use of conditioning, or lymphodepletion, before CAR-T cell infusion has proven to enhance the *in vivo* expansion of the construct, and both its engraftment and anti-tumoral function (190–192). The most commonly used drugs are cyclophosphamide (Cy) and fludarabine (Flu) in combination. Both, Cy and Flu, have shown to cause deleterious effects on ECs (173, 193) in *in vitro* assays. In fact, Flu has been observed to increase the incidence of CAR-T related toxicities (194, 195). Nevertheless, the individual impact of these drugs, *in vivo*, and the incidence of endothelial-related complications has to be elucidated.

Up to 80% of patients treated with CAR-T cell immunotherapy present CRS of any grade, clinically ranging from fever ± hypoxemia, hypotension, capillary leak and/or signs of specific-organ toxicity, depending on the severity of the case (196). Less incident, ICANS can be suspected by a wide range of symptoms and signs, such as headache, cognitive or motor impairment, delirium and seizures. Since ICANS is predisposed in the vast majority of cases by severe/early onset CRS (157, 197), the pathways involved in their development seem to be common. For this reason, the pathophysiology of CRS and ICANS will be reviewed altogether except when otherwise specified.

Clinically, different risk factors have been associated with an increased risk of developing CRS and ICANS: lymphodepletion schemes containing Flu, high burden/bone marrow involvement of the basal disease, and infusion of high doses of the CAR-T cell construct leading to high peaks of *in vivo* proliferation (188, 194). Biologically, elevations of proinflammatory cytokines (IL-6, interferon-γ, and TNF-α) after the construct infusion were firstly reported as the potential cause of CRS/ICANS in correlation with the clinical severity (187, 198).

Endothelial dysfunction appearing as a consequence of the mentioned cytokine storm, among other causes (199), has been hypothesized as a relevant pathway in the development of CAR-T cell-related toxicities (200). In the specific context of ICANS, the increased permeability within the endothelium of the blood-brain barrier was proven after observing the presence of CAR-T cells on the cerebrospinal fluid (157, 201–203). Recently, scores based on indirect biomarkers of endotheliopathy, such as EASIX or modified EASIX, have demonstrated to be reliable tools to predict the incidence or severe CRS and/or ICANS and their related decrease of the progression-free survival (204–207). Similar to other diseases with ED (112, 208, 209), innate immune activation and hemostasis imbalance are linked pathways also altered in CAR-T cell patients developing toxicities (210–213).

Coagulopathy is an underestimated adverse effect of CAR T-cell therapies (214) that usually derives from severe CRS or ICANS (157, 211). While isolated changes in coagulation parameters, such as elevation in D-dimer, increase in fibrinogen degradation product, decrease of fibrinogen levels and prolongation of activated partial thromboplastin time (aPTT), can be observed in a high proportion of patients (215, 216), the analytical and clinical phenotype of DIC occurs only in cases of high-grade toxicities and is related to an increase of the non-relapse mortality (215). More specifically, a recent study has described increased prothrombin time (PT) and aPTT, fibrinogen, D-dimer, factor VIII (FVIII) and VWF antigen levels in ≥grade 2 CRS. The manifestation of ICANS was associated with elevated PT, D-dimer, FVIII and VWF antigen levels and decreased fibrinogen and platelet count (207). Moreover, patients with high-grade ICANS were found to present higher levels of Ang-2 and VWF, lower levels of ADAMTS-13 metalloprotease and loss of VWF high molecular weight multimers than patients with lower severity grades (157, 198, 200). Although the consumptive mechanism seems to be the predominant one for the development of DIC, impairment of the liver function has also been described as an early indicator (217).

Recently, we have demonstrated that different circulating biomarkers of endotheliopathy, innate-immunity activation,

hemostasis alterations and fibrinolytic imbalance may be good early-predictors of severe CRS/ICANS (ST2, Ang-2, and NETs). Also, the use of some of these biomarkers could be a feasible discriminating tool for the differential diagnosis between CAR T-cell-related severe toxicities and sepsis (Ang-2, NETs, sC5b-9, VWF antigen, and PAI-1 antigen) (213).

11. Pharmacological armamentarium

Targeted treatments against the noxa responsible for endothelial activation and dysfunction is the ideal first line therapy, though, in most cases we do not have that kind of treatments available. In terms of pharmacological armamentarium targeting the endothelium, different options are under assessment. For instance, statins, vitamin C, direct oral anticoagulants, heparins, and N-acetylcysteine are cheap and safe drugs that have demonstrated a protective effect toward the endothelium by reducing the oxidative stress (218–224). The use of drugs able to restore the anti-inflammatory and anticoagulant properties of the endothelium, such as defibrotide, activated protein C, recombinant TM, and Ang-1 levels (an endothelium stabilizer molecule as opposed to Ang-2) have shown promising effects in *in vitro* and in murine models of endothelial dysfunction (225–231). However, disappointing results were found in clinical trials using activated protein C for severe sepsis, highlighting the complexity of the mechanisms involved in ED. In addition, the use of complement inhibitors, such as eculizumab and ravulizumab, in diseases in which terminal complement activation prevails like aHUS, has shown to play a prominent role (83, 147). Other compounds, like TNF-α, IL-1β, and ACE2 receptor blockers, ACE inhibitors or SSRIs, used in synergy with other treatments (i.e., those for CAR T-related toxicities, COVID-19 or major depression) could be worth exploring in clinical trials as potential useful novel therapies in the specific context in which ED is implied (232).

12. Conclusion and future perspectives

The endothelium is an endocrine organ that plays essential functions in maintaining homeostasis. It regulates the vascular tone, hemostasis and fibrinolysis; it shows anti-inflammatory and anticoagulant actions; and it participates in angiogenesis, among other functions. The failure of the endothelial adaptability to the different circulating stimuli, independently of their nature, may cause in the loss of endothelial integrity and function, which is critical for the development of cardiovascular disease.

Endothelial damage, and alterations of several linked pathways, are increasingly being proposed as a pathophysiological substrate for different pathologies and cell therapy complications. The knowledge generated during the last years has promoted the development of panels composed of ED biomarkers for the early prediction of these complications, to stratify their risk, and to facilitate their follow-up. In this regard, new and old therapeutic and prophylactic strategies focused on endothelial protection are being proposed. However, their impact on the incidence of complications and non-relapse mortality should be further explored.

More basic research is needed to elucidate the whole bunch of mechanisms by which the endothelium becomes dysfunctional in a variety of pathological conditions, and more investment in clinical assays is necessary to demonstrate the effect of potentially useful drugs to prevent and treat the endothelium. Additionally, considering that the endothelium is the biggest organ in the body, probably followed by the gastrointestinal tract, we are convinced that the future investigations on the endothelium should consider the crosstalk between both organs, in which the microbiome could be cornerstone (233).

Author contributions

MP: Conceptualization, Formal analysis, Investigation, Supervision, Writing – original draft, Writing – review and editing. AM-C: Writing – original draft, Writing – review and editing. MS: Writing – original draft, Writing – review and editing. SE-S: Conceptualization, Writing – original draft, Writing – review and editing. JM-S: Writing – original draft, Writing – review and editing. BD: Writing – original draft, Writing – review and editing. MR: Writing – original draft, Writing – review and editing. EG-O: Writing – original draft, Writing – review and editing. SF: Writing – original draft, Writing – review and editing. HV-C: Writing – original draft, Writing – review and editing. LY: Writing – original draft, Writing – review and editing. FC: Writing – original draft, Writing – review and editing. MN: Writing – original draft, Writing – review and editing. MD-R: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing.

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Pregnancy as a susceptible state for thrombotic microangiopathies

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Pregnancy and the postpartum period represent phases of heightened vulnerability to thrombotic microangiopathies (TMAs), as evidenced by distinct patterns of pregnancy-specific TMAs (e.g., preeclampsia, HELLP syndrome), as well as a higher incidence of nonspecific TMAs, such as thrombotic thrombocytopenic purpura or hemolytic uremic syndrome, during pregnancy. Significant strides have been taken in understanding the underlying mechanisms of these disorders in the past 40 years. This progress has involved the identification of pivotal factors contributing to TMAs, such as the complement system, ADAMTS13, and the soluble VEGF receptor Flt1. Regardless of the specific causal factor (which is not generally unique in relation to the usual multifactorial origin of TMAs), the endothelial cell stands as a central player in the pathophysiology of TMAs. Pregnancy has a major impact on the physiology of the endothelium. Besides to the development of placenta and its vascular consequences, pregnancy modifies the characteristics of the women's microvascular endothelium and tends to render it more prone to thrombosis. This review aims to delineate the distinct features of pregnancy-related TMAs and explore the contributing mechanisms that lead to this increased susceptibility, particularly influenced by the "gravid endothelium." Furthermore, we will discuss the potential contribution of histopathological studies in facilitating the etiological diagnosis of pregnancy-related TMAs.

KEYWORDS

women, pregnancy, endothelium, thrombotic microangiopathies, complement activation, angiogenesis factors, kidney biopsy, placental histology

1 Introduction

The terms "thrombotic microangiopathy" (TMA) or "TMA syndrome" (1, 2) encompasses a diverse array of clinical entities sharing a common histological presentation characterized by endothelial cell alterations and frequent fibrino-platelet thrombi restricted to the microvasculature (3). The biological manifestations of TMA are typically characterized by the concurrent presence of consumptive thrombocytopenia and microangiopathic hemolytic anemia (MAHA), both of which serve as reliable indicators alerting clinicians to the possibility of a TMA syndrome. The presence of one or more organ predominant dysfunctions resulting from the downstream ischemic effects of microvascular damage, has historically been used to distinguish the different TMA syndromes: predominant neurological impairment in thrombotic thrombocytopenic purpura (TTP), acute renal failure in hemolytic uremic syndrome (HUS), or hepatic cytolysis in the context of HELLP (Hemolysis, Elevated Liver enzymes, Low Platelet count) syndrome in pregnant women (4, 5). This clinical categorization

is susceptible to fallibility due to the presence of intragroup heterogeneity and potential overlap between distinct syndromes, thereby challenging precise differentiation (6–8).

The last 40 years have witnessed substantial advancements in elucidating the intricate mechanisms underlying TMAs (2, 9). In the 1980s, the discovery of a deficiency in a von Willebrand factor-cleaving protease, ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type1 motif– member 13), provided a pivotal breakthrough (10, 11). This deficiency was identified as a key contributor to the accumulation of unusually large von Willebrand factor multimers, leading to platelet aggregation and microthrombi formation characteristic of TTP (12, 13). Another series of significant advances emerged in the early 2000s, centered on the identification of abnormalities - genetic variants or acquired autoantibodies - affecting the regulation of the complement alternative pathway in patients with atypical HUS (aHUS) (14–16). This has emphasized the significance of disrupted regulation of complement, particularly via uncontrolled activation of the alternative C3 convertase and the ensuing stimulation of C5 convertase. Ultimately, the release of the C5a anaphylatoxin and most importantly of the C5b-9 terminal complement complex leads to the development/progression of endothelial lesions and thrombi (17, 18). Additional causal or associated factors of TMA (e.g., infection, medication, etc.) have also been identified with varying degrees of understanding of the mechanisms involved (2, 9, 19). These findings have enabled a more refined categorization of TMA subtypes based on their distinct etiological factors rather than relying solely on syndromic presentations. Consequently, the diagnostic and categorization of TMA now aspire to be grounded in physiopathological mechanisms, as outlined by the KDIGO guidelines (20).

This conceptual framework has forged pathways for the implementation of targeted therapeutic modalities, as exemplified by the utilization of plasma exchanges combined with or without anti-CD20 treatment in TMAs characterized by autoantibody involvement (e.g., cases of anti-factor H antibody-associated aHUS, ticlodipine-induced HUS, and immune-related TTP). Additionally, the deployment of C5 blockers in complement-mediated HUS (CM-HUS), and more recently, the integration of caplacizumab in the therapeutic armamentarium for TTP, attests to the precision and efficacy afforded by this paradigm. However, there are still many challenges. It is important to note that the mechanisms for most TMA cases are multifactorial and complex, with interactions between these factors contributing to the development and progression of the condition. Unraveling the intricate interplay between these factors and their roles in transitioning susceptibility to pathology could hold promise for the identification of diagnostic and follow-up markers, which are notably deficient apart from TTP, as well as for the development of additional targeted therapeutic strategies.

This review will focus on the specific context of pregnancy and the postpartum period, conditions that exemplify the inherent complexity of TMA pathophysiology and the challenges of deciphering the predominant causal factor. During pregnancy, intricate physiological adaptations occur within the vascular milieu, rendering the endothelial lining more vulnerable to disruptions in hemostasis. While the exact mechanisms are not fully elucidated for all TMA syndrome, this heightened susceptibility is attributed to a multifaceted interplay of hormonal, immunological, coagulation and hemodynamic factors, culminating in an environment conducive to dysregulated endothelial activation and subsequent TMA development, particularly in case of additional diseases or risk factors.

This review will examine the characteristics of TMA during pregnancy, refraining from detailed one-by-one descriptions and excluding discussions on management, as these have recently been comprehensively addressed (4, 5, 21). Instead, we will emphasize available tools for distinguishing pregnancy-related TMAs, with special attention to the potential contributions of histopathological analysis. Furthermore, this review aims to decipher the underlying mechanisms that promote susceptibility to TMA lesions during pregnancy and drive the transition from predisposition to pathological manifestations, with a particular focus on the influence of the "gravid endothelium."

2 Differentiating and diagnosing pregnancy related-TMAs

2.1 What TMA syndromes?

Pregnancy and the postpartum period, usually spanning up to 3 months post-delivery, are well-established significant risk factors for TMA (4, 5). TMA can manifest in diverse conditions, including exclusive pregnancy-specific patterns referred to as "pregnancy-specific TMAs" like preeclampsia (PE) and HELLP syndrome, and patterns associated with specific diseases or conditions, collectively termed "pregnancy-associated TMAs," the incidence of which rises within the pregnancy context (4, 5). Pregnancy-associated TMAs encompass TTP, CM-HUS, autoimmune disorders such as systemic lupus erythematosus and antiphospholipid syndrome (APS), notably the catastrophic APS (CAPS). Despite suggestions, it remains unconfirmed whether pregnant women with severe diffuse scleroderma have a higher incidence of renal crisis, a TMA-like disorder, compared to non-pregnant women (22, 23).

Moreover, both PE and HELLP syndrome may coincide or emerge as a result of pregnancy-associated TMAs, adding to the intricacy of diagnosis and management (24–27). Further complicating matters, TMA-like patterns may also be noted in case of obstetrical disseminated intravascular coagulation (DIC). This severe event, characterized by extensive thrombus formation (not limited to small vessels), is associated with various pregnancy complications, including acute peripartum hemorrhage, placental abruption, sepsis, PE/HELLP syndrome, retained stillbirth, amniotic fluid embolism, and acute fatty liver of pregnancy (28–30). Hence, any suspicion of TMA should prompt repeated analysis of platelet count, coagulation parameters (such as prothrombin time and fibrinogen and fibrin split products/D-dimer concentrations), while considering the pregnancy-associated intricacies of these metrics. This consideration has prompted certain researchers to advocate the use of pregnancy-specific diagnostic scoring systems for obstetrical DIC including fibrinogen concentrations, the PT difference and platelet count (31).

The biological criteria for TMA in pregnancy closely resemble those in the general population, except for the lower threshold of thrombocytopenia. Indeed, in the context of a healthy pregnancy, platelet count moderately decreases, gradually starting from the second trimester, reaching a nadir in the third trimester, while still remaining above 80×10^9/L. This benign gestational thrombocytopenia has been related to hemodilution due to increased plasma volume,

TABLE 1 Main features of pregnancy-specific TMAs and pregnancy-associated TMAs.

TMA syndromes in pregnancy	Epidemiology	Key-elements for diagnosis			Physiopathology	Treatment modalities
		Context/timing	Most discriminating clinico-biological symptoms	Impact of delivery		
PE with renal dysfunction	1.7% of all HDP 15% of all PE	From 20 weeks gestation to 4 weeks PP	new-onset HT and AKI [Δ] Ratio of sFLT1/PlGF > 85	Improvement in 48-72 h PP. Proteinuria may persist >1 year	Abnormal placentation with release of antiangiogenic markers, mediated primarily by sFlt-1 and sEng	Delivery, supportive care
HELLP	0.2–0.6% of all pregnancy	Previous PE (80% of HELLP)/T3 to early PP	Elevated liver enzymes AST or ALT > 70 IU/L	Improvement in 48-72 h PP	Few understood: Abnormal placentation (continuum with PE); Complement AP dysregulation; DIC	Delivery, supportive care
TTP	5.10^{-3} to 10^{-4}% of all pregnancy	TTP history/iTTP: T3, early PP; cTTP: from T1	ADAMTS-13 < 10% platelets < 30.10^{9}/L	No	Congenital or acquired ADAMTS-13 deficiency leading to accumulation of large VWF multimers	PEX, *caplacizumab**, IS (iTTP), plasma infusion (cTTP)
CM-HUS	4.10^{-3}% of all pregnancy	HUS history/from T3 to 3-months PP (80%)	Severe AKI Congenital or acquired abnormalities of complement system	No	Dysregulated activation of complement alternative pathway	PEX, C5 blocker
CAPS	1% of all APS/8% of all CAPS	Context of known APS or criteria +/within 4 weeks PP (80%)	± anti-cardiolipin ± anti-β2GPI antibodies ±lupus anticoagulant	No	Few understood: direct endothelial injury by aPL; dysregulated complement activation	Anticoagulation, PEX, IS
SLE	TMA in 17–24% of all lupus nephritis	Context of known SLE or LN	± Positive antinuclear ± anti-native DNA (anti-Ro/SSA)	No	Few understood: ADAMTS-13 activity deficiency (TTP-like syndrome); dysregulated complement activation; secondary APS	IS
DIC	0.03 to 0.35% of all pregnancy	Context of obstetrical complications	DIC (prolonged PT, thrombocytopenia, low fibrinogen)	No	DIC activates coagulation and triggers fibrinolysis	Cause-based treatment, supportive care

AID, auto-immune diseases; APS, antiphospholipid syndrome; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GIT, gastrointestinal tractus; HT, hypertension; HDP, Hypertensive disorders of pregnancy; IS, immunosuppression; TI, topoisomerase; PEX, plasma exchange; PlGF, placental growth factor; PP, postpartum; SLE, Systemic lupus erythematosus; SRC, Scleroderma renal crisis; T1, first trimester of pregnancy; T3, third trimester of pregnancy.

[Δ]The definition used in these reports is based on a serum creatinine 0.90 mmol/L and/or a 0.25% increase compared with baseline values.

**caplacizumab* is not approved for ongoing pregnancy (small molecular size, transplacental transfer). Nevertheless, it has shown success in pregnant women with acquired TTP, resulting in favorable outcomes (35, 36).

augmented platelet consumption in the utero-placental unit and potential heightened platelet clearance, and does not affect pregnancy outcome (32, 33). Diagnosis of pregnancy-related TMA is thus based on the following parameters: platelet count $<100 \times 10^9$/L, hemoglobin level < 10 g/dL, serum lactate dehydrogenase >1.5 times the upper limit of normal, undetectable serum haptoglobin, negative direct erythrocyte antiglobulin test, and presence of schistocytes on blood smear (5).

2.2 How do pregnancy-related TMAs differ?

The detection of MAHA in a pregnant or newly delivered patient constitutes a diagnostic and therapeutic emergency. In this context, algorithms for the management of pregnancy-related TMA are regularly updated, considering the challenge of urgent differentiation (5, 21, 34). Table 1 provides a concise overview of pregnancy-specific and -associated TMAs, focusing on the distinguishing features of different forms of TMA.

2.2.1 Context and timing

Though non-specific, the context (e.g., significant personal/family history, occurrence in conjunction with PE or postpartum hemorrhage) and the timing of TMA onset during pregnancy provide informative insights. PE, the most common cause of TMA during pregnancy, is diagnosed through the identification of new-onset hypertension after 20 weeks' gestation, concomitant with proteinuria and/or acute organ dysfunction (e.g., acute kidney injury (AKI), liver dysfunction, neurological features, hemolysis or thrombocytopenia, or fetal growth restriction) (37, 38). Although it is widely presumed that diverse subtypes of PE may exist, each likely driven by distinct pathophysiological mechanisms (39), there is no clearly delineated association between TMA features and a specific subtype of PE, particularly concerning its early or late onset. TMA is notably linked to severe forms of PE, especially the patterns associated with renal dysfunction and AKI (40–43). The distinction between PE complicated by TMA and HELLP syndrome remains subtle, especially in cases where HELLP complicates PE, and it is possible that a continuum exists between these conditions (4, 44, 45).

TTP and CM-HUS are rarer (occurring in approximately 1 in 20,000 to 200,000 pregnancies), but timely recognition is critical for improving maternal and fetal outcomes through targeted interventions. Pregnancy is a common circumstance for initial diagnosis or relapse of these diseases, unveiling ~20% of TTP and CM-HUS in childbearing-age women (46–51). While TTP is more common in the third trimester and early postpartum period (27), it can emerge as early as the first trimester, especially in case of congenital TTP (52, 53). The characterization of pregnancy-related HUS is even more limited; however, it predominantly arises during

the postpartum period, with a notable 80% of cases occurring between the immediate post-delivery phase and the fourth postpartum month in a European cohort (47), and 73% in a Spanish cohort (54).

While a history of autoimmune disease contributes to understanding causative factors, pregnancy can also serve as a trigger for these pathologies first diagnosed during pregnancy. Pregnancy-associated TMAs are observed in systemic lupus erythematosus, particularly involving anti-SSA/Ro antibodies associated with renal TMA in lupus nephritis (24, 55). Approximately 4–6% of CAPS occur during the third trimester of pregnancy or postpartum, and half of the patients had a history of APS (56–58).

2.2.2 Initial presentation

The first clinical and biological evaluation also lacks decisive factors but includes certain indicators. Blood pressure typically exceeds 140/90 mm Hg in PE/HELLP syndrome and CM-HUS cases, while it usually remains normal in TTP and is low in situations associated with obstetric severe complications and/or DIC. A recent study suggests that serum creatinine levels ≥1.9 mg/dL, LDH levels ≥1,832 units/L, or a combination of LDH levels ≥600 units/L and serum creatinine level ≥ 1.9 mg/dL can effectively differentiate CM-HUS from HELLP syndrome in the postpartum period (59). Furthermore, it has been suggested that a high LDH to AST ratio > 22 may indicate a higher likelihood of TTP rather than HELLP syndrome in a third-trimester pregnant patient suspected of having TMA (60, 61).

Renal failure may occur in all patterns of pregnancy-related TMAs, but a rapidly evolving AKI is more indicative of HUS or obstetric severe complications. Oligo-anuria may precede an increase in creatinine levels and serve as an early indicator of the extent of renal damage. Anuria may be a sign of renal cortical necrosis, a potential complication in both HUS and severe peripartum conditions, especially when linked to DIC in the latter case (62, 63). AKI is less common and relatively mild in TTP, but thrombocytopenia is more severe. The utilization of the PLASMIC score and the French score, developed to predict ADAMTS13 deficiency, can be helpful, although they have not been validated in pregnancy and may prove less discriminative in the setting of pregnancy (64). Renal involvement can be observed in both PE and HELLP syndrome. In a recent study, AKI was even more prevalent in HELLP patients than in those with preeclampsia (14.4% vs. 4.7%), likely due to higher rates of placental abruption or postpartum hemorrhage in HELLP syndrome (65). Acute tubular necrosis was the predominant finding in persistent AKI with HELLP syndrome, but coexisting TMA lesions are not uncommon (4/16 biopsies) (66). Liver function tests are likely to offer more discriminative diagnostic insights. While elevated liver enzyme levels serve as diagnostic criteria for both PE and HELLP syndrome (67, 68), they are considered a requisite for the diagnosis of HELLP syndrome according to the Mississippi classification (68). Moreover, the elevation of liver enzymes is notably more pronounced (6–8 times) in cases of HELLP syndrome compared to severe PE (69, 70).

2.2.3 Outcome

The progression over the initial 72 h can also retrospectively assist in distinguishing pregnancy-related TMA (5). Spontaneous improvement in hemolysis and thrombocytopenia following delivery is common in cases of PE or HELLP syndrome, in contrast to other pregnancy-related TMAs. A retrospective study that examined the dynamics of various biological parameters in 105 patients with immediate post-partum AKI found that changes in hemoglobin, haptoglobin, and liver enzymes were not effective discriminators. Conversely, the evolution of platelet count showed statistically significant differences between primary TMA-related AKI and other groups, including AKI associated with PE/HELLP syndrome or post-partum hemorrhage (71).

2.3 Are there any specific biomarkers?

The recommended initial workup for pregnant or postpartum women presenting with TMA is detailed in recent reviews (5, 21). This workup includes coagulation tests, quantification of ADAMTS-13 activity, investigation of autoimmune disorders (lupus anticoagulant, anticardiolipin antibodies, Beta-2-glycoprotein antibodies, antinuclear antibody) or metabolite deficiencies (vitamin B9, B12), exploration of complement levels (C3, C4, factor H, factor H autoantibody, factor I, factor B, and MCP/CD46 expression by flow cytometry), and, when available, measurement of sFLT1 and PlGF concentrations.

The most specific indicator is ADAMTS13 activity. An activity of less than 10%, definitively confirms TTP, while levels above 20% usually rule out TTP. Hence, it should be urgently assessed in all cases of pregnancy-MAHA. In contrast, there is currently a lack of specific biomarkers for distinguishing between CM-HUS, PE, or the HELLP syndrome.

2.3.1 Complement system

Quantitative levels of complement regulatory proteins lack both sensitivity and specificity to distinguish CM-HUS from other forms of pregnancy-TMA. Normal test results do not exclude the possibility of CM-HUS. During the acute phase, serum levels of C3, C5a, and sC5b-9 were within the normal range in ~50% of CM-HUS patients in two large series (72, 73). More recently, soluble C5b-9 levels were found elevated in only 21% of CM-HUS patients (74). The sensitivity of factor Ba exhibited better performance, with elevated levels found in 95% of CM-HUS cases. This study also identified urine Ba and soluble C5b-9 as potential biomarkers for CM-TMA, as both consistently display elevated levels in adults with aHUS in comparison to the highest levels observed in normal donors (74). There is currently no data available regarding these urinary biomarkers in the pregnant population.

Changes in plasma concentrations of complement proteins, including an elevation in complement activation products, have been documented in various types of pregnancy-related TMAs (27, 75, 76). Plasma levels of C3a, C4d, and sC5b-9 were 1.8-fold, 1.5-fold, and 1.2-fold higher in preeclamptic patients compared to healthy pregnant women (76). Elevated detection of complement activation products during pregnancy-related TMAs has been corroborated in other cohorts (77, 78) with some showing complement activation occurring as early as the first trimester (79).

Notably, pregnant women with elevated plasma factor Bb levels in early pregnancy had a heightened risk of later developing preeclampsia (80). In a study comparing the proteomic profiles in sera of patients who subsequently developed early-onset PE versus those with normal pregnancies, factor B was identified among the 12 proteins that showed differential expression (down-regulated with fold changes of −0.24) (81). In a similar approach, using blood samples collected immediately after the confirmation of PE diagnosis, the primary differential pathway

identified in early-onset severe preeclampsia was related to complement and coagulation (82). In the PROMISS cohort, including 487 pregnant women with SLE or APS, elevated complement activation products (Bb and sC5b-9) detected as early as 12–15 weeks into pregnancy were significantly associated with adverse pregnancy outcomes (fetal/neonatal death, preterm delivery <36 weeks because of placental insufficiency or preeclampsia and/or growth restriction <5th percentile). This association remained strong even after adjusting for demographic and clinical risk factors, with a particularly robust link observed in patients with APS (75).

Elevated plasma levels of sC5b-9 and factor Bb have also been documented in cases of severe delayed postpartum hemorrhage (83), as a potential consequence of significant endothelial stress. Other tests, less commonly used in clinical practice, have also demonstrated complement activation in other TMAs besides CM-HUS. Through the application of an *ex vivo* assay assessing C5b-9 formation on endothelial cells induced by patients' serum, enhanced C5b-9 deposition was observed in samples obtained from individuals with HELLP syndrome and preeclampsia (82, 84). This enhanced *ex vivo* C5b-9 deposition persisted up to 3 months postpartum, when patients were in clinical remission (84). Moreover, increased positivity of the modified Ham test was also noted in HELLP syndrome patients compared to those with normal pregnancies (85).

2.3.2 Angiogenesis factors

The imbalance between placenta-derived anti-angiogenic factors, notably the soluble fms-Like Tyrosine Kinase 1 (sFLT1) and soluble endoglin (sEnd), and angiogenic proteins as placental growth factor (PlGF), represents a critical event in the pathogenesis of both PE (86, 87) and HELLP syndrome (88). Thus, the measurement of these angiogenic and anti-angiogenic factors is increasingly used for the prediction or early diagnosis of these disorders. An sFlt-1/PlGF ratio above 85 before 34 weeks and 110 thereafter indicates possible PE or HELLP syndrome. In contrast, ratios below 38 suggest an alternative diagnosis with a high negative predictive value (89, 90). In a cohort of 1,117 patients presenting with PE symptoms, the median sFlt-1/PlGF level among those experiencing adverse outcomes, whether maternal (AKI, HELLP syndrome, pulmonary edema, DIC, cerebral hemorrhage, or eclampsia) or fetal, was significantly higher compared to patients without adverse outcomes (median 177 [IQR, 54–362] versus 14 [IQR, 4–62]). This suggests that incorporating the sFlt-1/PlGF ratio into the diagnostic process could aid in detecting adverse outcomes in women suspected of having preeclampsia (91).

Elevated sEnd levels were also detected in early-onset PE and HELLP syndrome, showing a strong correlation with serum levels of sFlt-1/PlGF (92, 93). Therefore, mean sEnd levels were elevated at delivery in 72 pathological pregnancies (67 pg./mL in PE and 76 pg./mL in HELLP), compared to 10 normal pregnancies (5 pg./mL), but there was no statistically significant difference between PE and HELLP. These findings pertain to HELLP syndrome in conjunction with preexisting PE, not isolated HELLP syndrome (94). In a study comparing sFlt-1/PlGF ratios among isolated PE, PE/HELLP, and isolated HELLP syndrome, the ratios significantly varied between the three groups. The highest levels were observed in the PE/HELLP group (287 [51–948]), whereas cases with isolated HELLP syndrome exhibited the lowest ratios (49 [3–405]) (95). PE/HELLP and isolated HELLP syndrome exhibit distinct angiogenic behaviors, suggesting that they are two distinct entities.

No direct association between an increase of angiogenic factors and the occurrence of pregnancy related-AKI or -TMAs has been documented. Recently, the endothelial cell-derived factor DEL-1 has been suggested as a potential diagnostic tool for HELLP syndrome in pregnancy-TMA, this factor being decreased in HELLP as compared to PE. Further prospective studies are needed for confirmation (96). Other emerging biomarkers have been identified, but no associations have been reported with PE associated with AKI or TMA (97). Few studies have investigated the levels of placental-derived angiogenic factors in other non-specific pregnancy-related TMAs. Some data have emerged in the context of TTP (98).

3 Does histopathological analysis offer insights into distinguishing pregnancy-related TMAs?

During the acute phase of a pregnancy-related TMA, conducting a biopsy of the affected organ is not a standard procedure, primarily due to the associated risk of hemorrhage. Thus, histological analysis cannot be utilized to guide the diagnosis and immediate therapeutic intervention. Nevertheless, it is valuable to summarize the limited available data to identify potential pathological variations based on the underlying cause of pregnancy-TMAs. The histological features of pregnancy-related TMAs are outlined in Table 2. Another aspect that will be discussed in this section is the potential relevance of placental histology. In cases of immediate postpartum TMAs, the placenta is accessible, and we will assess whether it can serve as a tool for distinguishing various patterns of pregnancy-related TMA.

3.1 Defining thrombotic microangiopathy: a pathologist's perspective

TMA lesions are traditionally defined as being confined to microvessels, including arterioles, capillaries, small arteries, with a diameter cutoff of <200 µm (3). When assessing capillary lesions under light microscopy, endothelial swelling is the most prevalent, followed by subendothelial widening and capillary wall thickening. Among arteriolar lesions, fibrinoid necrosis, mucoid intimal thickening and luminal thrombi are reported (Figures 1A,B). Intraluminal thrombi usually comprise fibrins and platelets, although their composition can vary according to the pattern of TMA (Table 2) (28, 44, 56, 58, 99–102). While endothelial changes are consistently evident, thrombi may not always be visualized, especially in instances of early or mild disease. Downstream from the thrombi, necrotic lesions can be observed, resulting from the ischemic process. Consequently, acute tubular necrosis lesions are frequently reported in association with renal TMA lesions in cases of HUS (103), and liver enzymes are elevated in women with HELLP syndrome due to microangiopathy with sinusoidal obstruction, leading to hepatocyte necrosis (44, 100). Immunofluorescence typically reveals fibrinogen deposits localized to thrombi and also, in mesangium, in glomerular capillary walls and in afferent arterioles. Nonspecific entrapment of IgM and complement components is usually observed in glomeruli and affected vessels in around 50% of specimens, primarily in glomerular capillary walls, mesangium, and afferent arterioles. Staining for IgG and IgA consistently yields negative results (104, 105).

None of these lesions are specific, and the histological diagnosis of TMA remains complex, mainly due to the absence of established criteria. Furthermore, the potential overlap between acute (defined as

TABLE 2 Histological features of pregnancy-specific TMAs and pregnancy-associated TMAs.

TMA syndromes in pregnancy	Target organ of TMA	Characteristics of TMA lesions	
		Morphologic changes/ location	Preponderant mechanisms of capillaries obstruction
PE	Kidney +++ (rare in others organs except in eclampsia patients (25%) or in necropsy: liver, heart, adrenal)	Endothelial vacuolization, hypertrophy of the cytoplasmic organelles. Thrombi rich in fibrin are infrequent (severe disease). No vasculitis/Primary glomerular lesions, rare in arterioles	Swelling of EC and accumulation of subendothelial electrodense deposits
HELLP	Liver +++ Kidney	Fibrin deposition with periportal hemorrhage and hepatocyte necrosis/ Sinusoids and portal tract capillaries, hepatic arteries, ± portal vein branches	Vasospasm, intraluminal fibrin and swelling of EC
TTP	CNS +++ Described in kidneys, adrenals, heart, liver, spleen. Few in GIT. Classically not in the lungs	Thrombi mainly consisted of aggregated platelets, relative absence of fibrin. No vasculitis, no fibrinoid necrosis/Diffuse in brain; Glomeruli, arterioles, and interlobular terminal arteries in kidney	Platelets thrombi rich in vWF and factor VIII Swelling of EC
CM-HUS	Kidney +++ Frequent: Colon, pancreas, myocardium, CNS Possible: adrenals, skin and lungs	EC swelling, subendothelial deposition of fibrinoid, thrombi rich in platelets with presence of fibrin. No vasculitis/ Glomerular capillaries, afferent arterioles ± intralobular arteries	Platelets thrombi Swelling of EC
CAPS	Kidney lung, CNS, heart, skin, liver, GIT	Microthrombi composed of fibrin, acute necrotizing vasculitis. In chronic cases, these lesions can be accompanied by/ small arteries and arterioles	Fibrin thrombi Intimal proliferation, EC swelling + frequent fibrous intimal hyperplasia
SLE	Kidney (except in TTP-like syndrome)	Microthrombi composed of fibrin, lesions related with LN (class IV ++ > III – V). Frequent immune complex deposits, leucocyte infiltration/ Glomeruli, small arterioles ± arteries	TTP like: platelets thrombi Other cases: fibrin thrombi
DIC	Most affected organs (descending order): kidney, liver, lung, heart, pancreas, adrenal gland, and GIT	Diffuse multiorgan bleeding, hemorrhagic necrosis, fibrin microthrombi in small vessels, and thrombi in medium and large vessels. No vasculitis	Fibrin thrombi

CNS, central nervous system; EC, endothelial cells; GIT, gastrointestinal tractus; LN, lupus nephritis; vWF, von Willebrand factor.

occurring within 2 months from the initial presentation) and chronic features further complicates the histological diagnosis. Approaches to standardized analysis are beginning to be proposed, as evidenced recently in cases of TMA in renal transplant recipients, where they incorporate histological, clinical, and biological data. In this specific context, fibrin thrombi in arterioles and/or glomerular capillaries (100%), arteriolar intimal edema/mucoid changes (95%), and mesangiolysis (82%) demonstrated the highest level of consensus among the panelists (106).

3.2 Contribution of renal pathology in pregnancy related-TMAs

The literature primarily provides renal pathology data for pregnancy-related TMAs, possibly due to the frequency of postpartum AKI and the feasibility of renal biopsies. Although there are some histological descriptions of organ involvement beyond the kidney (detailed in Table 2), most of these descriptions originate from post-mortem examinations (99–102), possibly introducing bias by representing the most severe clinical cases, which could overestimate the prevalence of diffuse TMA lesions.

The kidney serves as the principal target of TMA in conditions such as PE, HUS, and CAPS, suggesting a specific vulnerability of this organ to TMA lesion development. While various hypotheses and emerging data, notably concerning an elevated renal susceptibility to complement activation, have been proposed, our understanding of this renal vulnerability remains constrained (107, 108). As mentioned previously, renal TMA is less common in HELLP syndrome, with predominant tubular lesions reported in series associated with AKI (66). The presence of fibrin deposition in hepatic sinusoids with periportal hemorrhage and hepatocyte necrosis is well-documented in post-mortem examinations of individuals with PE associated with

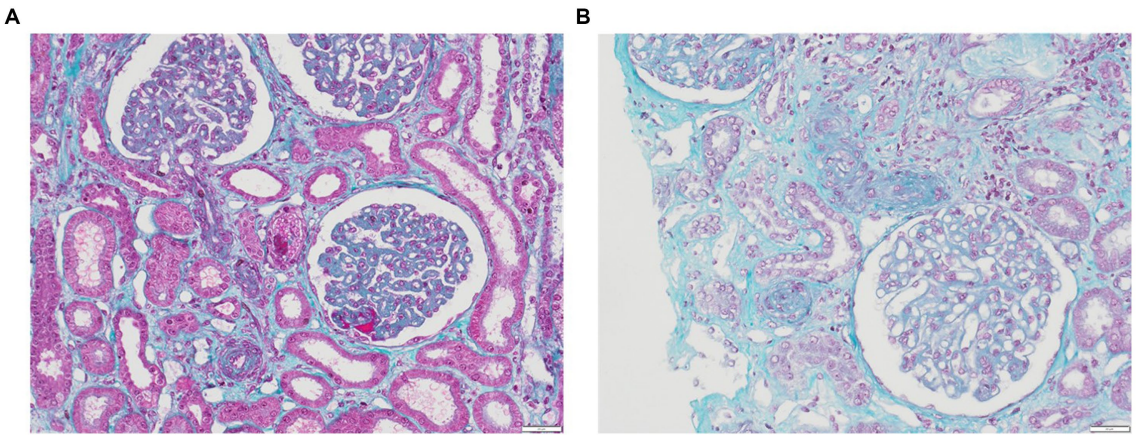

FIGURE 1
Renal TMA. **(A)** Glomeruli with GBM thickening due to subendothelial expansion, fibrin thrombi corresponding to acute TMA with swelling of EC in glomeruli and arterioles (trichrome stain, x20); **(B)** Acute TMA with arteriolar occlusions due to intimal edema and thrombosis (trichrome stain, x20).

HELLP syndrome. In contrast, these lesions are observed in liver needle biopsies in only 25% of eclampsia patients and are exceptionally rare in PE patients (44). These findings support the hypothesis of a clinical-pathological correlation between the extent of morphological changes and the severity of presentation, as well as adverse outcomes.

There is no documented correlation between the pathological features observed in renal biopsies and the underlying etiology of TMA. In non-gravid contexts, it has been suggested that the presence of isolated intimal mucoid edema without glomerular fibrin thrombi reduces the likelihood of CM-TMA in the setting of TMA associated with hypertensive emergencies (109); however, this remains a subject of controversy (103, 110). Additionally, some data suggest that renal arteriolar involvement is more widespread in aHUS and TTP, while glomerular involvement appears to be more prevalent in STEC-HUS (105). Nonetheless, it is important to note that the coexistence of these lesions is relatively common and has not been definitively linked to a specific underlying pathophysiological mechanism.

In gravid contexts, PE results in notable glomerular changes, termed "endotheliosis", characterized by glomerular enlargement and reduced capillary blood flow due to endothelial swelling. This substantial reduction in endothelial cell fenestrations has been also observed in others capillary networks, including, choroid plexus, and hepatic sinusoids (104). This phenomenon is closely associated with sFlt-1, which reduces circulation of vascular endothelial growth factor (VEGF), impacting placentation and endothelial cell function, especially in fenestrated endothelium (40, 111). However, this specific lesion is not unique to PE and has been observed in various other conditions, including placental abruption, parvovirus B19 infection, COVID-19, POEMS syndrome, and even uncomplicated pregnancies (112). The presence of arteriolar or arterial lesions should prompt consideration of underlying conditions, like hypertensive vasculopathy, or alternative diagnoses, such as CM-HUS (105). *Post partum* biopsies can provide information about healing process or progression. For example, in PE, the endothelial swelling, IgM and fibrin deposits typically resolve within 2 weeks of delivery. The subendothelial irregularities in the glomerular basement membrane may persist for months (105). Renal biopsies can also help in lupus patients due to potential clinical similarities between LN flares and PE (113, 114). In APL cases, the presence of acute TMA in glomerular and arteriolar lesions alongside chronic vascular changes aids in diagnosis (115).

3.3 Contribution of placenta pathology in pregnancy related-TMAs

In 2016, the Amsterdam Placental Workshop Group provided a consensus statement on placental lesion sampling and definitions (116). Presently, the accepted term is "maternal vascular malperfusion" (MVM), which encompasses a range of placental pathologic findings, including macroscopic features (fetal and placenta weights, infarcts, retroplacental hemorrhage) and microscopic findings (maternal decidual arteriopathy, distal villous hypoplasia, and accelerated villous maturation), Figures 2A–D. Maternal decidual arteriopathy comprises acute atherosis of decidual arteries, mural hypertrophy of arterioles within the placental membranes, abnormal persistence of mural smooth muscle within arteries at the placental basal plate, and the persistence of intramural endovascular trophoblast. The specific causes of MVM remain unclear, with potential factors including immunological and inflammatory elements, as well as pre-existing maternal susceptibility to microvascular dysfunction (117). MVM is described in PE and HELLP syndrome, and it also appears in instances of second-trimester spontaneous abortion, fetal demise, abruptio placentae, small for gestational age, preterm labor, preterm prelabor rupture of membranes, and maternal autoimmune diseases (117, 118).

Most of the placental histological data is centered around PE. Distinct lesions are predominant according the timing of PE during gestation, suggesting potential differences in underlying pathophysiological factors. Early-onset PE is characterized by more severe maternal MVM lesions, both macroscopic and microscopic (119). A positive correlation has been reported between the presence of decidual vasculopathy in vessels and adverse maternal and fetal outcomes (i.e., higher diastolic blood pressure, increased urinary protein levels, shorter gestational age, and lower birth

weight) (112). In contrast, late-onset PE placentas show normal or excessive growth, milder microscopic features like accelerated villous maturation and hypertrophic arteriopathy (119), and significantly fewer cases of failed spiral artery remodeling, acute decidual arteriopathy, and infarcts compared to early-onset PE placentas (120). Mural hypertrophy of placental arterioles was linked to the development of stage 2 hypertension during follow-up (121), emphasizing the impact of preexisting cardiovascular risk factors (e.g., diabetes, chronic hypertension, obesity) in late-onset PE pregnancies. A limited number of studies have compared placental histopathological features in severe PE cases with and without HELLP syndrome (122–124). Notably, Weiner et al., in their analysis of 287 placentas, reported that vascular and villous lesions indicative of maternal malperfusion were independently linked to HELLP syndrome (124).

Information regarding other pregnancy-related TMAs is limited. In a cohort of 91 pregnancies involving 47 TTP-patients, Scully et al. examined placental histology from 15 deliveries. Interestingly, untreated congenital cases exhibited distal villous hypoplasia, widespread placental ischemia with infarcts of various ages, and acute atherosis, while treated congenital cases showed normal placental features (27). Similar results were reported in a recent study involving 28 placentas from congenital TTP cases (125), emphasizing also the direct role of ADAMTS-13 deficiency in placental findings. Moreover, some research groups have examined placental deposits of complement activation products, which have been identified in conditions such as PE, HELLP syndrome, lupus, and APS, without conclusive discriminatory features among these pathologies at present (126–128).

The data is still quite limited, and a more systematic analysis of placentas from patients being treated for TMAs could provide valuable insights, particularly when combined with the recently suggested use of electron microscopy to study placental pathologies during pregnancy (129).

4 Endothelium in pregnancy

Endothelial cells play a pivotal role in the pathophysiology of TMA (18, 130), implying that the specific occurrence of these events during pregnancy and the postpartum period is, to some extent, associated with microvascular alterations characteristic of pregnancy. Investigating the intricate mechanisms contributing to this increased susceptibility is imperative for enhancing our understanding of the pathogenesis of pregnancy-associated TMA and, consequently, for developing effective preventive and therapeutic approaches to mitigate its consequences.

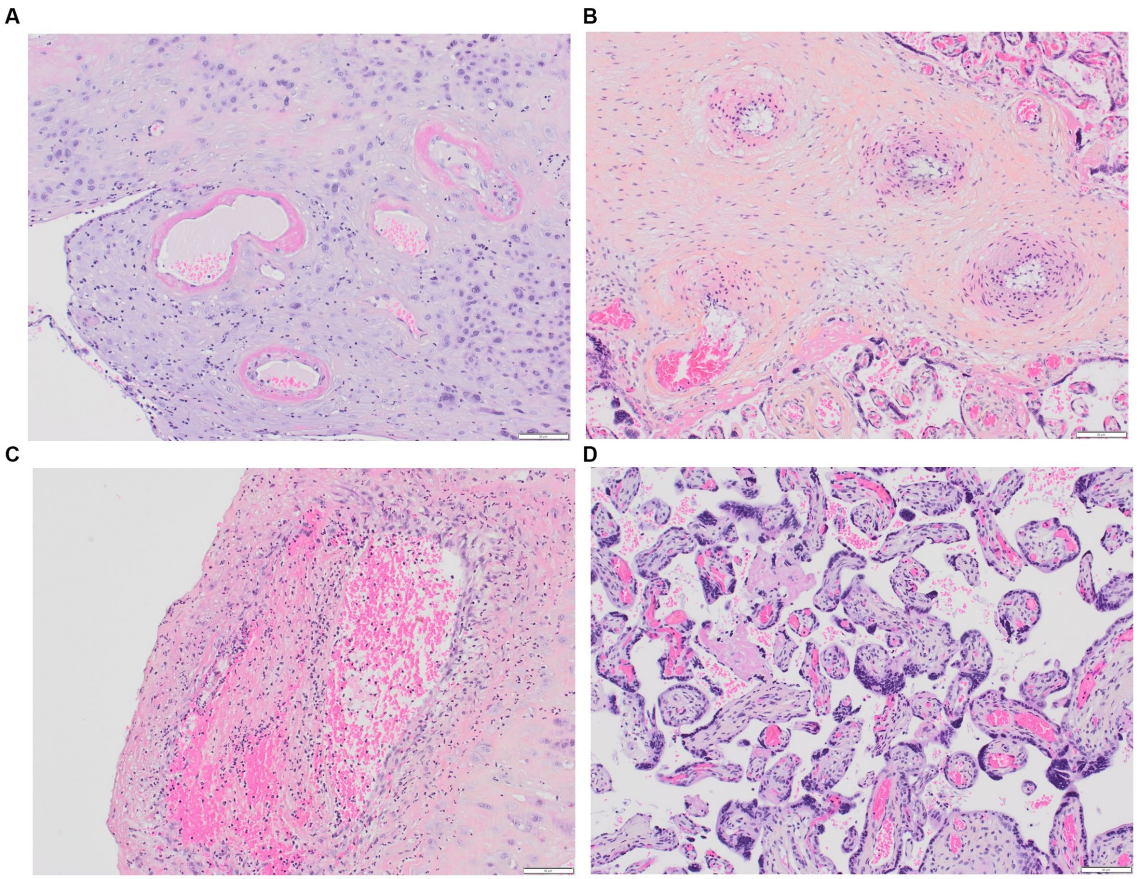

FIGURE 2
Placental MVM. **(A)** Maternal spiral arteries with acute atherosis and narrowed arteriolar lumens due to fibrinoid necrosis; **(B)** Recent maternal spiral artery thrombosis; **(C)** A section of free membranes showing decidual hypertrophic arteriopathy and residual smooth muscle; **(D)** This 23-week-gestation placenta exhibits accelerated villous maturation with small hypermature villi, increased syncytial knots, and intervillous fibrin.

4.1 Microvasculature heterogeneity, specificities of premenopausal women's endothelium

In contrast to large vessels with their three tunics, capillaries, the primary site for TMA with arterioles, consist of a thin endothelium, basement membrane, subendothelial connective tissue, and a few pericytes, with diameters ranging from 4 to 10 µm and an endothelial wall of about 0.5 µm. They operate at low intravascular pressures (0–25 mmHg, up to 50 mmHg in kidney glomeruli) and slow flow rates (<1 mm/s), facilitating efficient plasma and tissue exchanges (131).

The phenotype of microvascular endothelial cells varies from one organ to another to fulfill specific functions and respond to the different needs of various tissues (132–134). As early as 2003, the spatial heterogeneity among endothelial cells was supported by distinct transcriptomic profiles (135) and differential expression of protein surface markers (136, 137). Most recently, the advent of single-cell RNA sequencing technologies has revolutionized our understanding of endothelial diversity, revealing significant heterogeneity within the confines of a single organ or a blood vessel type, in both animal models and humans (138–140). The endothelium is a dynamic tissue with plasticity properties, making it also subject to temporal heterogeneity (141). Endothelial cells react and respond to various biomechanical and biochemical stimuli that differ between organs, such as hormones, as discussed below, contributing to gender-driven endothelial heterogeneity. Additionally, site-specific epigenetic modifications play a significant role in generating endothelial heterogeneity (142, 143).

Significant sex-based differences in endothelial cells have been documented (144–146), emphasizing the heightened endothelial protection in women of childbearing age, which is associated with a lower cardiovascular risk compared to males within the corresponding age group or postmenopausal women. The studies have primarily focused on exploring the involvement and interactions of circulating sex steroid hormones, their receptors, and sex steroid-independent mechanisms. Notably, estrogen receptor E2-mediated vascular benefits encompass inhibition of lipoprotein oxidation, reduction of atherosclerotic lesions, modulation of blood coagulation, inhibition of collagen accumulation in vessels, and adjustment of vascular tone (144, 147). Studies conducted in animal models involving ovariectomy and hormone supplementation therapy have contributed to the understanding of sex hormone-dependent effects, particularly those of estrogen, on endothelial function, including changes in endothelium-dependent dilation, and the respective roles of nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF). These investigations have predominantly concentrated on medium and large arterial vessels (e.g., aorta, carotid, mesenteric arteries) (148–150). For instance, small arteries from omental tissue in premenopausal women demonstrate increased sensitivity to bradykinin (151); premenopausal women have shown increased levels of progenitor ECs in comparison to men, alongside enhanced NO production essential for vascular homeostasis (152); the profile of endothelial-derived factors in childbearing women is associated with a less pronounced pro-oxidant environment, characterized by reduced levels of vascular reactive oxygen species and other oxidative stress markers (153, 154). Additionally, levels of endothelin-1, which play a role in cardiovascular and renal pathology, are generally higher in men than in women (155); plasma levels of VEGF have been found to be elevated in males compared to females and have shown a positive association with the advancement of atherosclerosis (156).

Collectively, these studies underscore the heightened endothelial protection observed in women of childbearing age, elucidating their diminished susceptibility to cardiovascular events. They also illustrate the importance of incorporating both genders into experimental protocols, also including *ex vivo* and *in vitro* studies. Nonetheless, questions and controversies debates, especially concerning the strict favorable vascular impacts of estrogen and its E2 receptor (157).

Furthermore, as previously emphasized, research on vascular sex differences has predominantly focused on macrovessels, while data on the microcirculation are limited (158). Some studies have demonstrated sex differences in the permeability or inflammation-induced disruption of the blood–brain barrier (159, 160), and circulating levels of products derived from the glycocalyx (e.g., syndecan-1, heparan sulfate, and hyaluronan) (161, 162). More answers may potentially come from single-cell RNA-seq analyses. Already, a single-cell RNA-seq analysis of mouse endothelial cells has further confirmed the presence of sex-specific differences in endothelial cells across various mouse tissues. Paik et al. found that markers of tissue-specific endothelial cells, enriched pathways, and endothelial subpopulations differed between male and female mice, particularly in the brain, heart, and lung. The Lars2 gene, encoding mitochondrial leucyl-tRNA synthetase, was notably more enriched in male than in female endothelial cells (163). While this comprehensive approach has yet to be undertaken in pregnant individuals or TMA patients, specific endothelial attributes associated with pregnancy have been recognized and may contribute to the heightened thrombogenicity of the gravid endothelium.

4.2 Specificities of the gravid endothelium

Vascular adaptations during pregnancy, even in healthy pregnancies, are associated with significant modifications in the characteristics and function of endothelial cells. For instance, glomerular capillary endotheliosis, originally associated to PE, has been detected in patients with gestational hypertension without proteinuria (> 1+ in 8/8, and > 2+ in 4/8 cases) and, as cited above, in women with healthy pregnancies (>1+ in 5/12, and > 2+ in 1/5 cases) based on renal biopsies conducted between 27 and 39 weeks of gestation (112). These findings suggest a continuum of endothelial changes that range from normal pregnancy to the development of PE. Multiple vascular changes take place during pregnancy, potentially heightening the vulnerability to thrombosis, and our primary focus in this context is on the specific factors involved in the pathophysiology of TMAs (Figure 3).

4.2.1 Complement activation

Complement activation via the three pathways - classical, lectin, and alternative - has been observed during normal pregnancy. It has been suggested that this activation is physiological, serving as a regulatory mechanism to facilitate the clearance of fetal placental debris and protect both the mother and developing fetus from pathogens (4, 82, 164, 165). The complement system plays a significant role in various stages of pregnancy, such as implantation, placentation, fetal development, and parturition (164, 166). This likely contributes

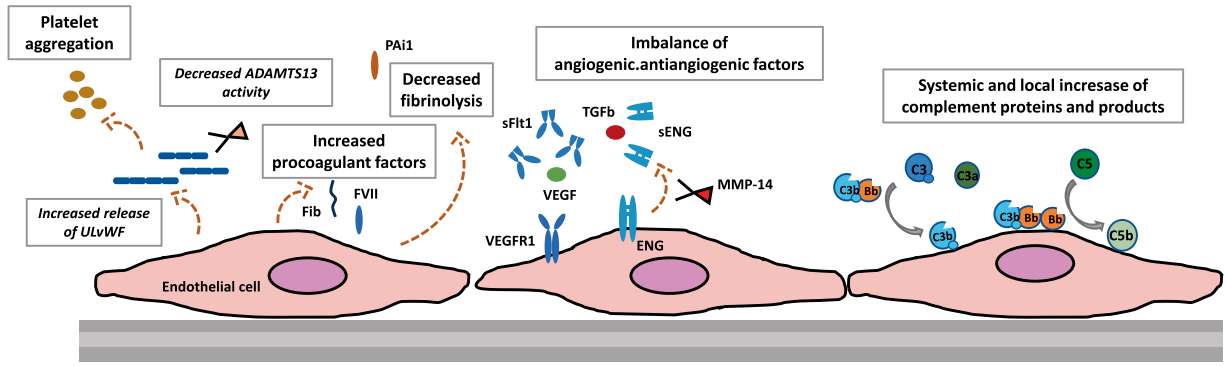

FIGURE 3
Gravid endothelium—pregnancy-induced adaptations. This figure highlights pregnancy-induced changes in the endothelium, emphasizing adaptations in complement activation, prothrombotic profile, and angiogenic state. These modifications underscore the complexity of the gravid endothelium, crucial for successful pregnancy outcomes.

to the variations in plasma levels and placental deposition/expression of complement components during this period.

Derzsy et al. reported significantly elevated levels of various complement proteins and activation products, including C4d (~2.75-fold), C3a (~8.8-fold), soluble C5b9 (~1.8-fold), C3 (~1.2-fold), and C9 (~1.3-fold), while C1-inhibitor concentrations were significantly lower (~0.8-fold), in healthy pregnant women sampled between 36 to 67 weeks of gestation ($n = 60$) compared with healthy non-pregnant women ($n = 59$). There was no significant modification in the alternative pathway fragment Bb (76). More recently, a study analyzing sequentially collected 733 plasma samples from 362 women with a normal pregnancy and 65 samples from non-pregnant women confirmed an increase in the levels of C3, C4, factor B, and mannose binding lectin, while the levels of C1q remained unchanged. The levels of C3a, and to a lesser extent, C5a, remained elevated throughout pregnancy, while soluble C5b-9 levels remained unchanged, suggesting that excessive complement activation does not progress beyond the C3 and C5 levels in normal pregnancy (79). Interestingly, the levels of factor H (FH), the principal regulator of the alternative pathway, whose default has been shown to be associated with a risk of developing CM-HUS, increased significantly, gradually up to 28 weeks of gestational age, and then remained stable (76, 79).

Several studies have shed light on the presence of complement activation product deposits and the expression of regulatory proteins within placentas from healthy pregnancy (164, 166). Notably, deposits of C3d and C9 have been reported in trophoblast basement membranes, along with staining for C1q, C3d, C4, C6, and C9 in normal uteroplacental spiral arteries (167–169). The presence of C5b-9 has also been documented in the normal term placenta, specifically within the decidua and the stroma of chorionic villi (170). These findings suggest that the placenta functions as a localized site of complement activation. Interestingly, it has been observed that human trophoblasts synthesize complement molecules such as C4, C3, and the late complement components (C6, C7, C8, C9) (171). Considering this complement activity, a notable presence of complement regulatory proteins has been observed within trophoblasts. Lokki et al. identified the presence of C4BP in syncytial bodies or regions with damaged syncytiotrophoblasts, as well as FH within tissue stroma (168), and trophoblasts have been recognized for their synthesis of FH (172). Some studies have also reported an elevated expression of membrane complement proteins (CD46, CD55, CD59) on extravillous trophoblasts during healthy pregnancies (164, 169).

These findings underscore the substantial involvement of complement activity in placental homeostasis. It is noteworthy that the current knowledge does not offer a concise rationale for the increased susceptibility to CM-HUS in the postpartum period. One hypothesis suggests that fetal-derived complement regulatory factors might exert a compensatory effect that diminishes after childbirth.

4.2.2 Prothrombotic profile

The heightened thrombotic risk during pregnancy is well-documented. When compared to non-pregnant women, there is a three to fourfold increase in the risk of arterial thromboembolism [e.g., stroke (173), myocardial infarction (174)], and a four to fivefold increase in the risk of venous thromboembolism (175). After childbirth, the risk of venous thromboembolism becomes even more significant, a twenty-fold increase. The primary cause of the heightened risk is hypercoagulability, which is believed to have developed as an evolutionary adaptation to safeguard women against the bleeding complications associated with miscarriage and childbirth. Hemostatic changes that lead to this hypercoagulable state during pregnancy encompass all facets of hemostasis, including primary hemostasis, coagulation, and fibrinolytic capacity. Numerous factors, including circulating factors, platelets, endothelial cells, and others, play critical roles in this process, particularly at the maternal-fetal interface. The Table 3 reports the per-pregnancy dynamic of important endothelial-derived factors.

An increase in platelet aggregation is documented during a healthy pregnancy (176, 177), although it is not consistently observed in all studies (178). These potential alterations in platelet aggregation coincide with a substantial two- to fourfold increase in von Willebrand factor levels, originating from endothelial Weibel-Palade bodies (179, 180). Concurrently, ADAMTS13 activity exhibits a noteworthy decline of 20–50%, persisting from the second trimester through delivery and into the early postpartum period (180, 181). This decline in ADAMTS13 activity is attributed to consumption resulting from elevated levels of von Willebrand factor and hormonal fluctuations, although it typically remains above 20% (179, 181). These physiological changes can exacerbate the low ADAMTS13 levels in individuals with congenital TTP to a critical level resulting in overt presentation of

TABLE 3 Main derived-endothelial factors involved in hemostasis and their dynamics during normal pregnancy.

	Prothrombotic factors	Function	Dynamic in healthy pregnancy	Antithrombotic factors	Function	Dynamic in healthy pregnancy
Primary hemostasis	*vWF*	Platelets aggregation, FVIII transport	↑	*ADAMTS-13*	Cleavage of vWF multimers	↓
	TXA2	Platelets aggregation, vasoconstriction	↓	*NO*	Inhibition of platelets activation, vasorelaxation, anti-inflammatory properties	↑
	PAF	Platelets aggregation, vasoconstriction	No data	*PGI2*	Inhibition of platelets activation, vasorelaxation	↑
Coagulation	*Tissue factor*	Binding to FVII, initiation of blood coagulation (extrinsic pathway)	↑	*TPFI/TFPI-2*	Inhibition of coagulation by binding to TF, factors Va, and Xa	→/↑
	PARs	Thrombin receptors	No data	*TM*	Formation of a thrombin complex enhancing protein C affinity	↑
				Heparan-sulfate	Binding to antithrombin	Few data, glycocalyx thinning
Fibrinolysis	*PAI-1*	Fibrinolysis inhibitor	↑	*t-PA*	Fibrinolysis activator	↑

NO, nitric oxide; PAI-1, plasminogen activator inhibitor type 1; PAF, platelet activating factor; PARs, protease activated receptor; PGI2, prostacyclin I2; t-PA, tissue plasminogen activator; TFPI, tissue factor pathway inhibitor; TM, thrombomodulin; TXA2, thromboxane A2; vWF, von Willebrand factor.

TTP. Other endothelial-derived factors contribute to the maintenance of hemostatic balance during a normal pregnancy. In comparison to non-pregnant controls, the ratio of prostacyclin (PGI2) to thromboxane A2 (TXA2) metabolites gradually increases from the second trimester onwards (182), and NO concentrations exhibit a significant elevation throughout pregnancy (183). It is noteworthy that these patterns diverge in the context of PE, particularly in severe cases (183–185).

Significant changes in blood coagulation factors during a healthy pregnancy are also documented and primarily attributed to hormonal fluctuations, particularly the increasing levels of estrogen as pregnancy progresses (147). Notably, plasmatic levels of factors VII, VIII, X, XII, and fibrinogen increase substantially, especially in the third trimester (33, 186–188). Furthermore, while endothelial cells typically express minimal tissue factor (TF) under physiological conditions (189, 190), maternal decidua and the placental syncytiotrophoblasts exhibit elevated levels of TF with a strong affinity for factor VII, initiating thrombin generation. During extravascular trophoblast invasion of the decidua, thrombin produced via TF expressed by decidual cells serves to prevent hemorrhage during the initial capillary breach and subsequent invasion and remodeling of spiral arteries and arterioles (191). While free protein S levels decrease by approximately 55%, other anticoagulant factors, such as thrombomodulin and tissue factor pathway inhibitor-2, exhibit increased expression in the placental vascular endothelium with advancing gestational age (33, 192, 193).

Regarding fibrinolysis, while levels of tissue plasminogen activator also rise during a normal pregnancy (194), concomitantly elevated levels of plasminogen activator inhibitor-1 (PAI-1) from endothelial cells and plasminogen activator inhibitor-2 (PAI-2) from the placenta, on the contrary, lead to a decrease in fibrinolytic capacity (188).

All these changes in hemostasis underscore the critical importance of achieving a precise balance for normal placental function and favorable pregnancy outcomes. While intricacies exist even at the level of the maternal-fetal-placental vascular beds (for instance, the TF/TFPI ratio is markedly elevated in syncytiotrophoblasts, whereas it is lower in HUVECs, implying a more procoagulant tendency of syncytiotrophoblasts) (193), the progression of human gestation involves a transition of endothelial cells toward a procoagulant state. This prothrombotic profile may endure until approximately 8 weeks postpartum.

4.2.3 Others actors

Additional modifications, which have the potential to impact susceptibility to TMA, are concomitant with a normal pregnancy. Placental microvascular endothelial cells undergo noteworthy fluctuations in their angiogenic state throughout pregnancy. The first and second trimesters are characterized by a predominance of angiogenic activity, facilitating rapid placental expansion. In contrast, as pregnancy progresses toward full term, there is a transition to a prevailing angiostatic condition, coinciding with placental growth arrest. Placental cells inherently possess the capacity for angiogenic regulation, engage in intricate interactions with perivascular cells, and demonstrate the ability to swiftly adapt and remodel in accordance with the specific demands of pregnancy. A longitudinal study reported the dynamic patterns of different angiogenesis factors of interest. sFlt1 levels remained relatively stable until weeks 29–30, after which they significantly increased, peaking at week 40 with a notable weekly increase of 643 pg./mL. In contrast, PlGF levels increased gradually up to weeks 29–30, followed by a steady decline of 14 pg./mL per week until week 40. The sFlt1:PlGF ratio decreased from weeks 9–12, remained consistently low from weeks 19–20 to 37–38, and then increased again toward weeks 39–40. Additionally, VEGF-A was detectable in only 8% of the samples during pregnancy but increased to 64% in the postpartum period (195). Therefore, the effective functioning of placental microvascular endothelial cells holds paramount importance for the successful progression of pregnancy (196).

Even more limited data exist regarding changes in the glycocalyx throughout pregnancy. Changes in glycocalyx thickness, along with reported shedding, have been documented and could potentially contribute to observed alterations in fluid balance during pregnancy (158, 197). Limited data is available regarding

changes in glycocalyx composition, especially concerning heparan sulfates. This aspect is of particular interest due to their anticoagulant action through binding to antithrombin III and their role as inhibitors of complement activation via FH binding. Interestingly, a recent transcriptomic analysis using microarrays revealed that among the 34 expressed proteoglycans in the placenta, syndecan-1 (SDC1) production is significantly the highest, and SDC1 is the most upregulated gene during the differentiation of trophoblast into syncytiotrophoblast (198). This suggests the existence of a pregnancy-specific glycocalyx that distinguishes itself from the glycocalyx of both adult and fetal endothelium and whose balance could contribute to the successful progression of pregnancy (199).

4.3 Overcoming endothelial regulation: from physiology to pathology in pregnancy

Therefore, the "gravid endothelium" is inherently more prone to activation, contributing to the favorable progression of pregnancy. The transition to a pathological state with the development of TMA lesions requires additional factors, as described in the concept of a "multiple-hits disease" (18, 200). These additional factors can be either genetic and/or acquired, and act to heighten susceptibility and/or induce endothelial stress. Their cumulative impact (with varying significance depending on the specific factor) will ultimately lead to an exceeding of the regulatory capacities involved in endothelial homeostasis.

Genome-wide association studies have not been conducted in women experiencing pregnancy-related TMAs. Such investigations have primarily been carried out in the context of PE, often without a clear differentiation of patient phenotypes. While more than fifty candidate genes have been associated with PE, no universally accepted susceptibility genes have emerged (41, 201, 202), possibly due to the polygenic nature of PE. In a study of 244,564 related women, Cnattingius et al. determined that PE has a heritability of over 50%, with 35% linked to maternal genetics, 20% to fetal genetics, and 13% to a 'couple' effect (203). Mutations and/or variants have been reported in the context of PE or HELLP syndrome, related to genes involved in angiogenesis, blood pressure regulation, coagulation, or complement activation (40, 202, 204, 205), underscoring the close pathophysiological connections with TMA. For instance, mutations or variations in Factor H, C3, or CD46 have been identified in cohorts of women with PE (206, 207). These findings indicate that a multifaceted interplay of susceptibility factors is likely, potentially resulting in distinct patterns of preeclampsia. Supporting this concept, Schuster et al. documented groups of patients exhibiting common gene and protein networks linked to severe preeclampsia (208).

The extensive range of environmental factors capable of increasing endothelial susceptibility cannot all be comprehensively reviewed here. In an illustrative example, placental hypoxia is worth mentioning to illustrate the interplay of factors involved in TMA. The placenta is a physiological immunological niche, especially under the influence of physiological hypoxia that contributes to immunological regulation (209). However, placental hypoxia has others consequences as the generation of oxidative stress and trophoblastic secretion of sFLT-1, promoting antiangiogenic activities and endothelial injury (210), alteration in placental metabolism favoring interruption of the glycocalyx susceptible to contribute to localized complement activation (199, 211, 212). Thus, a pathological placental hypoxia, as observed in the case of PE, represents a risk situation for TMA.

5 Conclusion

The susceptibility of the gravid endothelium to instigate TMA represents a pivotal facet within the realm of vascular pathology. During pregnancy, intricate physiological adaptations occur within the vascular milieu, rendering the endothelial lining more vulnerable to disruptions in hemostasis. This heightened susceptibility is attributed to a multifaceted interplay of hormonal, immunological, and hemodynamic factors, culminating in an environment conducive to dysregulated endothelial activation and subsequent TMA development. Elucidating the nuanced mechanisms underlying this heightened propensity is essential for advancing our comprehension of pregnancy-associated TMA pathogenesis and, subsequently, devising effective preventive and therapeutic strategies to mitigate its impact.

Author contributions

MF: Writing – original draft, Writing – review & editing. VG: Writing – original draft, Writing – review & editing. MS: Writing – original draft, Writing – review & editing. FP: Writing – review & editing. FF: Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Gender equity in hemophilia: need for healthcare, familial, and societal advocacy

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Hemophilia is a rare bleeding disorder caused by a genetic defect on chromosome X. It is inherited as an X-linked trait, and hence, it is more frequently diagnosed in males, whereas women have been traditionally considered only as carriers of the disease. However, the role of women in families of patients with hemophilia is pivotal. As mothers, sisters, daughters, and female partners of patients with hemophilia, they play a central role in the management of the patient, considering healthcare, social, and familial aspects, but they might be affected by the disease as well, particularly in regions where consanguinity is frequent. This paper aims to explore the involvement of women in hemophilia, including their carrier status, bleeding symptoms, treatment challenges, and psychosocial impact not only related to male patients, but also as patients affected with hemophilia themselves. We advocate health equity, equal access to healthcare for men and women with hemophilia and dedicated resources to improve the unique needs of the women dealing with hemophilia, ultimately leading to improved care and quality of life.

KEYWORDS

hemophilia, women, bleeding symptoms, carrier, treatment challenges, psychosocial impact, quality of life, gender-specific research

1 Introduction

Hemophilia is an X-linked recessive disorder caused by the deficiency in coagulation factor VIII (FVIII; hemophilia A) or IX (FIX; hemophilia B) (1). If left untreated, patients with severe hemophilia (i.e., FVIII or FIX <1 U/dL), may experience spontaneous musculoskeletal bleeding, which account for 80% of overall bleeding events, although intracranial hemorrhage and other life-threatening bleeding events may also occur (2). Recurrent joint bleeding (hemarthrosis) occurs most frequently in ankles, elbows, and knees and if the patient is not adequately treated, this could lead to irreversible joint damage (hemophilic arthropathy). Over the last decades, the scenario of hemophilia treatment has changed dramatically, thanks to the availability of novel replacement and non-replacement drugs for prophylaxis (2).

The value of prophylaxis in decreasing the number of bleeds has been already established, although the optimal trough factor level for personalized management of patients with hemophilia is still debated. In the past, a trough level of FVIII >1% was thought to be enough to protect joints from spontaneous bleeding but Manco-Johnson et al. (3) demonstrated that patients may still experience bleeding despite prophylaxis aiming to reach this trough level.

Current recommendations by the international hemophilia management guidelines, suggest a target minimum trough level of 3–5% to preserve joint function (4, 5). Nevertheless, den Uijl et al. (6) showed that patients with levels ≥10% still had, albeit very low, a risk of spontaneous joint bleeding, which was absent at levels ≥15%, findings that were recently confirmed by our group in a cohort of mild hemophilia A patients (7).

The improved availability of drugs and care pathways has increased awareness on the need of treatment also for women and girls with hemophilia (8). In X-linked recessive disorders, as males have only one copy of X-chromosome, the presence of an altered allele causes a clinically evident disease whereas females, called carriers, have a heterozygous state and a 50% chance to transmit the defective gene to a male or a female child (5). It has been described that in the family of each man with hemophilia, up to 5 potential carriers can be found and 1.6 of them are actually carriers (9). Like many X-linked disorders, the heterozygous state does not necessarily imply a disease-free state. Indeed, carriers of hemophilia may experience bleeding, especially when they have low factor levels. In the 2021 World Federation of Hemophilia (WFH) Report on the Annual Global Survey, on a total number of around 223.000 patients, the proportion of female patients was 3% for hemophilia A and 6% for hemophilia B, worldwide.[1] In a comprehensive study involving hemophilia patients from 139 treatment centers across the United States, it was reported that approximately 0.5% of female patients had severe hemophilia, whereas 1.4 and 18% were diagnosed as having moderate and mild hemophilia, respectively (10). In addition, some carriers experience bleeding symptoms even in the presence of normal factor levels, as defined by international standards FVIII/FIX >0.40 IU/mL (8).

Despite a bleeding tendency, diagnosis and management of hemophilia carriers or female patients with hemophilia frequently remain suboptimal and personalized comprehensive care plans are rarely proposed (11). Although the gender disparity is being increasingly recognized within the bleeding disorders community, comprehensive data on women with hemophilia are still limited since they are underrepresented in surveillance databases. Most clinical trials enroll patients with severe disease, predominantly males, while moderate and mild hemophilia patients are often excluded (12). As a consequence, widespread evidence gaps exist on the efficacy and safety of different treatment options for women, as most therapies have been developed and studied primarily in a male population.

In addition, female relatives to male patients with hemophilia play an important role in their lives, being often involved in the management of the patients as caregivers. Furthermore, being carriers themselves, they must face reproductive choices, often feeling guilty, with the awareness that they might pass on the condition. This emotional burden is influenced by various factors, including personal beliefs and societal attitudes surrounding genetic conditions. Female partners of patients with hemophilia are often the patient's caregiver but are rarely involved in dedicated programs of education on the real bleeding risk of their partners during everyday activities, including sexuality and awareness on reproductive choices.

In this brief review, we define the concepts of sex and gender, gender equality and equity, identify possible causes of health inequity for women with hemophilia and carriers of the altered allele, highlight the role of female relatives and partners to male patients with hemophilia and discuss possible solutions to promote health gender equity in hemophilia.

2 Defining sex, gender and gender equality

Sex and gender are conceptually different terms (Table 1). Sex is a multidimensional construct including a cluster of anatomical and physiological traits, such as external genitals, secondary sex characteristics, gonads, chromosomes, and hormones. These elements collectively distinguish organisms and contribute to the complex spectrum of biological diversity. It is typically assigned at birth by medical professionals as either male or female, based on the visual inspection of external genitals (13). However, in some cases sex traits may not fit with sex assigned at birth, as in the case of intersex individuals, who are born with biological sex characteristics, including chromosome patterns, gonads or genitals that do not fit typical binary notions of male or female bodies. In other cases, sex traits may not correspond to a single sex and may even change over time. For example, intersex individuals, which do not align with typical binary notions of male or female bodies. Therefore, sex is a complex concept that does not fit typical binary notions of male and female bodies.

Gender is a multidimensional construct that links a person's individual sense of self to cultural expectations about social status, characteristics, and behavior, which may differ across social contexts and in different countries (13). Cisgender is the term that defines people whose gender identity corresponds to the sex assigned at birth. Gender dysphoria is the feeling of discomfort that occurs when gender identity does not correspond to biological sex, whereas the term transgender describes individuals with a gender identity that does not match the sex they were assigned at birth. Transgender individuals are administered sex hormones and other hormonal medications with the purpose of aligning their secondary sexual characteristics with their gender identity (Table 1). There is limited data on transgender patients with hemophilia and the effects of gender-affirming treatments in these patients. Increased awareness is crucial for fostering inclusivity and personalized management approaches for these individuals (14). This paper focuses on women and girls affected by hemophilia, recognizing that the term may not encompass all people with the ability to menstruate.

Gender equality is the concept that envisages equal consideration for women, men and gender-diverse people. In turn, gender equity is the process to achieve gender equality, by recognizing that women and gender-diverse people are not in the same starting position as men due to historical, cultural and social disadvantages (Table 1). Gender equality requires equal enjoyment by women and men of socially valued goods, opportunities, resources, and rewards. In hemophilia, possible gender inequalities that disadvantage women could be the reduced access to healthcare for carriers both with and without symptoms, with consequent limited data on these cases, reduced access to genetic counseling and the psychosocial challenges arising from the sense of guilt experienced by individuals who are carriers of a genetic defect, particularly in developing countries (15).

Recently, the European Association for Hemophilia and Allied Disorders (EAHAD) and the European Hemophilia Consortium

1 https://www1.wfh.org/publications/files/pdf-2324.pdf

TABLE 1 Definitions regarding gender equality in health.

Term	Definition
Cisgender	People whose gender identity corresponds to the sex they were assigned at birth
Cross-sex hormone therapy	Also named gender-affirming hormone therapy, involves the administration of sex hormones or other hormone-based medications in transgender people, with the aim to align their secondary sexual features with their gender identity
Gender	The social differences between men and women, which depend on what a society believes are the roles, duties, responsibilities, rights, socially acceptable behavior, opportunities and status of women and men in relation to each other
Gender dysphoria	The feeling of discomfort or distress occurring when gender identity differs from biological sex
Gender equality	Fairness and justice in the distribution of benefits, power, resources and responsibilities between women and men. It recognizes that women and men have different needs, access to and control over resources, and that these differences should be addressed in a way that rectifies the imbalance between sexes
Equality	Is about ensuring that everyone has the same opportunities and maintain their health
Equity	Is about how public services meet the different needs of the population
Equity in health	The absence of unfair, avoidable, or remediable differences in health among different population groups
Intersex individuals	People who are born with biological sex characteristics, including chromosome patterns, gonads, or genitals, which do not fit typical binary notions of male or female bodies
Sex	The different biological and physiological characteristics of men and women, such as reproductive organs, chromosomes, hormones, etc.
Transgender	Individuals with a gender identity that does not match the sex they were assigned at birth

(EHC) developed a core set of practical principles of care (16). These principles of care acknowledge equitable access and quality of care for all individuals with bleeding disorders, irrespective of gender; the need of early and accurate diagnosis of bleeding disorders in women and girls; awareness of the additional challenges female patients with hemophilia face throughout life; provision of comprehensive care in a family-centered approach; inclusion of a dedicated obstetrician and gynecologist in the multidisciplinary team; education of female patients with bleeding disorders and their families regarding the menstrual cycle and its management; early recognition and optimal management of heavy menstrual bleeding; provision of pre-conception counseling and access to prenatal diagnosis (PND); planning of a patient-centered comprehensive management throughout pregnancy and the post-partum period; improvement of involvement of female patients with bleeding disorders in registries, clinical research and innovation. Therefore, promoting gender equality in all fields, including bleeding disorders, requires a concerted effort towards identifying and rectifying power imbalances, and empowering women with greater autonomy to manage their own lives.[2] Although only individuals can empower themselves, institutions can support the process at both the individual and collective levels. Finally, in addressing gender and health issues even in the field of bleeding disorders it is warranted to consider differences between women and men regarding age, ethnicity, geographical location, culture, education, sexual orientation, disability and socio-economic status.[3] To support researchers in properly addressing sex and gender issues whenever designing or reporting studies and to ensure inclusivity, a set of recommendations is available: the Sex and Gender Equity in Research (SAGER) guidelines (17). The SAGER guidelines propose a list of questions that can help authors preparing their manuscript and journal editors in evaluating submitted articles, then asking authors to improve reporting of sex and gender prior to peer review, if necessary.

3 Carriers and female patients with hemophilia

Women and girls carrying one copy of the *F8/F9* gene with the pathogenic variant of hemophilia on an X-chromosome are named carriers. Gene variant detection is the gold standard to identify female carriers. Unfortunately, genetic testing might not be available and thus the pathogenic mutation remains unknown. Appropriate genetic tests in girls or women with FVIII or FIX deficiency should be performed even if there is no known family history of hemophilia, due to the possibility of sporadic mutations, accounting for up to 30–50% of *de novo* cases (18, 19).

Rarely, women actually experience moderate/severe hemophilia due to the inheritance of an affected altered copy of *F8* or *F9* gene on X-chromosome from both parents thus leading to the complete expression of the hemophilia variant's severity, or one from an affected parent and the other from a *de novo* mutation, or due to X-chromosome abnormalities such as monosomy X (Turner syndrome, 45 X). Alternatively, carriers with a single affected X-chromosome may experience a skewed X-chromosome inactivation during X-inactivation, i.e., the random suppression of one of the two X-chromosomes, also known as lyonization (20, 21). In addition, a female carrier may experience abnormal bleeding even in the presence of normal factor levels, as defined by international standards of FVIII/FIX >0.40 IU/mL (22–24). Actually, factor levels are not good predictors of bleeding in these subjects (25). This may be partly due to an impaired response of FVIII to hemostatic changes (26, 27). As for FIX, an effective extravascular distribution may prevent from bleeding despite low levels of factor. Another study showed that even carriers of non-null variants could have very low factor levels and a

2 https://www.unfpa.org

3 https://www.paho.org/en/topics/gender-equality-health

severe clinical phenotype. Therefore, it is clear that the mechanisms that connect the type of underlying gene variant to the bleeding phenotype still need to be elucidated (21).

To improve diagnosis in women and girls with hemophilia and carriers, the International Society on Thrombosis and Hemostasis (ISTH) Scientific and Standardization Committee (SSC) approved a new nomenclature that defines five clinical categories for hemophilia carriers based on FVIII plasma levels: woman/girl with mild hemophilia when factor levels are >0.05 and < 0.40 IU/mL, moderate hemophilia when factor levels are 0.01–0.05 IU/mL, or severe hemophilia with factor levels <0.01 IU/mL. This nomenclature also considers the possibility that during the lifetime of women and girls the bleeding tendency may change. For cases with factor levels >0.40 IU/mL, the terms symptomatic or asymptomatic hemophilia carrier refer to the presence or absence of bleeding events (8).

The increased bleeding tendency in female patients with hemophilia of different severity or some carriers ranges from excessive mucocutaneous bleeding, bleeding from cuts and easy bruising, bleeding after major or minor surgery, to joint bleeding leading to arthropathy and subsequent poor quality of life or even life-threatening bleedings such as intracranial bleeds or hemoperitoneum (9, 28–32). In addition, women may experience sex-specific symptoms. The most common clinical manifestations in women and girls with hemophilia are heavy menstrual bleeding (limiting daily activities, physical exercise or social activities, lasting for more than 7 days, with the need to change tampon or pad every 2 h or less on heaviest days, or in the presence of clots), iron deficiency anemia and post-partum hemorrhages (33).

It has been shown that women and girls with hemophilia report lower quality of life compared to the general population due to the number of days of menstrual bleeding, its severity and impact on their lives (32).

4 Management and prevention of bleeding in female patients with hemophilia

Indeed, female patients with FVIII or FIX levels <0.40 IU/mL should be considered and managed as other patients with hemophilia, and clinicians should be aware that bleeding may also occur in carriers with FVIII/FIX levels of ≥0.40 IU/mL, with impact on their health-related quality of life.

Management of heavy menstrual bleeding envisages hemostatic agents such as tranexamic acid, desmopressin, which can be effective only in the case of hemophilia A or factor replacement therapy in more severe cases or in hemophilia B, hormonal therapies (such as oral contraceptive agents), or combinations, hormonal intrauterine devices (IUDs) and rarely surgical options (34–36). Personalized treatment options should be offered by the hemophilia care center based on age, fertility, other gynecological comorbidities, and patient's preference, also considering cultural and psychological aspects.

The reproductive health of female patients with hemophilia requires particular attention. When planning pregnancy, a carrier or woman with hemophilia already on hormonal treatment due to heavy menstrual bleeding, should be reminded of the possibility of bleeding recurrence upon discontinuation of this medication. Adequate levels of hemoglobin and ferritin should be ensured with oral and/or i.v. iron before pregnancy.

Clotting factor replacement is necessary for invasive procedures like PND and chorionic villus sampling (CVS). During pregnancy, a physiologic increase in FVIII level occurs in carriers of hemophilia A but it should be checked repeatedly. However, in hemophilia B carriers, a rise in FIX hardly occurs (37). Differently from hemophilia A carriers, where desmopressin can be used, hemophilia B carriers with low FIX levels can only be treated with factor replacement. Due to the maternal and neonatal bleeding risk, a proper pregnancy and delivery plan should be made and shared with the patient, the partner and other members of the multidisciplinary team, possibly before 32 weeks of gestation in case preterm delivery occurs. This plan should include the evaluation of the need of factor replacement to prevent post-partum bleeding, measures to prevent bleeding in possibly affected male newborns, pain management strategies and the use of tranexamic acid in the post-partum. Regional block anesthesia is feasible when factor levels are >50 IU/dL. If clotting factor levels are <50 IU/dL at 32–34 weeks of gestation, replacement treatment is administered to avoid bleeding during and after delivery, with the aim of a through level > 50 IU/dL for around 3–5 days after vaginal delivery and 7–10 days after cesarean section (38).

Instrumental delivery is not recommended, to avoid maternal bleeding risk, and is contraindicated in the case of an affected male newborn due to the risk of intracranial bleeding. In countries with good quality of care, the risk of intracranial bleeding in the newborn with hemophilia is 3.6% i.e., at least 40 times higher than non-affected newborns and is related to the method of delivery and the mother's awareness of being a carrier (39, 40).

Other types of bleeding and perioperative management of female patients with hemophilia should be managed based on the bleeding risk, severity of bleeding and severity of hemophilia, following the current international guidelines for hemophilia (5).

Non-replacement therapies may have a significant role in reducing bleeding complications with an easier approach compared to intravenous infusions. However, data on the efficacy and safety of these drugs in women with hemophilia are lacking (12).

We advocate for an increasing inclusion of female subjects in clinical trials, as well as the incorporation of female patients into future guidelines for the management of hemophilia.

5 Prenatal diagnosis and pre-implant evaluation

Women and girls with hemophilia, carriers and their partners must face important reproductive decisions, due to the possibility of having an affected child (41). Around 30% of women are unaware of their carrier status at the time of delivery, even if a positive family history is present (42, 43).

The education of future mothers is a cornerstone for quality-of-care improvement for hemophilia patients, as women play a key role in caregiving (44, 45). Even when previously aware of being a carrier, many women have described feelings of shock, sadness and grief whenever a son is diagnosed with hemophilia (46). Consequently, their family planning is often affected if the potential partner or family have limited understanding of the disease and its potential impact. This often leads to personal and familial psychosocial burden

particularly in developing countries, and the consequences, such as the cancellation of marriages or divorces, can introduce instability into women's lives (15).

An adequate genetic counseling is fundamental to support an informed reproductive decision. It is important to distinguish between information giving, education, and counseling. The purpose of genetic counseling is to provide the mother and the family with adequate information to make their own decisions regarding reproductive options and to provide support during the shared decision-making process also involving families and caregivers. Ideally, genetic counseling should be conducted before pregnancy, and for women who are already pregnant, it should be made available as early as possible. Studies investigating the experience of carriers throughout the reproductive decision-making and PND showed that the decision-making process depends on factors such as the severity of hemophilia of family members, their health-related quality of life, already having a child with hemophilia, living near a specialized medical center, having access to genetic and reproductive counseling and religious beliefs. Moreover, the cognitive and emotional aspects of this process are important as well (41, 47).

Molecular characterization, carrier detection and PND remain the key steps for planning and managing hemophilia carrier pregnancy. Current methods of PND envisage both invasive and non-invasive methods: CVS and amniocentesis are the most widely used technique for invasive PND, essentially accurate and precise methods for detecting affected males early in pregnancy. CVS could be performed between 9 and 12 weeks of gestation, thus providing early diagnosis compared to amniocentesis, which is traditionally performed around 14–16 weeks of gestation. Second trimester amniocentesis envisages that a result is available only after 17–18 weeks of gestation (48). Both techniques are considered gold standards in PND even at potential risk of miscarriage to the fetus. Miscarriage rates after CVS and amniocentesis are reported to be 0.2–0.8%, and 0.1–0.5%, respectively (49). Therefore, significant efforts have been made to obtain new starting material for non-invasive PND such as fetal cells and cell-free fetal DNA (cffDNA) present in the maternal circulation since early weeks of gestation.

During pregnancy, fetal sex determination is helpful when a specific sex is at risk of a severe genetic disease. Traditionally, prenatal fetal sex determination is performed by ultrasound, a non-invasive imaging method effective beyond 14 weeks of gestation with an accuracy >99% in cases of normal genitals (50), and by karyotype analysis following CVS and amniocentesis. Since the discovery in 1997 of cffDNA, i.e., small fragments of DNA (100-150 bp) released from apoptotic placental cells circulating in the mother's blood, male sex determination through analysis of cffDNA has been the first non-invasive prenatal test (NIPT) developed for clinical application. NIPT is still performed in worldwide healthcare systems thanks to its advantages such as early fetal sex determination (from 7 weeks of gestation) (51), safety – it is performed by a simple peripheral blood sample from the mother -, and high sensitivity and specificity (98.9 and 99.6%, respectively) (52). Fetal sex determination by NIPT has also reduced the need of invasive procedures for the definitive diagnosis by up to 50%, since they can be avoided in case of a fetus not at risk and it is also widely accepted for detecting common chromosome aneuploidies (13, 18, 21, X and Y), large deletions and duplications, as well as the common microdeletion syndromes. The development of NIPT for the fetal gender determination has also benefited PND in hemophilia and the current WFH guidelines suggest that pregnant women who are confirmed carriers of a variant in *F8/F9* coding gene may be offered non-invasive testing to determine the sex of the fetus through cffDNA analysis in the maternal blood (5).

NIPT for sex determination relies on the amplification of specific regions (SRY, DYS14, DAZ) of the male chromosome Y.

At present, due to the poor cffDNA amount and the high contamination by maternal DNA (>90%), NIPT cannot be used for a definitive diagnosis of X-linked inherited diseases, such as hemophilia. Studies are ongoing to develop strategies for routine non-invasive PND of X-linked diseases that may replace conventional invasive procedures in the future (53–56). Actually, this process does not exclude the very low probability to give birth to a female with hemophilia.

An alternative option to conventional PND methods for couples at risk of transmitting genetic disorders to their offspring is preimplantation genetic testing (PGT), a very early diagnosis performed on embryos obtained by *in vitro* fertilization (IVF) procedures before their transfer to the uterus.

PGT consists of a genetic analysis performed using 5–10 cells obtained via biopsy from an embryo at the blastocyst stage, before transfer to the uterus. It is mostly chosen by female carriers who do not want to consider pregnancy termination, in case of an affected fetus (57). PGT is legally restricted in many countries with several legal, ethical and social implications, however over the past decade, regulatory changes in various countries in the world have shown a growing acceptance of PGT. Unfortunately, such a procedure is available only in few expert Centers in the world.

Genetic counseling should provide support, information, and assistance in making informed choices regarding family planning and reproductive options. Management of pregnancy must be carefully discussed in a multidisciplinary team involving hematologists, gynecologists, anesthesiologists and a psychologist (58).

6 Psychosocial impact and quality of life

Apart from the psychosocial impact of reproductive choices that were previously discussed, women and girls with hemophilia and carriers experience gender-specific psychosocial issues.

Women are still often considered as simple carriers of inherited bleeding disorders, thus often limiting their access to healthcare and to have their diagnosis acknowledged within the healthcare systems (59).

Living with hemophilia can significantly impact the psychosocial well-being of women, leading to emotional distress, social isolation, and reduced quality of life. It is well known that psychological factors may influence health decisions and that such factors, as risk perception, perceived susceptibility, perceived consequences, and confidence in one's own physical and psychological resources might predict health behaviors.

Women with bleeding disorders often cope with their tendency to prioritize the care and interests of their children and family, downgrading their own needs. Joining a parent support group or patient organization can help provide new perspectives to face the challenges of the diagnosis while learning the importance of self-care and resilience (60, 61).

In order to increase awareness and contribute to the empowerment of women/girls with hemophilia and carriers, management should envisage psychological support since the diagnosis and discussion of the possibility of heavy menstrual bleeding and reproductive issues before pregnancy, possibly since puberty and eventually, about consequences of pregnancy (transmission of the disorder, consequences on the mother's health status, risks at childbirth).

It is crucial that healthcare professionals of women with bleeding disorders provide education to increase awareness on the incidence, severity, and impact of bleeding disorders not only to women affected and their families, but also to the medical community. In particular, obstetricians and gynecologists are often the first specialists that patients refer to when reporting prolonged bleeding. Education programs should also be extended to the hemophilia community, so that female relatives of patients with hemophilia can be offered the opportunity of carrier testing and counseling, and treatment for their bleeding as well (2). Hemophilia treatment centers should develop and enhance services to meet the physical and psychosocial needs of women with bleeding disorders and to make these services widely available.

Contrary to historical belief, female and male patients with hemophilia experience similar non-gender specific bleeding symptoms. Among these, musculoskeletal bleeding is increasingly described in women, and joint bleeding was reported in up to 9% in several studies (62–65). Male patients with hemophilia are at risk of fractures, low bone mineral density (BMD), osteopenia and osteoporosis. The pathophysiology of low BMD in hemophilia still needs to be elucidated, being possibly due to a multifactorial mechanism including functional impairment due to arthropathy, chronic infections and treatment and possibly a biological role of FVIII or hemostasis in general (66–69). However, data regarding the women with hemophilia and carriers are very limited and indicate a higher prevalence of osteoporosis compared to controls. This is particularly important, as female patients with hemophilia and carriers are exposed to sex-specific risk factors for osteoporosis such as menopause (70).

7 Female partners of patients with hemophilia

Not only mothers, but also female partners are often caregivers of male patients affected with hemophilia. Women dating patients with hemophilia often face difficulties in discussing the diagnosis with their affected partners and hemophilia related burden seems to influence many aspects of the partners' lives, in particular in family and relationships. The partner's quality of life is directly influenced by the patient's health (71).

Patients with hemophilia may have psychosocial problems such as depression, impaired relationship with the healthy partner or difficulties in sexual functioning with partner (72–74). In everyday life, even intimacy may be affected in patients with hemophilia, due to several physical problems such as hemophilic arthropathy leading to disability, risk of joint bleeding or hematomas of the iliopsoas or due to treatments or medications (75, 76). Many patients who received blood products before the implementation of viral inactivation procedures are affected by blood-transmitted diseases, such as human immunodeficiency virus (HIV) and hepatitis C virus (HCV) infections, further impacting intimacy and reproductive aspects (77, 78). Over the years, HIV infection has acted as an indirect source of stress for wives and partners of HIV seropositive patients with hemophilia (79).

Knowledge about intimacy, sexual health and difficulties with sexual activity in patients with hemophilia is extremely limited. In a pilot survey by Tobase et al. (80) examining sexual health 40% of patients reported that their bleeding disorder affected their sexual life. Thus, sexual health in this population requires greater attention (81, 82). Knowledge about sexuality in patients with hemophilia is important to inform physicians and stakeholders involved with policy development and comprehensive hemophilia care. Recommendations and guides for sexuality for patients with hemophilia are provided by patient associations.[4]

It is advisable that physicians and psychologists at the hemophilia treatment center also discuss these aspects of social lives with patients with hemophilia by also involving their partners, with the ultimate goal to improve health and well-being.

8 Discussion

The disparity of healthcare access for women with hemophilia and gender-specific issues are gaining growing attention in the community of hematologists taking care of bleeding disorders.

Although further efforts are needed to fully report annual bleeding rates and long-term complications and to evaluate quality of care in women with hemophilia and carriers, the adoption of standards that address symptom recognition is warranted, acknowledging for example that women can experience joint bleeds and pain due to arthropathy, and investigating the possibility of an increased risk of low BMD due to low FVIII or FIX levels.

Another critical issue for the future is the inclusion in trials and registries, not only of women and girls with hemophilia, but also of transgender individuals to collect as much evidence as possible and to inform decision in these cases. Finally, gender equality can be reached by also involving female partners of male patients with hemophilia in the discussion of health and psychosocial issues of these patients.

Collaboration between healthcare providers, researchers, and patient advocacy groups is crucial to enhance the care and support available for women with hemophilia, carriers and the female figures that support management of male patients with hemophilia. Similar action is required not only for women affected by hemophilia, but also for all women affected with bleeding disorders.

We advocate a paradigm shift in understanding and addressing the needs of this previously overlooked population and engaging a broader community to continue discussing, learning and taking actions that will help achieve a more equitable and inclusive society.

We aim to an improvement of health equity, equal access to healthcare for women and men with hemophilia and other bleeding disorders and dedicated resources to improve the unique needs of the women dealing with such diseases ultimately leading to improved care and quality of life.

We emphasize the importance of recognizing women as more than carriers and advocate for gender-specific research, improved

4 https://hemophilia.org.uk/wp-content/uploads/2020/01/sex_BD_web.pdf

diagnostic strategies and tailored treatment options. By acknowledging and addressing the unique challenges faced by women with hemophilia, healthcare professionals can optimize care and improve the quality of life of these individuals.

Author contributions

RG: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. IG: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. MM: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. SS: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. OR-L: Investigation, Writing – review & editing. FP: Conceptualization, Resources, Supervision, Writing – review & editing.

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Conflict of interest

RG advisory board of Bayer, Roche; speaker bureau/educational meetings Pfizer, SOBI, Takeda, Novo Nordisk; FP advisory board of CSL Behring, Biomarin, Roche, Sanofi, Sobi; speaker bureau/educational meetings Takeda/Spark.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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