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STATE OF THE ART

The intriguing role of platelets as custodians of brain-derived neurotrophic factor

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Abstract

A State of the Art lecture titled "Platelets and neurotrophins" was presented at the International Society on Thrombosis and Haemostasis Congress in 2023. Neurotrophins, a family of neuronal growth factors known to support cognitive function, are increasingly recognized as important players in vascular health. Indeed, along with their canonical receptors, neurotrophins are expressed in peripheral tissues, particularly in the vasculature. The better-characterized neurotrophin in vascular biology is the brain-derived neurotrophic factor (BDNF). Its largest extracerebral pool resides within platelets, partly inherited from megakaryocytes and also likely internalized from circulation. Activation of platelets releases vast amounts of BDNF into their milieu and interestingly leads to platelet aggregation through binding of its receptor, the tropomyosin-related kinase B, on the platelet surface. As BDNF is readily available in plasma, a mechanism to preclude excessive platelet activation and aggregation appears critical. As such, binding of BDNF to α2-macroglobulin hinders its ability to bind its receptor and limits its platelet-activating effects to the site of vascular injury. Altogether, addition of BDNF to a forming clot facilitates not only paracrine platelet activation but also binding to fibrinogen, rendering the resulting clot more porous and plasma-permeable. Importantly, release of BDNF into circulation also appears to be protective against adverse cardiovascular and cerebrovascular outcomes, which has been reported in both animal models and epidemiologic studies. This opens an avenue for platelet-based strategies to deliver BDNF to vascular lesions and facilitate wound healing through its regenerative properties. Finally, we summarize relevant new data on this topic presented during the 2023 International Society on Thrombosis and Haemostasis Congress.

KEYWORDS
brain-derived neurotrophic factor, hemostasis, neurotrophins, platelets, thrombosis

Imane Boukhatem, Samuel Fleury, and Georges Jourdi contributed equally to the work.

Essentials

- Neurotrophins are increasingly recognized as important players in vascular health.
- The brain-derived neurotrophic factor (BDNF) is highly concentrated within platelets.
- Release of BDNF from platelets into circulation protects against cardiovascular diseases.
- This opens up avenues for platelet-based strategies to deliver BDNF where it is needed.

1 | INTRODUCTION

In his seminal work, Bizzozero [1] concluded his treaty on platelets by saying, "It is hardly permitted to assume that elements, represented in the blood in such a constant fashion and great number, as it is the case for blood platelets, are active only under abnormal or pathological conditions. Their physiologic significance, therefore, remains to be investigated, as well as their origin and their possible relationship with other elements of the blood. It is not necessary to emphasize the difficulty of these tasks". Since that first insightful description of platelets in 1882, important strides have been accomplished in understanding their origin as derived from megakaryocytes. Their functions have evolved to include roles beyond hemostasis, such as host immunity, inflammation and wound healing, bone regeneration, angiogenesis, fetal development, and liver regeneration [2–9].

One fascinating area lies in the similarities between platelets and neurons [10]. The first comparison that can be made is that platelets contain multiple neurotransmitters including serotonin, dopamine, γ-aminobutyric acid, glutamate, and adrenaline [11–15]. Platelets are known to uptake serotonin from blood circulation through the serotonin transporter *SERT* and then store serotonin in their dense granules through the vesicular monoamine transporter 2 located on their granular membrane, in a similar fashion to neurons [16]. Based on this shared mechanism, platelets have been proposed as a peripheral and readily accessible cellular model to reflect the serotonin dynamics in neurons more than 45 years ago [17]. Similar to platelet α-granules, neurons contain protein-filled large dense-core vesicles, which they release upon activation. Neuronal small dense-core vesicles rather contain neurotransmitters, *ADP*, *ATP*, and serotonin, comparable with the content of platelet dense granules [10]. In addition to neurotransmitters, platelets also contain many proteins with known activities in neuronal health and in hemostasis. For instance, the brain-derived neurotrophic factor (BDNF) is a neural growth factor that also induces platelet aggregation [18]. Amyloid β and its precursor protein is another example of a neuroactive protein mainly known in the neurotoxic amyloid plaques of Alzheimer's disease, but that can also induce platelet aggregation [19]. Interestingly, platelet levels of such proteins were found to be dysregulated in neurologic diseases, such as Alzheimer's disease and major depressive disorders [20,21]. Increasingly, studies suggest a link between platelets and neurons, even postulating possible active regulation by platelets of brain function, although the exact mechanisms remain to be fully elucidated [10,22].

In this State of the Art review article, we propose to delve into the interplay between the neurotrophic system and platelet function, with a particular focus on the better-characterized neurotrophin in platelet biology, BDNF.

2 | NEUROTROPHINS AND THEIR RECEPTORS

Discovered in the early 1990s, neurotrophins are a family of proteins that play a crucial role in the development, survival, and function of neurons. There are 4 neurotrophins: the nerve growth factor (NGF), the BDNF, the neurotrophin-3 (NT-3), and the neurotrophin-4-5 (NT-4/5). Neurotrophins are synthesized as larger proteins, known as proneurotrophins (proBDNF, proNGF, proNT-3, and proNT-4/5) [23], which are proteolytically cleaved by different convertases such as furin, MMP3, and MMP7 into mature proteins [24]. They are essential for the development of the nervous system, and they exert their effects by binding to their specific receptors on the cell surface: the high-affinity tropomyosin-related kinase (Trk) receptors and the low-affinity p75 neurotrophin receptor ($p75^{NTR}$). NGF was the first to be discovered and binds the receptor TrkA. BDNF is the ligand for the TrkB receptor along with NT-4/5, and NT-3 binds the TrkC receptor. While each neurotrophin binds to a specific Trk receptor, the low-affinity receptor $p75^{NTR}$ can bind all neurotrophins and can act as a coreceptor for the Trk receptors (Figure 1) [25]. The Trk receptors signal through kinase-dependent pathways and are involved in the regulation of proliferation, differentiation, survival, axonal and dendritic growth, and synapse formation, whereas the $p75^{NTR}$ receptor acts as a modulator of function, by promoting cell death and apoptosis, or cell survival and inflammation, depending on the cellular context and available coreceptors [26]. These processes have been reviewed in detail elsewhere [26,27].

In addition to their well-studied role in the nervous system, neurotrophins and their receptors are increasingly recognized as important mediators in numerous other biological systems. In the cardiovascular system, they play a role in the developing heart and vessels [28–30] and the overall regulation of angiogenesis [31]. The better-characterized neurotrophin in this context, BDNF, has been reported to be expressed in most cardiovascular structures, including endothelial cells [32], vascular smooth muscle cells, macrophages [33], and fibroblasts [34], as well as in other organs and tissues such as the kidney, the liver, the lung, and the stomach [35]. However, platelets are by far the largest peripheral reservoir of BDNF, containing

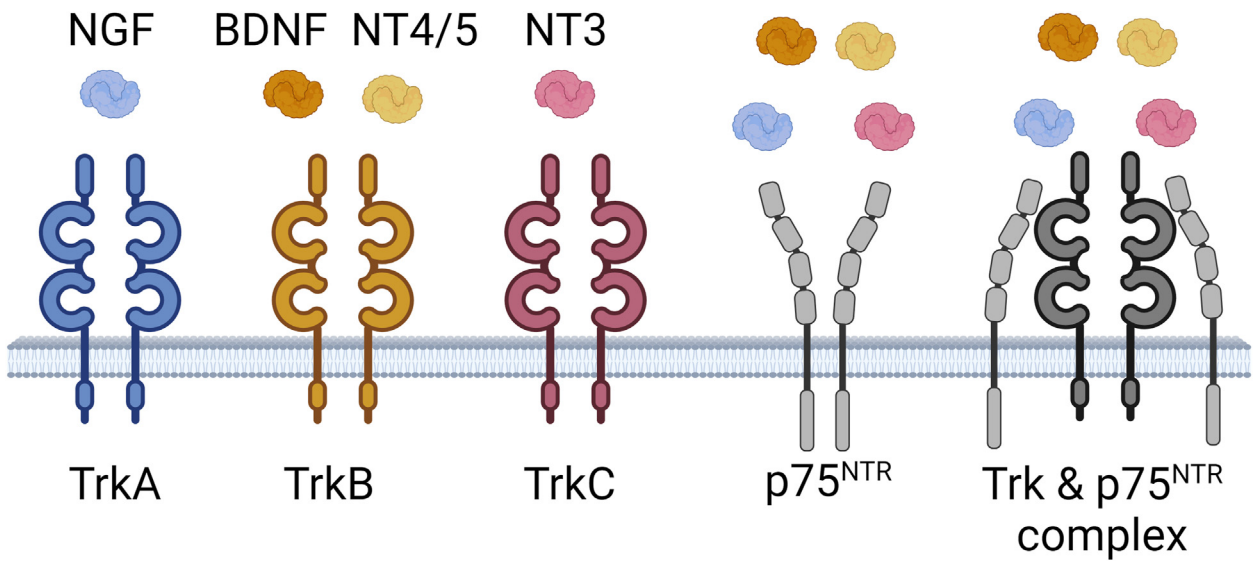

FIGURE 1 A schematic representation of the 4 known neurotrophins and their canonical receptors. Neurotrophins bind to the tropomyosin receptor kinase (Trk) family of receptors with the highest affinity. Nerve growth factor (NGF) specifically binds to TrkA, brain-derived neurotrophic factor (BDNF) and neurotrophin (NT) 4/5 (NT4/5) preferentially bind to TrkB, whereas NT3 binds to TrkC. All neurotrophins also have a low-affinity receptor, namely the p75 neurotrophin receptor ($p75^{NTR}$). Trk and $p75^{NTR}$ receptors can also form a complex that has a higher affinity for neurotrophins than Trk and $p75^{NTR}$ receptors alone.

approximately 2.50 to 15.75 ng of BDNF per 2.5×10^8 platelets [36,37].

3 | ORIGIN OF THE PLATELET-BORNE BDNF

The presence of BDNF in platelets was first reported in 1990 by Yamamoto and Gurney [38] and further described in more detail in 2002 by Fujimura et al. [36]. While its presence in platelets is not disputed, its origin has been more elusive to ascertain. The first to address this question were Fujimura et al. [36], who hypothesized that BDNF binds to a recognition site on the platelet surface where it can be internalized. Using radiolabeled BDNF (^{125}I-BDNF), they reported the likely presence of 2 classes of receptors: a very high–affinity site with a *Kd* of 130×10^{-15} M, corresponding to 80 sites/platelet, and a moderately high–affinity site with a *Kd* of 20 nM, corresponding to 3450 sites/platelet [36]. However, they were not able to show the presence of TrkB or $p75^{NTR}$ on the platelet surface, albeit data were not shown [36]. Other studies have shown both megakaryocytes and platelets to express the TrkB and the $p75^{NTR}$ receptors, but their function remains largely unknown [39,40]. BDNF internalization assays have not been reported by other groups, but this hypothesis is plausible as BDNF is not the first neuromodulator to be reported to be internalized by platelets. Indeed, platelets have an important reservoir of serotonin that they internalize from circulation into dense granules, which they release upon activation [41]. Tachykinins are another neuromodulator stored in platelets and released upon activation [42]. Further study is required to better understand the potential mechanisms of BDNF internalization by platelets.

Another compelling hypothesis is that BDNF could be inherited by platelets from their precursor cells, megakaryocytes. Fujimura et al. [36] have looked at BDNF content in immortalized megakaryoblast cell lines, *DAMI* and MEG-01, where they reported BDNF to be absent. Recognizing that these megakaryoblast cell lines lacked the hallmarks of mature megakaryocytes [43], Tamura et al. [44] have attempted to reproduce these experiments in MEG-01 cells incubated with thrombopoietin to induce maturation. They have found MEG-01 to produce BDNF in these conditions and have postulated that it may serve to potentiate cell proliferation [44]. Later, a study by Chacón-Fernández et al. [45] convincingly established the presence of BDNF in megakaryocytes derived from CD34+ cells of human origin and absence from megakaryocytes of murine origin, mirrored by the almost complete absence of BDNF from mouse platelets [46]. The relative contribution to the BDNF platelet store from megakaryocytes vs via internalization from the bloodstream remains however to be determined.

4 | PLATELETS AS ACTIVE RESERVOIRS OF BDNF

While the origin of the high levels of BDNF in platelets remains to be more fully established, several studies have demonstrated that BDNF is secreted upon platelet activation [36–38,47]. The first evidence of the platelet origin of circulating BDNF was the observation of much higher BDNF levels in serum (as high as 100- to 200-fold) as compared with plasma (1-10 ng/mL), suggesting that clotting released large quantities of the neurotrophin into circulation [47]. The pathways involved in BDNF release from platelets were further studied in

purified systems, where many classical agonists were able to induce secretion of appreciable levels of BDNF from intraplatelet stores into their milieu [36,37,44]. In 2001, Tamura et al. [48] identified 2 different pools of BDNF contained within human platelets, one being in the α-granules and the other being cytoplasmic. Accordingly, a large fraction of platelet BDNF is released upon activation while the rest remains within the platelet [37,48]. Tamura et al. [48] also reported differential secretion of BDNF between protease-activated receptors (PAR)-1 and -4, with PAR-1 activation resulting in higher BDNF secretion (37% of total platelet content) than PAR-4 activation (3.8% of total platelet content). Fujimura et al. [36] further reported that low shear was less effective than high shear in inducing platelet release of BDNF, but the highest proportion of release was seen with calcium ionophore and thrombin-activated washed platelets. Similar results were found by Le Blanc et al. [37], reporting approximately equal BDNF secretion among a PAR-1 agonist, collagen, *ADP*, and arachidonic acid, with each agonist leading to a 60% to 70% decrease in intraplatelet BDNF levels and a parallel increase in plasma BDNF levels following platelet aggregation.

Although the opposing roles of mature BDNF to its precursor form, proBDNF, have been reported in the nervous system, few studies have looked at the expression of proBDNF in circulation. Notwithstanding, proBDNF can readily be measured in both platelets and plasma, albeit in strikingly different ratios [37]. In platelets, intracellular distribution of proBDNF shows a diffuse pattern, mainly within the cytoplasm, whereas BDNF appears to be concentrated in granules. Perhaps not surprisingly, intraplatelet proBDNF levels do not decrease upon platelet activation, suggesting that proBDNF is not secreted by platelets nor cleaved to BDNF upon platelet activation [37]. Furthermore, proBDNF levels are similar (around 1 ng/mL) in plasma and serum, suggesting that it is not secreted by platelets [37,49,50]. When added to the observation that proBDNF concentrations are roughly 10-fold higher in plasma than in platelets, this suggests that platelets do not contribute to a significant degree to the circulating pool of proBDNF. As a consequence, platelet activation strongly but transiently modifies the BDNF-to-proBDNF ratio, from approximately 10 molecules of proBDNF to each molecule of BDNF at resting state to approximately 2.5 to 3 molecules of proBDNF to BDNF upon platelet activation [37].

5 | SUPPORTING ROLE OF BDNF IN PLATELET FUNCTION

The vast release of BDNF into the bloodstream begs the question of physiological function, both within the vessel and beyond. To study the biological relevance to platelet function, Boukhatem et al. [18] have isolated platelets from the bloodstream of healthy volunteers and exposed washed platelets to increasing concentrations of BDNF. Unlike NGF, which shares many of the physicochemical characteristics of BDNF but targets a distinct Trk receptor, BDNF induced platelet aggregation in a concentration-dependent manner, an aggregation that was inhibited with the addition of a blocking antibody against BDNF [18]. Through the activation of a truncated TrkB receptor and recruitment of *Rac-1*, protein kinase C, and *PI3K/Akt* pathways, BDNF was able to prime platelets to synergistically act with classical agonists at low concentrations (40 nM) and induced complete biphasic aggregation at higher supraphysiological concentrations (125 and 370 nM) that in part relied on amplification from secondary mediators [18]. Given that BDNF is readily detectable in human plasma, a regulatory mechanism to prevent excessive platelet activation appears critical.

In further *in vitro* experiments, Jourdi et al. [51] have shown that as little as 25% of autologous plasma was sufficient to completely abolish platelet aggregation in response to exogenous BDNF. The mechanism necessary to preclude untimely BDNF-induced platelet activation in the bloodstream and to limit BDNF-mediated platelet activation to the sites of vascular injury appears to be strongly dependent on α2-macroglobulin (α_2M), a broad-spectrum protease inhibitor in circulation. Indeed, molecular docking analyses have revealed that BDNF binds to α_2M, thus preventing its stable binding to TrkB on the platelet surface [51]. This interaction was confirmed experimentally, with BDNF pull-down shown to be immunoreactive against α_2M. Native α_2M inhibited BDNF-induced platelet activation and aggregation in a concentration-dependent manner. This inhibitory effect was specific, as native α_2M had no effect on platelet aggregation induced with other platelet agonists, nor did albumin or fibrinogen have an impact on BDNF-induced platelet responses. Given that native α_2M is present at micromolar concentrations in circulation while BDNF is present at nanomolar concentrations, it is likely that the vast majority of circulating BDNF is physiologically bound to α_2M [51–53]. This may represent a strategy to stabilize the circulating neurotrophin, protect it from proteolysis and potential clearance pathways, and prevent circulating BDNF from activating platelets. Local BDNF release from activated platelets after injury might overcome the inhibition, thus resulting in platelet aggregation where it is needed (Figure 2) [54].

6 | BDNF AS A MODULATOR OF CLOT MORPHOLOGY

Apart from its effect on platelet function, BDNF has been shown to interfere with fibrin clot formation and lysis. Indeed, Amadio et al. [55] have shown that BDNF reduces the fibrin fiber network density in a concentration-dependent manner, most probably by binding to the fragment 15 to 66 (within the heparin-binding domain) of the fibrinogen β chain. BDNF was also shown to enhance clot lysis and prolong both thrombin and reptilase clotting time in citrated plasma samples [55]. This suggests that BDNF can further influence fibrin clot formation by a mechanism independent of its binding with fibrinogen β chain, a mechanism that remains to be elucidated. Additionally, BDNF was reported to alter the viscoelastic properties of the fibrin clot *in vitro*, resulting in decreased maximum clot firmness. Transposing these data *in vivo*, Amadio et al. [55] reported a negative correlation between plasma BDNF levels and the maximum clot firmness in samples from patients with coronary artery disease (CAD). Exogenous

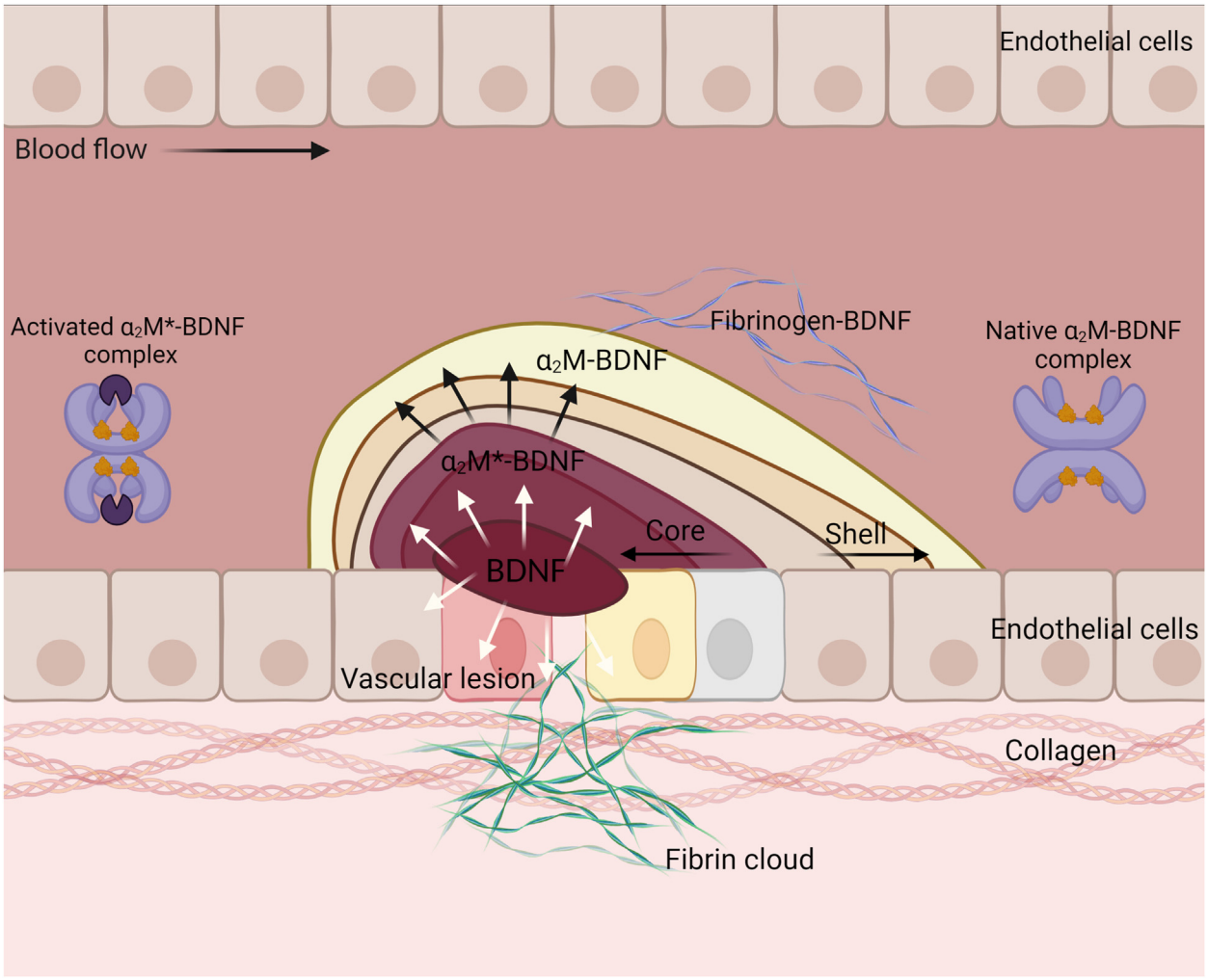

FIGURE 2 Working core-and-shell model of the role of brain-derived neurotrophic factor (BDNF) in platelet function and hemostatic plug formation. Adapted from the core-and-shell model from Stalker et al. [54]. The hemostatic plug consisting of an inner region known as the core and an outer region known as the shell, with their specificities, allows for a postulated working model of BDNF in platelet function and platelet plug formation. In the core, where platelet aggregates are dense and thrombin levels are high, platelets are maximally stimulated, releasing important quantities of BDNF, which can diffuse to support platelet activation and aggregation and to support endothelial healing along the vascular lesion. Excess BDNF is captured by α2-macroglobulin (α_2M*), which is activated by the high protease levels within the core, and targets excess BDNF for elimination. Further from the core, BDNF binds to fibrinogen and contributes to the thrombus architecture of a more loosely composed, plasma-permeable shell. Native α_2M binds to the remaining BDNF, protecting the neurotrophin from degradation and potentially transporting the protein to a distal site.

addition of BDNF to these plasma samples markedly modified their fibrin clot profile, restoring a physiological clot morphology [55]. This supports a physiological function of BDNF in modulating clot firmness and susceptibility to lysis (Figure 2).

7 | THE ROLE OF BDNF IN VASCULAR HEALTH

Beyond an autocrine/paracrine role in hemostasis and thrombosis, BDNF has been postulated to target other cells within the vasculature. While of great interest, this has been covered in detail elsewhere [31,56]. Overall, current studies suggest that BDNF secreted by platelets could contribute to endothelial health and regeneration by the promotion of endothelial cell survival, recruitment of hematopoietic precursors, and possibly vascular smooth muscle cell migration [57–60]. These effects should be considered together when extrapolating to BDNF-dependent clinical outcomes in vascular health [31].

Since the late 1990s, a plethora of epidemiologic studies have shown BDNF to be associated with outcomes in cardiovascular disease (CVD; Table) [52,55,61–81]. While there is some conflicting literature, in general, higher levels of BDNF have been found to be protective against adverse cardiovascular outcomes. One of the largest-scale studies stemmed from the Framingham cohort and evidenced an inverse relationship between the circulating serum BDNF levels and CVD risk and mortality in 3687 participants, with

TABLE A selection of studies associating variations in circulating brain-derived neurotrophic factor levels with cardiovascular diseases.

Study design	Patient characteristics	Healthy control characteristics	Sample type	Circulating BDNF levels	Reported outcomes	BDNF level variations in patients vs controls	Reference
Coronary artery disease							
Prospective study	38 unstable angina patients (mean age, 66 y; 25 women), 45 stable effort angina patients (mean age, 65 y; 30 women)	24 non–coronary artery disease patients (mean age, 62 y; 9 women)	Plasma	Controls: 1297 (654-1856) pg/mL Unstable angina: 1304 (802-1689) pg/mL Stable effort angina: 1267 (781-1706) pg/mL	Unstable angina, stable effort angina	No difference in plasma BDNF levels among the 3 groups. The difference in BDNF plasma levels between the coronary artery and aorta was significantly greater in unstable angina patients than in stable effort angina patients or controls	[33]
Longitudinal study	188 patients (mean age, 85 y; 117 women)	-	Plasma	[a]Women: 718 (582-885) pg/mL Men: 567 (416-772) pg/mL	CVD mortality	Low BDNF plasma levels were associated with greater all-cause mortality in women. No association between plasma BDNF and mortality in men and serum BDNF and mortality in either sex. Serum BDNF was significantly higher in women than in men	[61]
Prospective cohort study	3687 participants (mean age, 65 y; 2068 women)	-	Serum	23.5 ± 8.3 ng/mL	Angina pectoris, coronary insufficiency, myocardial infarction, stroke (ischemic or hemorrhagic) or transient ischemic attack, incident heart failure, intermittent claudication, or death secondary to CVD	BDNF inversely associated with CVD risk and mortality	[62]
Prospective study	143 patients (mean age, 68 y; 39 women)	91 controls (mean age, 63 y; 42 women)	Plasma	Controls: 1361 (884-1846) pg/mL Patients: 937 (679-1263) pg/mL	Stable coronary artery disease	Lower BDNF plasma concentrations in patients compared with controls. Low BDNF levels were associated with adverse cardiovascular events at 12-month follow-up in patients	[63]
Retrospective study	41 coronary heart disease patients (mean, 64 y)	21 healthy controls (mean, 59 y; 0 women)	Plasma	[b]Controls: 208.90 ± 25.13 pg/mL Patients: 126.83 ± 15.39 pg/mL	Coronary heart disease	Lower BDNF plasma levels in patients in comparison with controls	[55]

(Continues)

TABLE (Continued)

Study design	Patient characteristics	Healthy control characteristics	Sample type	Circulating BDNF levels	Reported outcomes	BDNF level variations in patients vs controls	Reference
Cross-sectional retrospective study	673 patients (mean age, 66 y; 158 women)	607 controls (mean age, 64 y; 352 women)	Plasma	Controls: 866 (483-1.563) pg/mL Patients: 784 (458-1253) pg/mL	Coronary artery disease	Lower BDNF plasma levels in patients in comparison with controls	[52]
Cerebrovascular disease							
Prospective study	87 patients (mean age, 72 y; 42 women)	-	Plasma	9.96 ± 5.21 ng/mL	First-in-life complete ischemic stroke	Reduced BDNF concentration in acute phase of stroke is a factor for poor prognosis in terms of patients' functional status in the medium term (ie, within the following 90 d)	[64]
Prospective study	514 patients (total cohort of 600 patients; mean age, 56 y; 215 women)	514 matched controls	Serum	[a]Controls: 23.9 (23.1-24.8) ng/mL Patients: 18.3 (17.0-19.7) ng/mL	Acute phase of ischemic stroke	Lower serum BDNF levels in stroke patients in comparison with controls. Low BDNF levels are associated with poor long-term functional outcome	[65,66]
Prospective study	270 patients (median age, 65 y; 126 women)	100 healthy subjects (median age, 65 y; 47 women)	Serum	Controls: 28.1 (18.1-36.8) ng/mL Patients: 22.1 (14.5-27.5) ng/mL	Acute ischemic stroke	Lower serum BDNF levels in stroke patients in comparison with controls. Negative correlation between serum BDNF level and stroke infarct volume	[67]
Prospective study	75 patients (mean age, 73 y; 41 women)	28 healthy subjects (mean age, 69 y; 20 women)	Serum	Patients: 3.89 ± 2.05 ng/mL Controls: 14.9 ± 4.7 ng/mL	Acute ischemic stroke	Lower serum BDNF levels in stroke patients in comparison with controls	[68]
Prospective study	75 patients (median age, 69 y; 31 women)	56 healthy subjects (median age, 43 y; 27 women)	Serum	Controls: 12 ng/mL (8-15) Patients: 14 ng/mL (10-17; visually deduced)	Acute ischemic stroke	Higher serum BDNF levels in stroke patients in comparison with controls	[69]
Prospective study	116 patients (mean age, 72 y; 49 women)	40 healthy subjects (mean age, 68 y; 17 women)	Plasma	Patients: 4.1 pg/mL (2.73, 9.24) Controls: 5.59 pg/mL (2.57, 8.38)	Acute ischemic stroke	No difference in plasma BDNF levels between stroke patients and controls	[70]
Prospective study	52 patients (aged 52-74 y; 29 women)	12 healthy subjects age- and sex-matched	Serum	Patients: 648.22 ± 94.66 pg/mL Controls: 1349.0 ± 1054.62 pg/mL	Acute ischemic stroke	Lower serum BDNF levels in stroke patients in comparison with controls	[71]

(Continues)

TABLE (Continued)

Study design	Patient characteristics	Healthy control characteristics	Sample type	Circulating BDNF levels	Reported outcomes	BDNF level variations in patients vs controls	Reference
Cross-sectional study	53 patients (mean age, 66 y; 29 women)	-	Serum	Depressive patients: 751.35 ± 642.86 pg/mL Nondepressive patients: 729.39 ± 500.90 ng/mL	Acute ischemic stroke	No significant difference in serum BDNF level between depressive and nondepressive patients	[72]
Prospective study	216 patients (mean age, 66 y; 99 women)	-	Serum	Depressive patients: 8.1 (5.6-9.4) ng/mL Nondepressive patients: 13.7 (10.4-16.5) ng/mL	Acute ischemic stroke	Serum BDNF level is an independent predictor of poststroke depression at 3 mo	[73]
Prospective study	50 patients (mean age, 66 y; 22 women)	-	Plasma	Patients with discharge (>10 d): 9736.61 ± 2873.95 pg/mL Patients with discharge (≤10 d): 9715.00 ± 2561.15 pg/mL	Acute ischemic stroke	Plasma BDNF levels are associated with better clinical prognosis	[74]
Prospective longitudinal study	38 patients (mean age, 71 y; 15 women)	-	Serum	Nontreated patients: 9.2 ± 1 ng/mL Treated patients: 11.4 ± 0.8 ng/mL (mean ± SEM)	Acute ischemic stroke	Higher serum BDNF levels in recombinant tissue plasminogen activator-treated than nontreated patients. Serum BDNF levels do not predict stroke outcome at 90 d	[75]
Multicenter prospective study	552 patients (mean age, 52 y; 209 women)	-	Serum	Patients at inclusion: 3.5 ng/mL Patients at 1 y of follow-up: 3.8 ng/mL (mean; visually deduced)	Acute ischemic stroke	No association of serum BDNF level with functional outcome and residual lesion at 1 y of follow-up. Lower serum BDNF levels at 1 y in comparison with baseline (ie, 24 h from symptom onset)	[76]
Prospective study	204 patients (median age, 64 y; 94 women)	100 normal controls matched for sex, age, and BMI	Serum	Patients at admission: 13.4 (9.2-16.9) ng/mL Controls: 24.5 (17.2-27.5) ng/mL	Acute ischemic stroke	Low serum BDNF levels at admission associated with poor short-term (at 3 mo) functional outcome and mortality	[77]

(Continues)

TABLE (Continued)

Study design	Patient characteristics	Healthy control characteristics	Sample type	Circulating BDNF levels	Reported outcomes	BDNF level variations in patients vs controls	Reference
Prospective study	40 patients (median age, 63 y; 18 women)	40 controls (median age, 63 y; 19 women)	Serum	Patients: 19.14 ± 4.87 ng/mL Controls: 27.34 ± 4.28 ng/mL	Acute ischemic stroke	Lower serum BDNF levels in stroke patients in comparison with controls	[78]
Prospective study	100 patients (median age, 69 y; 45 women)	50 controls (median age, 65 y; 28 women)	Serum	Nonpoststroke depression patients at admission: 6.3 (6-7.5) ng/mL Poststroke depression patients: 5.6 (4.3-5.7) ng/mL Controls: 5.3 (4-5.7) ng/mL (visually deduced)	Acute ischemic stroke	Higher serum BDNF levels on day 1 in nonpoststroke depression patients than in normal controls. Lower serum BDNF levels on day 1 in poststroke depression patients than in nonpoststroke depression patients. Serum BDNF on day 1 after admission predicts the risk of subsequent poststroke depression	[79]
Prospective study	75 patients (median age, 64 y; 31 women)	-	Serum	Nontreated patients at 6 wk: 23.16 ± 5.13 ng/ml Atorvastatin-treated patients at 6 wk: 32.95 ± 6.14 ng/mL	Acute ischemic stroke	Serum BDNF levels significantly elevated by 6 wk after stroke in atorvastatin-treated compared with nontreated patients. Increased serum BDNF level associated with improved functional recovery	[80]
Pulmonary embolism							
Prospective study	90 patients (mean age, 63 y; 39 women)	55 age- and sex-matched controls (mean age, 60 y; 24 women)	Plasma	Controls: 644 (576-784) pg/mL Patients: 403 (252-582) pg/mL	Pulmonary embolism	Lower BDNF concentrations in patients compared with controls. Plasma BDNF negatively correlated with pulmonary embolism severity score. Among patients, those who died had lower BDNF levels compared with those who survived	[81]

BDNF results are expressed as mean ± SD or median (IQR) unless otherwise specified.

BDNF, brain-derived neurotrophic factor; BMI, body mass index; CVD, cardiovascular disease.

[a]Data are reported as geometric mean (95% CI).

[b]Data are reported as mean ± SEM.

participants in the highest quintile of serum BDNF being significantly protected in comparison with those in the lowest quintiles [62]. Other studies have reported higher CVD incidence and/or poor recovery outcomes associated with low BDNF levels in circulation [55,64,65,67,82]. A recent systematic review and meta-analysis has revealed lower serum BDNF levels in stroke patients compared with healthy controls, along with a negative correlation between stroke severity and BDNF levels [83]. The mechanisms potentially underlying these associations are not well-understood and include modulation of underlying cardiovascular risk factors. For example, circulating BDNF has been shown to be negatively associated with triglyceride, low-density lipoprotein cholesterol, and fibrinogen levels and positively associated with high-density lipoprotein cholesterol levels in CVD patients [84]. There are also associations between BDNF levels and food uptake and adiposity, metabolic activity of adipose tissue, and risk of diabetes, all known to influence risk of future cardiovascular events [85–87].

Some of the strongest evidence of the importance of BDNF to vascular health has come from studying the impact of its genetic variations on CVD. While there are many reported single nucleotide polymorphisms in the human *BDNF* gene, the best-characterized variant is *rs6265*. It is a functional variant in the exon 2 of the *BDNF* gene, which is located on the short arm of chromosome 11 (11p13) and is comprised of 11 exons and 9 functional promoters. The *rs6265* single nucleotide polymorphism results in a valine to methionine substitution at position 66 (Val66Met; g.27658369C>T) in the prodomain of the protein. It shows marked differences in allele frequency between world populations, with the derived Met allelic frequency ranging from 0 to 72% across populations [88]. The Met allele has been shown to reduce neuronal activity–induced BDNF secretion by approximately 25% due to impaired intracellular trafficking secondary to decreased binding to sortilin (a *Vsp* 10-p domain sorting receptor family member) [89–92]. A Mendelian randomization analysis performed in the CAD Genome-Wide Replication and Meta-Analysis consortium (>22,000 CVD cases and >60,000 controls) revealed that the T allele of *rs6265* encoding for Met was associated with 0.772 ng/mL higher serum BDNF levels and that higher serum BDNF levels were associated with a decreased risk of future cardiovascular events and mortality [62]. Similarly, evaluation of a large cohort in the Catheterization Genetics study from Duke University showed that homozygous subjects of the Val allele had a higher risk than Met allele carriers for clinical CVD [93]. The Val/Val genotype was also found to be associated with greater CVD incidence and severity in a cohort of 5510 patients of European origin, while no association of the Met allele with CVD was observed in another European cohort [93,94]. Many other studies reported significant association between the Met allele and CVD incidence and/or poor recovery outcome either in mice or in humans [95–99]. Together, these findings suggest that lower BDNF levels, which can, in part, be ascribed to genetic variations, are associated with adverse cardiovascular risk.

8 | BDNF: CONNECTING BLOOD, BRAIN, AND HEART?

While CVD and dementia share common risk factors, the mechanistic pathways underlying the heart-brain connection are not clearly understood. There is accumulating evidence that platelets could be used as biomarkers of cognitive health and could potentially be used as vectors of neurotrophic factors.

In a study on 1385 participants, divided roughly equally between those with an established CAD and those without, Bélanger et al. [52] investigated whether BDNF levels intercede in the relationship between platelet activation and cognitive function and whether this relationship is moderated by the presence of CAD. Whereas platelet hyperactivity is generally deleterious in CAD and is associated with worse cognitive outcomes, platelets also release vast amounts of BDNF upon activation, a neurotrophin protective against cognitive decline. Using mediation modeling, BDNF levels were found to be mitigating the association between platelet hyperactivity and cognitive decline, with BDNF having a weaker protective effect on cognitive functions in participants with CAD than in controls [52]. This suggests that platelet-released BDNF may provide a protective effect against cognitive decline. In support of this, Weinstein et al. [100] have shown in the original Framingham cohort (5209 participants aged 28-62 years with normal cognitive performance at recruitment) that lower serum BDNF levels preceded the onset of cognitive impairment over 10 years of follow-up. As serum BDNF levels are a reflection of platelet stores of BDNF [38,47], this opens the possibility that boosting circulating BDNF levels could lead to better cognitive outcomes.

9 | INTERNATIONAL SOCIETY ON THROMBOSIS HAEMOSTASIS CONGRESS REPORT

Several abstracts bridging the gap between the brain and the circulation were presented at the International Society on Thrombosis and Haemostasis Congress 2023 in Montreal. We will briefly discuss them in this section.

In her Alan Giles Memorial Lecture, Katerina Akassoglou presented innovative research linking the brain and blood together. Akassoglou's work demonstrated that when the blood-brain barrier is disrupted, fibrinogen has the potential to leak into the brain through the damaged vessels, where it induces neuroinflammation, promotes neural toxicity, and inhibits myelin repair. This fibrinogen leakage is one of the contributors to the cognitive decline associated with stroke and neurodegenerative diseases such as Alzheimer's disease.

Eyileten et al. [101] have analyzed microRNA (miRNA) levels of patients with ischemic stroke compared with similar controls. Interestingly, they have found a number of miRNAs to be modified in

ischemic stroke, with top upregulated miRNAs being associated with serum BDNF levels, supporting a role of serum BDNF in stroke outcomes. They concluded that miRNAs and their regulated pathways have a potential diagnostic and prognostic utility in neurovascular diseases.

In their study, Boukhatem et al. [102] showed the presence of different neurotrophins in human platelets. Indeed, platelets were shown to express a precursor form of NGF (proNGF) and of NT-3 (proNT3), as well as their putative Trk receptors (TrkA and TrkC). However, in contrast to BDNF, NGF and NT3 failed to induce platelet activation and aggregation, speaking to the question of the role of TrkA, TrkC, and the proneurotrophins in human platelets.

Similarly, Fleury et al. [103] have investigated the involvement of the low-affinity receptor for BDNF, namely $p75^{NTR}$, in platelet responses to BDNF. The study highlighted high variability in the levels of $p75^{NTR}$ expression, with a 10-fold difference between the lowest and highest values in platelets. The study showed that inhibition of the $p75^{NTR}$ receptor with a pharmacologic agent hindered platelet responses to BDNF in a minority of participants, suggesting a limited role for $p75^{NTR}$ in BDNF-induced platelet aggregation.

Exploring the question of the bioavailability of BDNF in plasma and its association with BDNF-induced aggregation, Jourdi et al. [104] presented that as little as 25% of plasma completely abolished platelet responses to BDNF. This inhibition was explained by reduced bioavailability of BDNF through binding to α_2M and preventing BDNF from binding to its receptor, TrkB, thus limiting the platelet responses to BDNF.

In another study to explore the contribution of BDNF to hemostasis and thrombosis *in vivo*, Jourdi et al. [105] explored the effect of sex on BDNF-induced platelet aggregation in rats. No differences were observed between male and female rats in terms of circulating BDNF levels. However, platelets pretreated with BDNF were significantly more sensitive to collagen and PAR-4 activation, but this effect was only seen in female animals. Intriguingly, blood clot firmness in the presence of BDNF was reduced, but also only in females. This sex-specific effect requires further investigation.

Finally, Boukhatem et al. [106] presented their study on the megakaryocytic origin of platelet BDNF. CD34+ cells isolated from adult peripheral blood were differentiated into megakaryocytes, where BDNF expression and its role were investigated. CD34+-derived megakaryocytes expressed BDNF, proBDNF, and their receptors TrkB and $p75^{NTR}$. During megakaryopoiesis, BDNF was released only at later stages by mature megakaryocytes, in line with previous reports [44,45]. Together, these data suggest a megakaryocytic origin of platelet and circulating BDNF.

10 | FUTURE DIRECTIONS

Circulating and platelet-derived BDNF is believed to be involved in an array of physiological and possibly pathological processes. Nonetheless, a multitude of questions remain to be investigated and are crucial for a better understanding of the platelet BDNF field.

The apparent paradox of BDNF being released upon platelet activation and further promoting platelet responses to classical agonists while being reported as potentially protective against CVDs postulates a possible duality in its effect on hemostasis and thrombosis. Indeed, it could be argued that BDNF secretion is beneficial when it occurs in the context of bleeding, where more sustained platelet activation and secretion of inflammatory, chemiotactic, and angiogenic factors could further contribute to wound healing and vascular regeneration. Increased BDNF levels in circulation on the other hand could lead to reduced vascular burden, either through lifestyle factors such as regular exercise or through genetic predisposition, as well as potentially reduced thrombotic burden through facilitated formation of more porous thrombi. Overall, this apparent paradox remains a challenge to the field that highlights the need for studies to better understand the interplay among the different factors known to affect peripheral BDNF levels.

Most studies that have investigated BDNF in platelets have looked at the importance of its release to hemostasis and thrombosis [18,102,106]. However, the report of the presence of BDNF and its receptors in megakaryocytes and their ability to release BDNF into their surrounding milieu prompts further investigation into the role of BDNF in the bone marrow. Indeed, BDNF has been shown to have a significant impact on the differentiation, development, and survival of various types of stem cells such as differentiation of hematopoietic stem cells into endothelial cells and that of mesenchymal stem cells into neural cells [107,108]. Future investigations should consider whether megakaryocyte-released BDNF acts on other TrkB-expressing cell types in the bone marrow niche.

Circulating BDNF levels are altered in multiple neurologic and neurodegenerative afflictions. Although it is not clear whether dysregulation of BDNF levels is an integral part of these pathologies or a result of the latter, the negative association between low BDNF levels and cognitive disorders has led scientists to consider BDNF as a potential therapeutic agent. While invasive cerebral injection of BDNF yielded good results in animal models, its therapeutic potential comes up short mostly due to BDNF's poor physiochemical properties, especially its excessively short half-life and its inability to effectively cross the blood-brain barrier [109–111]. Together with the emergence of nanomedicine, the idea of encapsulating BDNF in nanovectors has arisen [112]. Recently, Arora et al. [113] have shown that the intravenous injection of BDNF plasmid contained within brain-targeting liposomes alleviated amyloid β production and plaque load in an Alzheimer's mouse model. These results suggest that nanovectors could be an efficient way to work around the limiting physiochemical attributes of BDNF.

While wild-type mice lack significant expression of BDNF in their platelets, Want et al. [46] have generated a transgenic mouse line that expresses BDNF in platelets at levels similar to those found in humans. These transgenic mice displayed less retinal ganglion cell dendrite loss following optic nerve crush compared with controls not expressing BDNF in their platelets [46]. These results suggest that platelets, which store impressive amounts of BDNF and secrete it at convenience, could be used as a vector, allowing targeted

BDNF delivery to the central nervous system. While the underlying mechanisms remain unknown, transport of BDNF via platelet-derived extracellular vesicles is currently being considered [46]. Similarly, platelet-inspired carrying nanovectors are also under investigation. Currently developed as a tool to support thrombus formation, these particles are engineered to mimic important platelet physiological and functional characteristics and ultimately support hemostasis [114,115]. However, such particles can cross the blood-brain barrier and have recently been used to successfully deliver fumarate to the brain in a mouse model of multiple sclerosis [116,117]. As such, platelet-like nanoparticles and perhaps platelets themselves could represent an interesting vector to deliver BDNF to the brain, a postulate ripe for further *in vivo* investigation.

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AUTHOR CONTRIBUTIONS

I.B., S.F., and G.J. designed the article and wrote the first draft of the manuscript. M.L. supervised the research group and critically revised the manuscript.

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