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REVIEW

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Apelin-13 as a novel diagnostic laboratory biomarker in thromboembolic disorders: a review of literature with prospective insights

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Abstract

Thromboembolic disorders, including deep vein thrombosis (DVT) and pulmonary embolism (PE), are major global health concerns, causing significant morbidity and mortality. Early diagnosis is crucial for effective treatment and improved patient outcomes. Recent research has identified Apelin-13, a bioactive peptide in the apelin family, as a promising diagnostic biomarker for Thromboembolic disorders. Apelin-13 supports vascular health by regulating protease balance through plasminogen activator inhibitors and modulating endothelial cell function. Additionally, it plays a vital role in coagulation, with elevated levels associated with an increased risk of clot formation, suggesting its utility in predicting thrombosis risk, particularly in preoperative evaluations. Findings indicate that the Apelin-13 pathway shows significant promise as a biomarker for Thromboembolic disorders, underscoring its potential therapeutic applications and the need for further investigation. This review synthesizes current literature on thromboembolic disorders and associated laboratory biomarkers, with a particular focus on Apelin-13. It examines Apelin-13's role in disease mechanisms, its physiological functions, and its potential as a diagnostic biomarker in thromboembolic conditions.

Keywords Apelin-13, Biomarker, Thromboembolic, Thrombosis, Embolism, Cardiovascular, Hematology

Introduction

Thromboembolic (TE) disorders, which include deep vein thrombosis (DVT), pulmonary embolism (PE), and stroke, are major worldwide health problems because they have high rates of illness and death [1]. Prompt and precise diagnosis is essential for efficient management and treatment of many disorders. Nevertheless, existing diagnostic approaches frequently depend on a blend of clinical evaluations, imaging modalities, and recognized biomarkers, which may not consistently offer enough sensitivity or specificity. This has stimulated continuous investigation into innovative biomarkers that could augment diagnostic precision and better patient outcomes [2].

Recently, Apelin-13, as a biologically active peptide of the apelin family, has been considered an attractive biomarker in TE disorders [3]. The precise role of Apelin-13 in the development and diagnosis of TE disorders has yet to be completely understood despite its involvement in these important physiological processes [3–5].

This review examines the physiological role of Apelin-13 and its potential as a diagnostic biomarker for TE disorders. It reviews published studies to understand its function and efficacy, as well as its potential to enhance current diagnostic methodologies for treating

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TE conditions, complementing or improving existing treatments.

A short overview of thromboembolism disorders

The term "thromboembolism disorders" (TE) refers to a group of conditions characterized by the formation of blood clots, known as thrombi. These clots can obstruct blood vessels, leading to the occurrence of emboli, which happen when a clot travels through the circulatory system and lodges in a different location [6].

Venous thromboembolism (VTE) is a TE event that occurs in the venous system, which is divided into DVT and/or PE [7]. It is the third leading cause of vascular death after heart attack and stroke [8, 9]. The most concerning fact about this disorder is that 60% of VTE cases are hospital-acquired, making VTE the most common preventable cause of death in hospitalized patients [10, 11].

It seems that variable factors such as age, gender, race, and medical conditions have caused differences in the incidence of VTE in geographic regions [12].

The American Heart Association estimates the total number of VTE cases in the United States to be about 1,220,000 in 2021 [13]. Overall, the reported incidence of VTE in Europe and the United States is estimated to be about 1–2 per 1,000 people per year [14]. Among the US adult population, the lifetime risk of VTE is estimated to be 8% [15].

Thrombotic embolism is a dangerous disorder in which a thrombus develops, usually as a result of vascular damage, blood stasis, or hypercoagulability, and then breaks apart to move through the circulation as an embolus [16]. When an embolus becomes trapped in a smaller blood channel, it obstructs blood flow, causing tissue or organ damage from ischemia. The disorder comprises three primary processes: thrombus development, embolization, and vascular occlusion, and is regulated by conditions identified by Virchow's triad [16, 17]. Timely diagnosis and understanding of these pathways are required for effective thrombotic embolism therapy to avoid serious consequences.

VTE leads to the formation of thrombi, which are structures rich in platelets, red blood cells, leukocytes, and fibrin, in almost all venous districts, especially in the valve pockets and dilated sinuses of the lower extremities [18]. The formation and release of thrombus, known as Virchow's triad, includes blood flow stasis, hypercoagulability, disruption, and damage to endothelial function, which is an understandable way to represent the pathophysiology of VTE [19]. When the mentioned factors are present, the coagulation cascade, which is the series of enzymatic reactions, is activated, which initially leads to the formation of fibrin and the stabilization of platelets, leading to the growth and expansion of the thrombus and finally obstruct the blood flow [20, 21].

VTE is often multifactorial, And the risk factors are conditions and behaviors that increase the possibility of blood clot formation and cause disruption of blood flow and movement and blockage of blood vessels. Traditional risk factors for VTE include conditions of immobility and prolonged inactivity, such as long-haul flights [22, 23], hospitalization [24], recent surgeries, especially knee and arthroplasty and abdominal and hip surgeries [25, 26], trauma, and fractures which cause restriction in movement [27–29], male sex [30], old age [31], obesity [32, 33], smoking [34], family history, and genetic conditions that affect blood clotting, such as Factor V Leiden (FVL) mutation [35], Prothrombin G20210A Gene Mutation (PGM) [36], and Plasminogen Activator Inhibitor-1 [37].

Also, disorders and diseases that cause hypercoagulability, including diabetes [38], cancer [39], antiphospholipid syndrome [40, 41], use of Oral Contraceptives and Hormonal Replacement [42, 43], pregnancy, thrombophilic disorders, chronic kidney disease, autoimmune disorders [44, 45], sickle cell anemia [46], and infection, including with viruses such as SARS-CoV-2 [47].

Thromboinflammation

Thromboinflammation is a complex process that involves the bidirectional relationship between thrombosis (blood clotting) and inflammation processes. In this state, inflammatory responses can trigger thrombosis, while thrombotic events can further exacerbate inflammation [48]. This vicious cycle can lead to severe complications in various diseases, including cardiovascular conditions and COVID-19 [49, 50].

Thromboinflammatory biomarkers play a pivotal role in the interplay between inflammation and thrombosis, with key contributors including Tissue Factor (TF), Plasminogen Activator Inhibitor-1 (PAI-1), Tumor Necrosis Factor-alpha (TNF-α), interleukins (IL-6, IL-1β), Angiopoietin, adhesion molecules (VCAM1, ICAM), and complement components (C3b, C5a) [51, 52].

TF initiates coagulation and is linked to hypercoagulable states such as those observed in severe COVID-19, while elevated PAI-1 impairs fibrinolysis [50], fostering thrombosis in conditions like Idiopathic Multicentric Castleman Disease (iMCD) [53]. TNF-α and interleukins drive pro-inflammatory and prothrombotic responses [54], while markers like Angiopoietin, VCAM1, and ICAM reflect endothelial activation. Complement system components amplify thromboinflammation, highlighting the interconnected roles of these biomarkers in vascular pathology [53].

Recognizing the role of thromboinflammation and its biomarkers is crucial in clinical care. Elevated levels of these markers can aid in diagnosing patients at higher risk of developing blood clots, while also

providing insights into disease severity and potential outcomes, especially in conditions like stroke and COVID-19 [55]. This understanding opens doors to targeted treatments, such as using statins to lower platelet cholesterol and tissue factor activity, offering promising new approaches to managing thromboinflammatory conditions [56].

Diagnostic biomarkers in TE

Although an optimal and clear strategy for the diagnosis of TE has not been determined, several new biomarkers for the diagnosis of TE have been identified and used in recent years, and more are under active investigation, ultimately leading to significant progress in the diagnosis of TE [57, 58]. A combination of biomarkers with the Wells score [59], can increase the diagnostic accuracy of TE in patients and guide decisions about further diagnostic interventions.

Some of the biomarkers utilized in the diagnosis of TE include D-dimer, P-selectin, and fibrin degradation products (FDP) [58, 60, 61]. These biomarkers, together with clinical history, physical examination, and imaging modalities, can aid in the diagnosis and monitoring of TE events.

D-dimer

D-dimer molecules are produced when cross-linked fibrin is broken down in the context of fibrinolysis [62, 63]. D-dimer measurement, along with clinical decision rules (CDRs), such as the Wells criteria [64], and the Geneva score [65], are valid and practical methods for the roll-out of VTE in patients [66, 67]. The Wells criteria and Revised Geneva score are clinical tools used to assess the probability of DVT or PE. They guide diagnostic testing and resource utilization, often combined with D-dimer testing and clinical judgment, to evaluate patients with suspected PE [68, 69]. D-dimer is generally a test with high sensitivity but low specificity. Many conditions lead to elevated D-dimer levels, including pregnancy, infection, malignancy, and post-surgical conditions [70, 71]. Also, even partial activation of the coagulation cascade involves increased fibrinolysis, resulting in elevated D-dimer levels [72], and thus can cause false-positive D-dimer results [62].

D-dimer testing may be contraindicated in certain conditions, such as when there is a high pre-test chance of PE or DVT and imaging is necessary regardless of the test result. Furthermore, situations such as pregnancy and cancer can boost D-dimer levels on their own, potentially leading to false results if utilized without further diagnostic procedures [73].

Serum Profılın-1

Serum Profilin-1's role in regulating the actin cytoskeleton and platelet function makes it a possible biomarker in TE disorders. Profilin-1 regulates actin polymerisation, which is an important component of platelet activation and aggregation, and hence plays a role in cellular processes that influence thrombogenesis [74]. Profilin-1 overexpression may enhance platelet reactivity and help in the formation of blood clots. Hence, greater protein serum levels have been associated with an increased risk of thrombosis. Profilin-1 has the potential to serve as a marker of endothelial dysfunction and vascular injury. These two elements are critical for the development of TE events such as PE and DVT. Profilin-1 may be useful in the management, risk assessment, and early detection of TE diseases [74–77].

A recent study evaluated the utility of serum Profilin-1 as a diagnostic and prognostic biomarker for PE. Among 102 PTE patients and 64 healthy controls, Profilin-1 levels were significantly higher in PE patients (median 2878 pg/mL vs. 579 pg/mL, $p < 0.001$), with a diagnostic sensitivity and specificity of 76.47% and 79.69%, respectively. Higher Profilin-1 levels were also associated with mortality, showing 90.91% sensitivity and 71.25% specificity for prognosis at levels ≥ 3292.1 pg/mL. The study findings suggest that Profilin-1 is a promising biomarker for PE diagnosis and prognosis [75].

Troponin

Troponin, primarily known as a cardiac biomarker for diagnosing myocardial infarction (MI), may also increase in TE conditions like DVT and PE [78]. Elevated troponin levels in PE are a result of right heart strain caused by increased pulmonary artery pressure. These elevated levels can help assess the severity of the embolism, as they are associated with right ventricular dysfunction. Higher troponin levels in PE patients are linked to worse clinical outcomes and increased mortality risk, making troponin a valuable prognostic biomarker for identifying high-risk patients and guiding therapeutic decisions [78, 79]. While troponin's role in PE prognostication shares some similarities with its use in cardiac events, understanding the nuances in its elevation patterns, magnitude, and clinical context is crucial for accurate interpretation and optimal patient management [80, 81]. Clinicians must consider the possibility of PE in patients with elevated troponin, especially when the clinical picture is not typical for acute coronary syndrome.

Soluble P-selectin

P-selectin is an adhesion glycoprotein found in the α-granule of platelets and the Weibel-Palade body of the

endothelium. Cell surface P-selectin helps leukocytes adhere to the thrombus, and platelet P-selectin helps to bind more leukocytes and propagate venous thrombosis [82, 83]. Studies support the possible involvement of P-selectin in the pathogenesis of TE [60, 84, 85]. In conclusion, it has been proposed as a biomarker with a diagnostic function for VTE. This biomarker, when combined with a Wells score ≥ 2, soluble P-sel > 90 ng/ml has shown a positive predictive value of 100% [86, 87].

Factor V Leiden

The Factor V Leiden mutation is a genetic variation that results in a mutant Factor V protein that is resistant to deactivation by activated protein C, increasing the likelihood of blood clot formation. This mutation significantly raises the risk of developing TE illnesses such as DVT and PE [88, 89]. Genetic testing can detect Factor V Leiden, a biomarker that allows for the early detection of persons who are prone to clotting difficulties. This information is crucial for risk classification and guiding preventive measures in individuals at high risk of TE events [88, 90].

FDP (fibrin degradation products)

Fibrin Degradation Products (FDP) are biomarkers that reflect the breakdown of fibrin, a crucial component of blood clots, and are, therefore, strongly linked to ET illnesses [61, 91]. Elevated FDP levels indicate enhanced fibrinolytic activity, which happens when the body attempts to remove blood clots and is important in detecting disorders such as DVT and PE [92]. High FDP levels indicate continuous clot production and breakdown, implying active TE processes. Clinically, FDP testing helps to diagnose and monitor the severity of TE episodes, as well as guide treatment approaches such as anticoagulant medication [92]. Some studies have shown that FDP is involved in the development and progression of atherosclerosis and thrombosis [93, 94].

Apelin family and its subtypes

The apelin family is a set of peptides synthesized from the apelin gene that plays an important role in a variety of physiological and pathological processes [95]. These peptides work as endogenous ligands for the apelin receptor (APLNR or APJ), a G protein-coupled receptor implicated in cardiovascular function, metabolism, and homeostasis [96].

Apelin peptides are derived from a 77-amino acid precursor known as preproapelin, which is processed into multiple bioactive isoforms of varying length [97]. These include Apelin-36, the longest active form; Apelin-17, a shorter and more powerful fragment; and Apelin-13, which has been intensively examined because of its great stability and activity. Additionally, [Pyr1]-Apelin-13, a pyroglutamated version of Apelin-13, has increased bioactivity and resistance to enzymatic degradation [97, 98].

Apelin is a natural ligand for the G protein-coupled receptor APJ, so the name apelin comes from the APJ Endogenous Ligand. The apelin/APJ system is widely distributed in vivo. The gene encoding Aplin (APLN), which encodes a 77 amino acid propeptide, is located on chromosome Xq25-26.1 [97]. Preproapelin is cleaved from its C-terminus to produce a mature apelin peptide (apelin-36,−17,−12, and −13), the latter of which also exists as a pyroglutamyl form, [Pyr1]apelin-13 [99]. Each group of subtypes has a different activity, and shorter subtypes lead to the activation of the APJ receptor more effectively; because of this, the activity of Apelin-13 and Apelin-17 is much stronger than that of Apelin-36, and the different binding affinities for their receptor determine the different APJ signaling pathways activated in cells [100, 101].

Apelin-13 and its physiological function

Apelin-13, the most biologically active isoform of the apelin family, is an endogenous peptide derived from the apelin gene (APLN). Among all apelin subtypes, Apelin-13 has the highest affinity for the Apelin receptor (APJ), a G protein-coupled receptor, through which it regulates various physiological processes in the body. Its biological significance makes it a key player in pathways involving cardiovascular function, fluid homeostasis, and energy metabolism [102–105].

Apelin-13 plays a diverse role in physiological processes, including reducing inflammation in cardiovascular and metabolic diseases [106], promoting vasodilation, regulating blood pressure, and enhancing heart function [107], making it a promising therapeutic target for conditions such as heart failure, hypertension, and metabolic disorders [108]. It supports angiogenesis, aiding tissue repair and growth [105], and improves insulin sensitivity while regulating glucose and lipid metabolism, contributing to obesity and diabetes management [104]. Apelin-13 also helps maintain fluid balance by influencing water and electrolyte levels, impacting blood volume and kidney function [109]. Additionally, it has roles in the central nervous system, including neuroprotection, appetite regulation, and stress response [110], as well as in reproductive processes like ovulation and placental development [104].

Apelin-13 is an important regulator of endothelial cell activity and vascular health. It has a wide range of actions on endothelial cells, contributing to a variety of physiological and pathological conditions [111]. Apelin-13 has been demonstrated to protect endothelium cells, especially when they are injured or under stress. Apelin-13 inhibits hydrogen peroxide-induced cell death in

endothelial cells by lowering intracellular reactive oxygen species (ROS) levels. This protective effect implies that Apelin-13 may aid to preserve endothelium integrity in the face of oxidative stress [112, 113].

Most studies agree that apelin/APJ's action on the cardiovascular system is due to its similarity to that of the angiotensin receptor. In the cardiovascular system, Apelin-13 leads to a decrease in blood pressure and modulates the contraction of heart tissue and blood vessels [114–116]. Also, about the effect of Apelin-13 on changes in blood pressure and heart rate studies have been conducted, and a decrease in blood pressure and an increase in heart rate have been reported [117, 118].

Apelin-13 can protect the heart against ischemic damage and reperfusion both in vivo and in vitro. this effect is associated with inhibiting apoptosis and ferroptosis and stimulating autophagy. Apelins also protect the heart from ischemic damage and reperfusion [119]. Also, in 2016, an in vitro study showed that Apelin-13 treatment may reduce myocardial fibrosis caused by myocardial infarction, and this cardioprotective effect may be mediated through the regulation of NF-κB signaling [120].

Apelin-13 plays a multifaceted role in vascular and organ health, influencing endothelial function and offering therapeutic potential. It promotes vasodilation in well-managed chronic kidney disease patients and may enhance the body's natural ability to break down clots [121]. In vitro studies suggest that Apelin-13 can reverse endothelial-to-mesenchymal transition (EndMT) and associated kidney fibrosis, offering renoprotective benefits by suppressing the TGFβ/Smad/CEBPA pathway [122]. However, Apelin-13 also increases MLC phosphorylation in vascular smooth muscle cells, leading to vasoconstriction and potentially worsening hypertension when the endothelium is damaged by ADMA [123]. Additionally, it has been shown to reduce pulmonary vascular permeability in mice with acute lung injury, likely through AMPK activation to improve mitochondrial function and autophagy [124]. These findings emphasize Apelin-13 as a complicated vascular regulator.

Figure 1 represents the physiological function of Apelin in the human body, and Table 1 presents animal studies investigating its physiological role.

Cellular-Molecular mechanism of Apelin-13 in thrombosis

Apelin-13 is also involved in the regulation of various physiopathological mechanisms such as apoptosis, inflammation, angiogenesis, oxidative stress, and energy metabolism [133].

In CVDs, Apelin shields the heart by curbing fibrosis, obstructing hypertrophy and clotting, boosting contractility, and alleviating inflammation and oxidative stress. It not only shrinks infarction size but also fosters

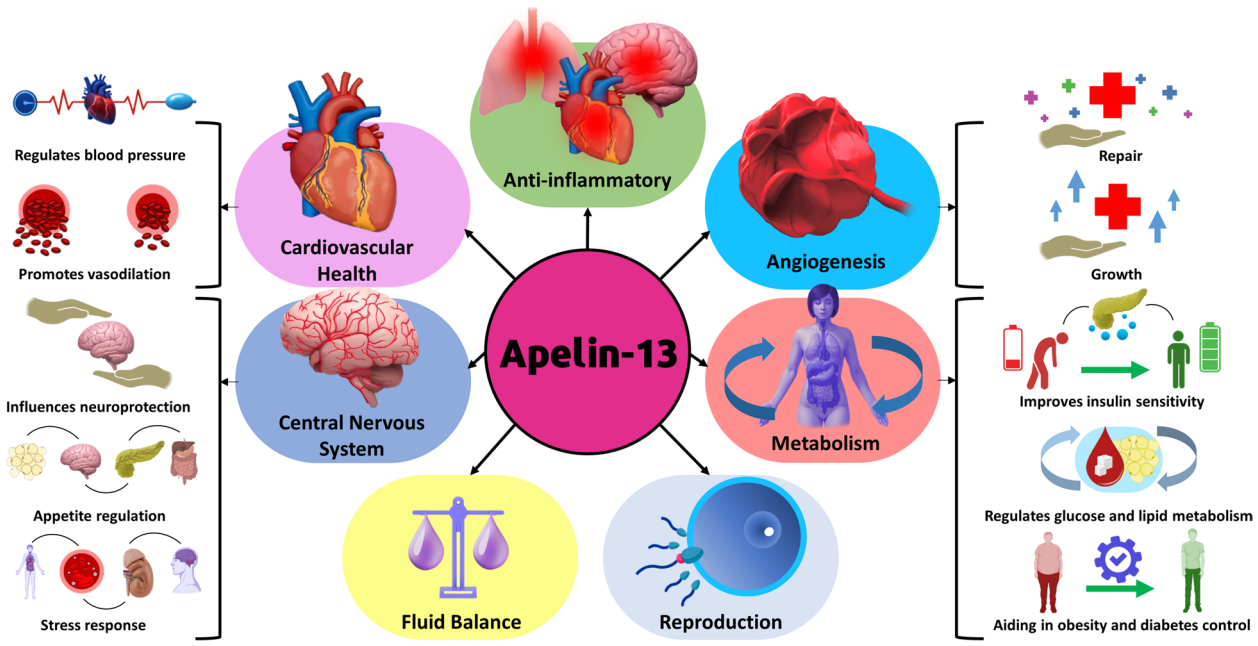

Fig. 1 The main physiological functions of Apelin-13. Key physiological functions of Apelin-13: (1) Anti-inflammatory effects in cardiovascular and metabolic diseases, (2) cardiovascular health via vasodilation, blood pressure regulation, and heart function improvement, (3) promotion of angiogenesis for tissue repair, (4) regulation of metabolism, improving insulin sensitivity and glucose-lipid balance, (5) fluid balance control affecting blood volume and kidney function, (6) neuroprotective roles in the central nervous system, including appetite and stress regulation, and (7) contributions to reproductive processes like ovulation and placental development

Table 1 Animal studies investigating the physiological role of Apelin-13

Species	Target of evaluation	Dose/Duration of Apelin-13	Method	Result	Conclusion	Ref.
Male Wistar rats	Cardiac muscle tissue	10 nmol/kg/d 5 days	I: Blood sampling was conducted at specified intervals post-myocardial infarction to assess serum levels of LDH, CK-MB, MDA, and NO. II: Myocardial infarct size and hemodynamic function were measured on day 14.	I: Reduces infarct size and serum biomarkers. II: Elevates heart rate and NO levels. III: No notable blood pressure differences were observed.	Apelin has long-term cardioprotective effects against myocardial infarction by attenuating cardiac tissue damage and lipid peroxidation and increasing NO production.	[125]
Male SD rats	Pulmonary artery	10 nmol/kg/d 3 weeks	mPAP, RVHI, lung tissue morphology, and LC3 protein detection, as well as mRNA levels of P62 and Beclin-1, and expressions of LC3, LC3-II/I, and P62 were measured.	I: Decreased mPAP II: Reduction of RVHI	Apelin may prevent pulmonary arterial hypertension by inhibiting autophagy.	[126]
ApoE-male/- rat	Carotid vascular endothelial cells	2 mg/kg/d 3 weeks	Atherosclerotic plaque formation was induced in the carotid artery, and Mice were treated with apelin-13 (2 mg/Kg/day) or vehicle for the last 3 weeks	I: Decreased inflammatory response. II: Reduction of total cholesterol. III: Decreased low-density lipoprotein. IV: Reduction of free fatty acid	Apelin-13 did not alter the lesion size but stabilized atherosclerotic plaques and improved lipid profiles. Activating the Aplin system reduces plaque vulnerability.	[127]
Albino rats	System evaluation	2×6×10−8 mol/Kg/d from 6th to 20th day of pregnancy	I: Measurement of mean arterial blood pressure, total urine protein, serum urea, creatinine, NO, ET-1, IL-6, and MDA II: istopathological investigation of kidney tissue	Maintain uterine perfusion pressure	Apelin treatment notably enhanced blood pressure, urine proteins, serum urea, creatinine, ET-1, IL-6, NO levels, and MDA while improving kidney histoarchitecture in the treated group.	[128]
Healthy albino rats	Renal vessel	(6×10−8 mol/kg body-weight/twice d) day 6 to 20 of gestation	I: blood pressure and urine protein at GD 0, 10, and 18 were determined II: serum apelin, PLGF, VEGF, sFlt-1, sEng, IFN-γ, and IL-10 levels and serum SOD enzyme and CAT activities estimated III: Placental histopathological examination performed.	I: reduction of blood pressure II: Increase in ejection fraction III: Decreased proteinuria	the protective role of apelin in preeclampsia pathogenesis is examined. Apelin may confer benefits through angiogenic balance restoration, antioxidant enhancement, and inflammation inhibition.	[129]
Male Wistar rats	Cerebral	Three treatment groups (MCAO + apelin-13 at 10, 20, 40 µg/kg)/ injected 5 min before reperfusion.	I: Neural loss and infarct volume were assessed using Nissl and TTC staining. II: Neurological deficits were scored by modified criteria. III: Serum NO was measured colorimetrically	Apelin-13 (20, 40 µg/kg) reduced neural death, infarct volume, and sensory-motor issues ($p < 0.05$) and normalized serum NO levels at 20 µg/kg ($p < 0.05$) in MCAO groups.	Apelin-13 IV injection improves sensory-motor balance by reducing neural degeneration and restoring serum NO levels, effectively treating ischemic stroke.	[130]

Table 1 (continued)

Species	Target of evaluation	Dose/Duration of Apelin-13	Method	Result	Conclusion	Ref.
Male Wistar rats	Cerebral	50 ng/kg, 10 µl per rat/ 2 h MCAO followed by 24 h reperfusion.	Neurological deficits and infarct volume were assessed with TTC staining. MPO activity and cytokines were measured by PCR, while APJ, Iba1, GFAP, and HMGB1 were evaluated using immunohistochemistry and western blot.	Apelin-13 in I/R rats improved neurological function, reduced infarct size, and lowered MPO, IL-1, TNF-, and ICAM-1 levels. APJ expression increased, while apelin-13 decreased Iba1, GFAP, and HMGB1, indicating less microglial and astrocytic activation.	apelin-13 exhibits neuroprotective properties for neurons in the context of ischemia/reperfusion by mitigating neuroinflammatory responses.	[131]
Male SD rats	lung	1 mg/kg of Apelin-13 was added in 5 ml saline/pumping time of 10 min	The effects of apelin-13 on LIRI were assessed histologically via H&E staining, and lung edema was measured by the wet/dry weight ratio. UCP2 protein expression and mitochondrial changes were evaluated using western blotting and electron microscopy.	Group IR showed lung damage with increased IL-1b, IL-6, and TNFa levels, indicating oxidative stress. In contrast, apelin-13 in group APL reduced these effects, with lower ROS and higher UCP2 expression.	Apelin-13 protects against lung ischemia-reperfusion injury by reducing edema, inflammation, oxidative stress, and mitochondrial dysfunction.	[132]

NO: nitric oxide; BP: blood pressure; LDH: lactate dehydrogenase; CK-MB: creatine kinase-MB; MDA: malondialdehyde; mPAP: Mean pulmonary artery pressure; RVHI: right ventricular hypertrophy index; IL-6: interleukin-6; ET-1: endothelin-1; GD: gestation days; PLGF: placental growth factor; VEGF: vascular endothelial growth factor; sFlt-1: soluble fms-like tyrosine kinase-1; sEng: soluble endoglin; IFN-γ: interferon-gamma; IL-10: interleukin-10; SOD: superoxide dismutase; CAT: catalase; IRI: ischemia/reperfusion injury; TTC: triphenyltetrazolium chloride; MCAO: middle cerebral artery occlusion; MPO: myeloperoxidase; TNF: tumor necrosis factor; IL-1: interleukin-1; ICAM-1: intercellular adhesion molecule-1; APJ: apelin receptor; Iba1: ionized calcium-binding adapter molecule-1; GFAP: glial fibrillary acidic protein; HMGB1: high mobility group box 1; ROS: reactive oxygen species; SD: Sprague-Dawley;

revascularization post-myocardial infarction (MI). Nonetheless, cardiovascular ailments coincide with diminished apelin and heightened Angiotensin-Converting Enzyme 2 (ACE2) levels in the bloodstream [134].

In an animal study, the Apelin-deficient mice exhibited advanced heart failure (HF) and demonstrated diminished tail-bleeding time alongside heightened thrombus formation, a condition that intravenous Apelin-13 administration can facilitate [135]. This in vivo experiment cannot rule out the possibility that plasma apelin or apelin from ECs near thrombosis may be involved in platelet function, but in vitro, apelin inhibits Ca2 + mobilization mediated by collagen and thrombin receptor activation. Additionally, the APJ is expressed on the outer membrane of resting human platelets, and when platelets become active, the APJ is released with platelet-derived microparticles [136]. The depressed aggregation that apelin caused can be offset by L-NAME, suggesting that apelin's antithrombotic effects are mediated by platelet-derived nitric oxide (NO). The research on the antithrombotic effect of apelin has just started, but it undoubtedly provides a new avenue for the regulatory role of apelin in the cardiovascular system [134].

In vascular diseases, the apelin pathway enhances NO levels, reduces reactive oxygen species (ROS) production, and mitigates the RAS system by promoting ACE2 expression and inhibiting AT1R signaling through APJ heterodimerization. ACE2 functions in a feedback loop to cleave apelin peptides while converting Ang II into vasoprotective Ang (1–7) via the RAS system. This pathway promotes angiogenesis, restores endothelial integrity, and inhibits platelet activation [137, 138]. ACE2 degrades not only native apelin peptides but also other peptidases, including NEP and KLKB1. Consequently, metabolically stable apelin analogs and non-peptide APJ agonists are proposed as alternatives to regulate the apelin pathway in vascular diseases [134].

Recent research indicates that Apelin-13 inhibits foam cell formation in cultured cells through the PI3K/Beclin-1 autophagic pathway [139]. Apelin mitigates TAC-induced aortic adventitial remodeling and fibrosis by suppressing miRNA122/CTGF/NFAT5 and LGR4/β-catenin signaling pathways [140]. Apelin mitigates obesity from diet by improving vascular integrity, and statins exhibit atheroprotective properties by activating the apelin/APJ pathway [141, 142]. It is significant to note that apelin inhibits platelet activation induced by thrombin and collagen, functioning as a robust antithrombotic agent [135]. Thus, it protects against thrombotic events.

The Apelin mechanism in preeclampsia operates by enhancing hemodynamic parameters and promoting angiogenesis while concurrently reducing ROS, proteinuria, and both renal and hepatic injury [105, 143]. Apelin increases insulin sensitivity, endothelial NO synthase (eNOS), AMPK, and Akt in insulin resistance. It also reduces inflammatory markers, adiposity, and glucose levels [144]. The mechanism of apelin in kidney disease operates through enhancing Akt/eNOS, renal perfusion, and catalase levels while concurrently diminishing apoptosis, TGF-ß, inflammation, and oxidative stress [121]. The apelin pathway in pulmonary arterial hypertension (PAH) operates by enhancing the levels of eNOS, BMPR2, and miR-424/503 while simultaneously reducing the proliferation and migration of pulmonary artery smooth muscle cells (PASMC) and fibroblasts, as well as alleviating right ventricular overload [145, 146]. The Apelin mechanism in erectile dysfunction operates by augmenting intracranial pressure, phosphorylated eNOS, and matrix metalloproteinase (MMP) activity while concurrently diminishing ROS, apoptosis, penile fibrosis, and the differentiation of fibroblasts [147]. In the aforementioned pathological states, the enhancement of the apelin pathway—achieved through the administration of native apelin peptides, apelin analogs, or non-peptide APJ agonists facilitates an increase in eNOS-mediated NO production, mitigates oxidative stress, decreases the secretion of inflammatory mediators, protects against apoptosis, and inhibits pathological remodeling.

In summary, it can be stated that Apelin exhibits a significant antagonistic effect on the action of vasoconstrictors, a phenomenon which is achieved through the inhibition of the formation and activity of reactive oxygen species, while simultaneously promoting the enhanced synthesis of NO, a vital signaling molecule in the vascular system [148, 149].

Figure 2 represents the biochemical pathways of how apelin acts.

Apelin-13: a novel diagnostic biomarker in TE

Studies have shown that serum levels of Apelin-13 are elevated in individuals with thromboembolism, suggesting its involvement in the prothrombotic cascade and potential use as a diagnostic biomarker for this condition [5, 150, 151].

In recent studies, the sensitivity and specificity of Apelin-13 as a biomarker for diagnosing TE diseases have been considered. The cutoff point for Apelin-13 in diagnosing TE disorders can vary depending on the study and the population being examined. However, many studies aim to identify a threshold concentration that provides the best balance between sensitivity and specificity for diagnosing conditions like VTE or PE [5, 152].

Apelin-13 levels in serum can be measured using various methods, with enzyme-linked immunosorbent assay (ELISA) being the most widely used due to its balance of specificity, sensitivity, and ease of use. Radioimmunoassay

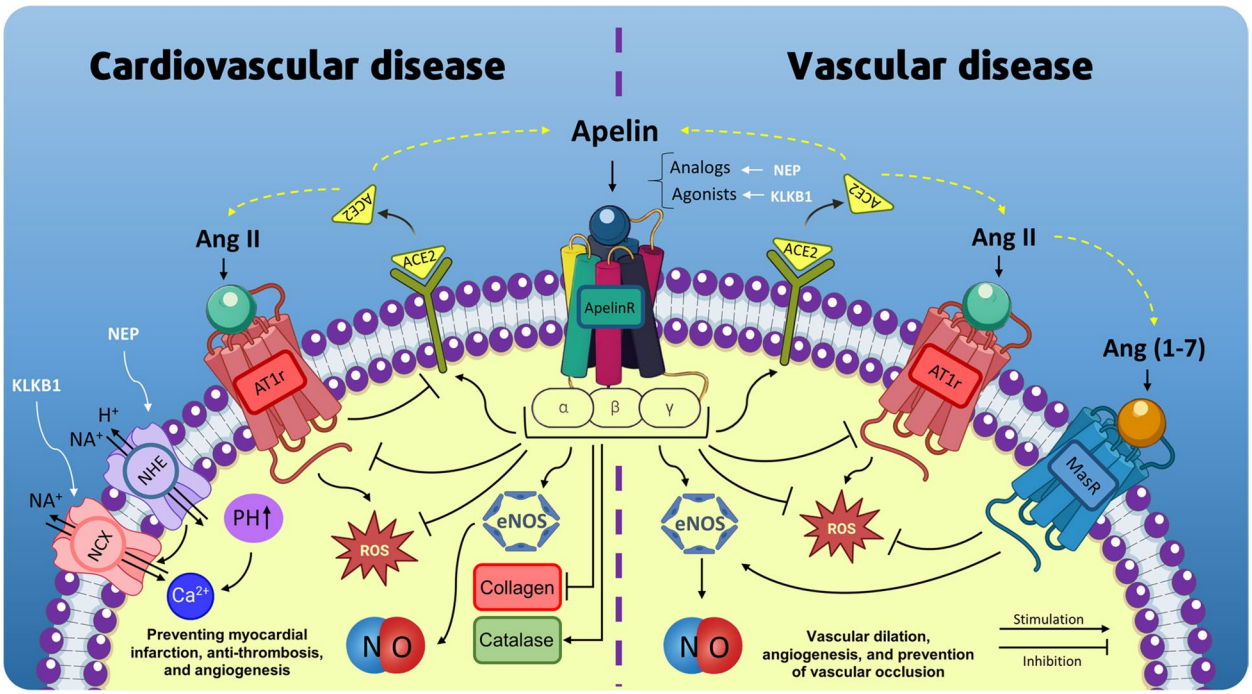

Fig. 2 Cellular and molecular mechanisms of Apelin action in cardiovascular and vascular diseases. Apelin enhances ACE2 expression and inhibits AT1R signaling, reducing reactive oxygen species (ROS) production. This promotes the conversion of Ang II to Ang (1–7), a cardio-protective factor that stimulates nitric oxide (NO) production via eNOS and the MasR pathway. These processes lead to blood vessel dilation, angiogenesis, and prevention of vascular occlusion

(RIA) is another immunoassay option, though it is less commonly employed. For highly precise and specific measurements, liquid chromatography-mass spectrometry (LC-MS) is a powerful alternative, offering exceptional accuracy for Apelin-13 quantification [77, 153]. Accurate measurement of Apelin-13 in serum requires careful consideration of several factors. Proper sample handling, including collection, storage, and processing, is essential to prevent degradation. The analytical method must have high specificity to distinguish Apelin-13 from other apelin isoforms and sufficient sensitivity to detect physiologically relevant concentrations. Standardization using appropriate standards and quality controls is crucial for reliable quantification. Additionally, pre-analytical variables such as fasting status, time of day, and other physiological factors should be accounted for in the study design to ensure accurate and consistent results [77, 153].

Apelin-13 demonstrated superior sensitivity and specificity in comparison to D-dimer, indicating its prospective role as a novel diagnostic biomarker for PE. Additional investigations within this domain are imperative to validate these results and to explore the clinical applicability of Apelin-13 as a diagnostic instrument for PE. Apelin-13 has the potential to facilitate earlier diagnosis and prompt initiation of therapeutic interventions in affected individuals across various age demographics and genders [5]. In the investigation conducted by Kartas et al., the optimal cut-off value for Apelin-13 in both the patient and control cohorts was determined to be 1579 ng/ml through the utilization of receiver operating characteristic (ROC) curve analysis. The sensitivity of the assay was recorded at 92.7%, while the specificity was noted to be 96.7%. This investigation elucidated that the concentration of Apelin-13 in serum may serve as a novel diagnostic biomarker for individuals afflicted with PTE [151].

Recent studies have shown that serum levels of Apelin-13 are associated with adverse outcomes in patients with reduced ejection fraction HF and may be useful biomarkers in determining the prognosis of these patients [154, 155]. Also, preclinical and clinical studies have shown that disturbances in the Aplin pathway, contribute to the development and pathophysiology of diseases, especially CVDs [156, 157].

Considering the aforementioned roles of apelin, current developments in this field also focus on harnessing its therapeutical potential. Applications in a broad array of disorders, such as preeclampsia [105, 143], insulin sensitivity [158], insulin resistance [144], atherosclerosis [156, 159], aortic aneurysms [160], MI, HF [156], kidney disease [121], PAH [145], and ED [147], have been explored with promising results.

In a recent study, 52 participants suspected of PE were examined. Findings from CT angiography classified participants into two groups: those with PE and those without. The serum D-dimer and Apelin-13 biomarkers were then analyzed and compared between the groups. The researchers concluded that Apelin-13 serum levels may act as a novel diagnostic biomarker for PE [5].

Also, in another recent research, 124 individuals were examined, comprising 94 PE cases and 30 healthy controls. PE was identified using chest computed tomography angiography. Post-diagnosis, Apelin-13 levels were assessed via enzyme-linked immunosorbent assay (ELISA). The study revealed no significant variance in Apelin-13 levels between patient and control groups or among death risk categories, indicating it is not a reliable biomarker for PE diagnosis [150].

In a separate investigation conducted in recent years, 53 patients with PE and 35 healthy controls were examined. The control group comprised healthy volunteers undergoing routine health checks. The serum levels of Apelin-13 were measured in both groups, followed by a comparative analysis. The researchers found significantly higher Apelin-13 levels in PE patients. These results suggested that apelin may be a valuable biomarker and therapeutic target for acute PE in future research [152].

In another study, 142 patients were analyzed, comprising 82 with PE and 60 controls. Serum Apelin-13 levels were assessed via venous blood samples using an ELISA kit. The findings indicate that serum Apelin-13 may serve as a novel diagnostic biomarker for PTE. Additionally, Apelin-13 levels were elevated in patients with DVT. These findings imply the potential use of Apelin-13 as a biomarker for acute PTE and DVT in future research [151].

Some biomarkers can be used to diagnose patients with TE, such as D-dimer, BNP, troponin, etc., but due to the widespread use of D-dimer, in most studies, this biomarker has been compared with Apelin-13 [5, 150, 152].

The quantity of serological biomarkers employed in the identification of PTE remains limited. D-dimer represents a non-invasive biomarker frequently utilized for the diagnosis of PTE, indicating the presence of endogenous fibrinolytic activation [161]. The sensitivity of D-dimer exhibits a considerable degree, whereas its specificity remains relatively low [162, 163]. Recent research indicates that Apelin-13 demonstrates superior sensitivity and specificity in patient diagnosis, suggesting its potential as an effective, novel biomarker [5, 151].

Table 2 presents a summary of human studies investigating the role of Apelin-13 as a biomarker for TE conditions.

Table 2. Diagnostic biomarker studies of Apelin-13 in TE.

Challenges and limitations

There exist multiple constraining variables. The investigators contend that investigations encompassing more extensive patient cohorts will unequivocally elucidate the correlation between PTE and Apelin-13. These findings, along with the significance of Apelin-13, are anticipated to enhance clinical methodologies in the future diagnosis of PE [151]. Research related to the relationship of Apelin-13 with TE disorders is limited; many of them are the first time to deal with this issue in different dimensions.

Prudence must be employed when interpreting plasma concentrations of apelin, particularly in individuals exhibiting diminished glomerular filtration rate (GFR), as the elimination of numerous peptides is influenced, and an elevation in arginine vasopressin (AVP) may intrinsically lead to a reduction in apelin levels [134].

The sample population size that was analyzed in this investigation was rather restricted. This limitation may elucidate the absence of statistically significant differences in serum Apelin-13 levels between the cohort of patients who survived and the subgroup of those who did not survive within the entire population. Nevertheless, the researchers contend that the constrained size of the study does not appear to affect the fundamental observations derived from the research detrimentally [165].

The abbreviated half-life of native apelin peptides, approximately 5 min, is attributable to swift proteolytic degradation facilitated by specific peptidases [166, 167], including ACE2 [118], plasma kallikrein (KLKB1) [168], and neprilysin (NEP) [169]. This phenomenon, in conjunction with the desensitization of the APJ [170], significantly limits the translational potential of this biological system, thereby necessitating an investigation into apelin analogs that exhibit resistance to endogenous degradation, as well as the development of non-peptide receptor agonists that demonstrate enhanced efficacy [134].

Conclusion and future directions

Table 3 represents a summary of the role, mechanism of action, diagnostic potential, challenge, and clinical application of Apelin-13.

Apelin-13 is a peptide involved in cardiovascular and metabolic regulation. Due to its multifaceted role in vascular biology and disease pathology, it holds promise as a modern diagnostic laboratory potential biomarker for TE disorders, including PE, DVT, stroke, etc [107, 171]. Its ability to modulate endothelial function, inhibit platelet aggregation, and influence the coagulation cascade positions it as a valuable tool for early diagnosis, risk assessment, and monitoring of thrombotic diseases. Its combination with other markers like

Table 2 Diagnostic biomarker studies of Apelin-13 in thromboembolic disorders

Author / Ref.	Patients' basic characteristics	Results	Conclusion
Selimoglu et al. 2016 [152]	- Case: 53 patients with PE (median 57 y) - Control: 35 healthy volunteers (median 53 y)	The serum level of Apelin-13 in the PE group (76.94 ± 10.70 ng/mL) was reported to be significantly higher than the control group (50.01 ± 7.13 ng/mL; $P < 0.001$)	Apelin-13 levels are elevated in patients with PE. Apelin may be a new biomarker and a possible potential therapeutic target in patients with acute PE.
Karataş et al. 2018 [151]	- Case: 82 patients with PTE (age: 71.0 ± 14.9) (22 with DVT, 60 without DVT) - Control: 60 (age: 66.2 ± 8.1)	Serum Apelin level: - PTE group: 2219.4 +/- 65.2 ng/mL DVT (+) group: 2495.8 +/- 738.0 ng/mL DVT (-) group: 2118.0 +/- 496.8 ng/ml - Control group: 1234.7 +/- 35.5 ng/mL	The amount of serum Apelin-13 increased in patients with acute PTE and DVT. Apelin-13 can potentially be a novel biomarker in patients with acute PTE and DVT.
Wang, X., et al. 2020 [164]	- Before propensity score matching: Case: 244 AIS patients (age: 60.55 ± 11.86) Control: 167 healthy (age: 59.66 ± 6.74) - After propensity score matching: Case: 110 AIS patients (age: 60.59 ± 12.65) Control: 110 healthy (age: 59.27 ± 6.89)	Serum Apelin-13 levels were significantly decreased in patients with PTE versus healthy controls. - Follow-up: A 3-month follow-up indicated a significant link between apelin-13 and mortality or major disability. At 1-year follow-up, patients with high apelin-13 levels demonstrated a lower incidence of stroke and combined events.	Low serum apelin-13 levels were associated with increased severity, higher risk of death or major disability at 3 months, and a higher incidence of recurrent stroke and combined events at 1 year in acute ischemic stroke patients, highlighting its potential as a prognostic biomarker.
Goidescu, C.M., et al. 2021 [154]	- Case: 53 with HF (age: 67.94) - Control: 13 (age: 55.38)	14 patients (24.52%) exhibited adverse clinical progression. I: ACE2 levels exceeding 4000.75 pg/mL II: apelin-13 levels below 402.5 pg/mL correlated with poor clinical outcomes. II: only ACE2, Apelin-13, NT-proBNP, and hsCRP presented statistically significant values, indicating that they can predict evolution.	Elevated ACE2 levels (> 4000.75 pg/mL) and low apelin-13 levels (< 402.5 pg/mL) were identified as independent predictive biomarkers of poor clinical outcomes in patients with reduced ejection fraction heart failure, highlighting their prognostic significance in disease progression.
Gergics, M., et al. 2023 [165]	124 critically ill patients (64 men, 60 women, median age: 70 (59–78) years)	Serum apelin-13 levels were lower in non-survivors compared to survivors. Notable positive correlations existed between apelin-13 and CRH, significantly elevated in surviving non-septic patients ($p < 0.05$ for both).	Higher serum apelin-13 levels were strongly correlated with survival in critically ill patients, serving as an independent prognostic marker alongside free cortisol, particularly in non-septic cases.
Baykal et al. 2024 [150]	- Case: 94 patients with PE (mean age: 68 y) - Control: 30 (mean age: 61.5 y)	Patients with PE showed elevated HIF-1 alpha levels compared to controls, with significantly higher levels in the high-mortality risk group, while NGAL levels were also elevated in high-mortality risk patients; however, Apelin-13 levels showed no significant differences across groups.	Apelin-13 was ineffective as a biomarker for differentiating PE patients from healthy controls.
Mehrban et al. 2024 [5]	52 individuals suspected of PE (22 men and 29 women, mean age 63.67 ± 10.00) - Case: 22 patients with PE - Control: 30 patients without PE	The D-dimer cutoff point was 500 ng/ml, with 95.5% sensitivity and 43.3% specificity. The Apelin-13 cut-off point was 58.50 ng/ml, with 90.9% sensitivity and 90% specificity. The mean level of Apelin-13 was significantly higher in patients with PE (49.8 to 73.11 ng/L) ($p < 0.001$)	Serum Apelin-13 and D-dimer levels significantly increased in patients with PE, (Apelin-13 showing promising sensitivity and specificity at a threshold of 58.50 ng/ml)

ϖ Abbreviations: pulmonary group (PE), PTE: pulmonary thromboembolism (PTE); acute ischemic stroke (AIS); heart failure (HF)

Table 3 Summarize of Apelin-13

Aspect	Details
Biomarker	Apelin-13
Type of Molecule	Peptide, a subtype of the Apelin family
Role in TE disorders	Potential novel biomarker for the diagnosis of TE disorders, including DVT, PE, and stroke.
Diagnostic Significance	are associated with endothelial function and cardiovascular health, making it a candidate for identifying TE conditions.
Mechanism of Action	interacts with the APJ, influencing vascular tone, blood pressure, and endothelial cell proliferation.
Current Research Status	Ongoing studies are exploring the utility of Apelin-13 in improving the sensitivity and specificity of TE diagnosis.
Advantages over Existing Biomarkers	It may offer enhanced diagnostic accuracy in conjunction with existing biomarkers and clinical assessments.
Challenges	Requires further validation in large-scale clinical trials to establish its diagnostic utility and standardize its measurement.
Clinical Applications	Potential use in early diagnosis, risk stratification, and monitoring of TE disorders.

TE Thromboembolism, *PE* Pulmonary Embolism, *DVT* Deep Vein Thrombosis, *APJ* Apelin receptor

D-dimer could enhance diagnostic precision, making it a valuable tool in clinical practice [5].

Exploring the potential of combining Apelin-13 with additional biomarkers may improve diagnostic accuracy for conditions such as PE, DVT, and other TE diseases. While Apelin-13 shows promise as a diagnostic tool, additional research is essential to fully understand its mechanisms and validate its application in a wide range of clinical settings and diverse populations.

To establish its diagnostic and prognostic value in clinical practice, further clinical research and validation studies are necessary to clarify findings and evaluate their clinical significance. Future investigations should focus on larger sample sizes and diverse populations to compare its specificity and sensitivity with traditional markers like D-dimer.

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Declarations

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References

1. Wendelboe A. J.I. Weitz 2024 Global Health Burden of venous thromboembolism. Arterioscler Thromb Vasc Biol 44 5 1007–11.
2. Liederman Z, Chan N, Bhagirath V. Curr Challenges Diagnosis Venous Thromboembolism J Clin Med, 2020. 9(11).
3. Yang P, et al. [Pyr(1)]Apelin-13((1–12)) is a biologically active ACE2 metabolite of the endogenous Cardiovascular peptide [Pyr(1)]Apelin-13. Front Neurosci. 2017;11:92.
4. Sun X, et al. Impact of Apelin-13 on the development of coronary artery Ectasia. Acta Cardiol Sin. 2020;36(3):216–22.
5. Mehrban A, et al. Evaluation of elevated serum apelin-13 and D-dimer concentrations in individuals diagnosed with pulmonary embolism. Int J Emerg Med. 2024;17(1):48.
6. Arnold MJ. Thromboembolic Disease. Prim Care. 2024;51(1):65–82.
7. McManus RJ et al. *Thromboembolism.* BMJ Clin Evid, 2011. 2011.
8. Klemen ND, et al. Mortality risk associated with venous thromboembolism: a systematic review and bayesian meta-analysis. Lancet Haematol. 2020;7(8):e583–93.
9. General OotS, National Heart L, Institute B. *The Surgeon General's call to action to prevent deep vein thrombosis and pulmonary embolism.* 2008.
10. Sidney S, et al. Age-adjusted mortality rates and age and risk–associated contributions to change in heart disease and stroke mortality, 2011–2019 and 2019–2020. JAMA Netw open. 2022;5(3):pe223872–e223872.
11. Grande Ratti MF, et al. Incidence of hospital-acquired venous thromboembolic disease. Rev Fac Cien Med Univ Nac Cordoba. 2018;75(2):82–7.
12. Lutsey PL, Zakai NA. Epidemiology and prevention of venous thromboembolism. Nat Reviews Cardiol. 2023;20(4):248–62.
13. Virani SS et al. *Heart disease and stroke statistics-2021 update: a report from the American Heart Association.* 2021.
14. Wendelboe AM, Raskob GE. Global burden of thrombosis: epidemiologic aspects. Circul Res. 2016;118(9):1340–7.
15. Bell EJ, et al. Lifetime risk of venous thromboembolism in two cohort studies. Am J Med. 2016;129(3):339. e19-339. e26.
16. Watson C, et al. Venous thromboembolism: review of Clinical challenges, Biology, Assessment, Treatment, and modeling. Ann Biomed Eng. 2024;52(3):467–86.
17. Kushner A, et al. *Virchow triad*, in *StatPearls [Internet]*. StatPearls Publishing; 2022.
18. Saha P, et al. Leukocytes and the natural history of deep vein thrombosis: current concepts and future directions. Arterioscler Thromb Vasc Biol. 2011;31(3):506–12.
19. Piazza G. Beyond Virchow's Triad: does cardiovascular inflammation explain the recurrent nature of venous thromboembolism? London, England: SAGE Publications Sage UK; 2015. pp. 102–4.
20. Smith SA, Travers RJ, Morrissey JH. How it all starts: initiation of the clotting cascade. Crit Rev Biochem Mol Biol. 2015;50(4):326–36.

21. Alias S, Lang IM. Coagulation and the vessel wall in pulmonary embolism. Pulm Circ. 2013;3(4):728–38.
22. Martinelli I, et al. Risk of venous thromboembolism after air travel: interaction with thrombophilia and oral contraceptives. Arch Intern Med. 2003;163(22):2771–4.
23. Parkin L, et al. Air travel and fatal pulmonary embolism. Thromb Haemost. 2006;95(05):807–14.
24. Rogers MA, et al. Triggers of hospitalization for venous thromboembolism. Circulation. 2012;125(17):2092–9.
25. Konstantinides SV, et al. The Task Force for the diagnosis and management of acute pulmonary embolism of the European Society of Cardiology (ESC). 2019 ESC guidelines for the diagnosis and management of acute pulmonary embolism developed in collaboration with the European Respiratory Society (ERS): the Task Force for the diagnosis and management of acute pulmonary embolism of the European Society of Cardiology (ESC). Eur Respir J. 2019;54(3):1901647.
26. White RH, Zhou H, Romano PS. Incidence of symptomatic venous thromboembolism after different elective or urgent surgical procedures. Thromb Haemost. 2003;90(09):446–55.
27. Horner D, et al. Individual risk factors predictive of venous thromboembolism in patients with temporary lower limb immobilization due to injury: a systematic review. J Thromb Haemost. 2019;17(2):329–44.
28. Mahmoud SS, Esser M, Jain A. Thromboembolic events in pelvic and acetabulum fractures: a systematic review of the current literature on incidence, screening, and thromboprophylaxis. Int Orthop. 2022;46(8):1707–20.
29. Ma J, et al. Incidence and risk factors of preoperative deep venous thrombosis in closed tibial shaft fracture: a prospective cohort study. Archives of Orthopaedic and Trauma Surgery; 2020. pp. 1–7.
30. Cosmi B, et al. Sex, age and normal post-anticoagulation D-dimer as risk factors for recurrence after idiopathic venous thromboembolism in the Prolong study extension. J Thromb Haemost. 2010;8(9):1933–42.
31. Akrivou D, et al. Pathophysiological aspects of aging in venous thromboembolism: an update. Medicina. 2022;58(8):1078.
32. Borch K, et al. Abdominal obesity is essential for the risk of venous thromboembolism in the metabolic syndrome: the Tromsø study. J Thromb Haemost. 2009;7(5):739–45.
33. Puurunen MK, et al. Epidemiology of venous thromboembolism in the Framingham Heart Study. Thromb Res. 2016;145:27–33.
34. Mi Y, et al. Venous thromboembolism has the same risk factors as atherosclerosis: a PRISMA-compliant systemic review and meta-analysis. Medicine. 2016;95(32):e4495.
35. Bertina RM, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature. 1994;369(6475):64–7.
36. Poort SR et al. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. 1996.
37. Zhang Q et al. Plasminogen activator inhibitor-1 (PAI-1) 4G/5G promoter polymorphisms and risk of venous thromboembolism–a meta-analysis and systematic review. Vasa, 2020.
38. Bai J, et al. Diabetes is associated with increased risk of venous thromboembolism: a systematic review and meta-analysis. Thromb Res. 2015;135(1):90–5.
39. Agnelli G, et al. Apixaban versus dalteparin for the treatment of acute venous thromboembolism in patients with cancer: the Caravaggio study. Thromb Haemost. 2018;118(09):1668–78.
40. Garcia D, Erkan D. Diagnosis and management of the antiphospholipid syndrome. N Engl J Med. 2018;378(21):2010–21.
41. Bucci T, et al. Cardiac and vascular features of arterial and venous primary antiphospholipid syndrome. The multicenter ATHERO-APS study. Thromb Res. 2022;209:69–74.
42. Abou-Ismail MY, Sridhar DC, Nayak L. Estrogen and thrombosis: a bench to bedside review. Thromb Res. 2020;192:40–51.
43. de Bastos M et al. Combined oral contraceptives: venous thrombosis. Cochrane Database Syst Reviews, 2014(3).
44. Ahlehoff O, et al. Cutaneous lupus erythematosus and the risk of deep venous thrombosis and pulmonary embolism: a Danish nationwide cohort study. Lupus. 2017;26(13):1435–9.
45. Aviña-Zubieta JA, et al. The risk of deep venous thrombosis and pulmonary embolism in primary Sjögren syndrome: a general population-based study. J Rhuematol. 2017;44(8):1184–9.
46. Folsom AR, et al. Prospective study of sickle cell trait and venous thromboembolism incidence. J Thromb Haemost. 2015;13(1):2–9.
47. Burn E, et al. Venous or arterial thrombosis and deaths among COVID-19 cases: a European network cohort study. Lancet Infect Dis. 2022;22(8):1142–52.
48. Stark K, Massberg S. Interplay between inflammation and thrombosis in cardiovascular pathology. Nat Rev Cardiol. 2021;18(9):666–82.
49. d'Alessandro E, et al. Thrombo-inflammation in Cardiovascular Disease: An Expert Consensus Document from the third Maastricht Consensus Conference on thrombosis. Thromb Haemost. 2020;120(4):538–64.
50. Obeagu EI, Obeagu GU. Thromboinflammation in COVID-19: unraveling the interplay of coagulation and inflammation. Med (Baltim). 2024;103(28):e38922.
51. Klavina PA, et al. Dysregulated haemostasis in thrombo-inflammatory disease. Clin Sci (Lond). 2022;136(24):1809–29.
52. Patel D, et al. Biomarkers of thrombo-inflammatory responses in pulmonary embolism patients with Pre-existing Versus New-Onset Atrial Fibrillation. Clin Appl Thromb Hemost. 2021;27:10760296211014964.
53. Lossos C, et al. Idiopathic Multicentric Castleman Disease results in endothelium activation and Coagulation Dysregulation Via tissue factor and PAI-1. Blood. 2023;142:5414.
54. Hot A, Lenief V, Miossec P. Combination of IL-17 and TNFα induces a pro-inflammatory, pro-coagulant and pro-thrombotic phenotype in human endothelial cells. Ann Rheum Dis. 2012;71(5):768–76.
55. Luo Y, et al. The role of Thrombo-inflammation in ischemic stroke: focus on the Manipulation and clinical application. Mol Neurobiol; 2024.
56. Mack A, Vanden Hoek T, Du X. Thromboinflammation and the role of platelets. Arterioscler Thromb Vasc Biol. 2024;44(6):1175–80.
57. Chan NC, Weitz JI. Recent advances in understanding, diagnosing and treating venous thrombosis. F1000Res, 2020. 9.
58. Anghel L et al. From Classical Laboratory parameters to Novel biomarkers for the diagnosis of venous thrombosis. Int J Mol Sci, 2020. 21(6).
59. Wells PS, et al. Value of assessment of pretest probability of deep-vein thrombosis in clinical management. Lancet. 1997;350(9094):1795–8.
60. Fenyves BG, et al. Plasma P-selectin is an early marker of thromboembolism in COVID-19. medRxiv; 2021.
61. Moresco RN, et al. Association between plasma levels of D-dimer and fibrinogen/fibrin degradation products (FDP) for exclusion of thromboembolic disorders. J Thromb Thrombolysis. 2006;21(2):199–202.
62. Schefold JC, et al. Renal function–adjusted d-Dimer levels in critically ill patients with suspected thromboembolism. Crit Care Med. 2020;48(4):e270–6.
63. van Es N, et al. Wells rule and D-dimer testing to rule out pulmonary embolism: a systematic review and individual-patient data meta-analysis. Ann Intern Med. 2016;165(4):253–61.
64. Wells PS, et al. Evaluation of D-dimer in the diagnosis of suspected deep-vein thrombosis. N Engl J Med. 2003;349(13):1227–35.
65. Le Gal G, et al. Prediction of pulmonary embolism in the emergency department: the revised Geneva score. Ann Intern Med. 2006;144(3):165–71.
66. Crawford F et al. D-dimer test for excluding the diagnosis of pulmonary embolism. Cochrane Database Syst Reviews, 2016(8).
67. Karny-Epstein N, Abuhasira R, Grossman A. Current use of D-dimer for the exclusion of venous thrombosis in hospitalized patients. Sci Rep. 2022;12(1):12376.
68. Zhang NJ, et al. Automated Pulmonary Embolism Risk Assessment using the Wells Criteria: Validation Study. JMIR Form Res. 2022;6(2):e32230.
69. Shen JH, et al. Comparison of the Wells score with the revised Geneva score for assessing suspected pulmonary embolism: a systematic review and meta-analysis. J Thromb Thrombolysis. 2016;41(3):482–92.
70. Rafee A, et al. D-Dimer in the diagnosis of deep vein thrombosis following total hip and knee replacement: a prospective study. Annals Royal Coll Surg Engl. 2008;90(2):123–6.
71. Chan W-S, et al. A red blood cell agglutination D-dimer test to exclude deep venous thrombosis in pregnancy. Ann Intern Med. 2007;147(3):165–70.
72. Tritschler T, et al. Venous thromboembolism: advances in diagnosis and treatment. JAMA. 2018;320(15):1583–94.
73. Linkins LA, Takach Lapner S. Review of D-dimer testing: good, bad, and Ugly. Int J Lab Hematol. 2017;39(Suppl 1):98–103.

74. Paszek E et al. Profilin 1 and Mitochondria-Partners in the Pathogenesis of Coronary Artery Disease? Int J Mol Sci, 2021. 22(3).
75. Erdem E, et al. A new biomarker in the diagnosis and prognosis of pulmonary thromboembolism: serum profilin-1. Heliyon. 2024;10(17):e37102.
76. Allen A, Gau D, Roy P. The role of profilin-1 in cardiovascular diseases. J Cell Sci, 2021. 134(9).
77. Zhang Y, et al. Serum proteome profiling reveals heparanase as a candidate biomarker for chronic thromboembolic pulmonary hypertension. iScience. 2024;27(2):108930.
78. Bonfanti L et al. Diagnostic significance of combining D-dimer with high-sensitivity cardiac troponin I for improving the diagnosis of venous thromboembolism in the emergency department. Acta Bio Medica: Atenei Parmensis, 2021. 92(5).
79. Jakubiak GK. Cardiac troponin serum concentration measurement is useful not only in the diagnosis of Acute Cardiovascular events. J Pers Med, 2024. 14(3).
80. Janisset L et al. Cardiac biomarkers in patients with Acute Pulmonary Embolism. Med (Kaunas), 2022. 58(4).
81. Long B, et al. An emergency medicine approach to troponin elevation due to causes other than occlusion myocardial infarction. Am J Emerg Med. 2020;38(5):998–1006.
82. Jacobs B, Obi A, Wakefield T. Diagnostic biomarkers in venous thromboembolic disease. J Vascular Surgery: Venous Lymphatic Disorders. 2016;4(4):508–17.
83. Myers DD, et al. P-selectin and leukocyte microparticles are associated with venous thrombogenesis. J Vasc Surg. 2003;38(5):1075–89.
84. Mosevoll KA, et al. Altered plasma levels of cytokines, soluble adhesion molecules and matrix metalloproteases in venous thrombosis. Thromb Res. 2015;136(1):30–9.
85. Antonopoulos CN, et al. The role of soluble P selectin in the diagnosis of venous thromboembolism. Thromb Res. 2014;133(1):17–24.
86. Vandy FC, et al. Soluble P-selectin for the diagnosis of lower extremity deep venous thrombosis. J Vascular Surgery: Venous Lymphatic Disorders. 2013;1(2):117–25.
87. Ramacciotti E, et al. Evaluation of soluble P-selectin as a marker for the diagnosis of deep venous thrombosis. Clin Appl Thromb Hemost. 2011;17(4):425–31.
88. Price DT, Ridker PM. Factor V Leiden mutation and the risks for thromboembolic disease: a clinical perspective. Ann Intern Med. 1997;127(10):895–903.
89. Kujovich JL. *Factor V Leiden thrombophilia.* 2018.
90. Stefely JA, et al. Marked factor V activity elevation in severe COVID-19 is associated with venous thromboembolism. Am J Hematol. 2020;95(12):1522–30.
91. Omer AE, Muddathir ARM, Eltayeb LB. Measurement of fibrin degradation products (FDPs) among patients with cardiovascular diseases: a significant target for prognosis. J Biochem Technol. 2021;12(4–2021):23–8.
92. Moresco RN, et al. Association between plasma levels of D-dimer and fibrinogen/fibrin degradation products (FDP) for exclusion of thromboembolic disorders. J Thromb Thrombolysis. 2006;21:199–202.
93. Fukujima MM, et al. Fibrinogen as independent risk factor for ischemic stroke. Arq Neuropsiquiatr. 1997;55:737–40.
94. Corban MT, et al. Elevated levels of serum fibrin and fibrinogen degradation products are independent predictors of larger coronary plaques and greater plaque necrotic core. Circ J. 2016;80(4):931–7.
95. Antushevich H, Wójcik M. Review: apelin in disease. Clin Chim Acta. 2018;483:241–8.
96. Murali S, Aradhyam GK. Structure-function relationship and physiological role of apelin and its G protein coupled receptor. Biophys Rev. 2023;15(1):127–43.
97. Lee DK, et al. Characterization of apelin, the ligand for the APJ receptor. J Neurochem. 2000;74(1):34–41.
98. Read C, et al. International Union of Basic and Clinical Pharmacology. CVII. Structure and pharmacology of the apelin receptor with a recommendation that Elabela/Toddler is a second endogenous peptide ligand. Pharmacol Rev. 2019;71(4):467–502.
99. Habata Y et al. *Apelin, the natural ligand of the orphan receptor APJ, is abundantly secreted in the colostrum.* Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 1999. 1452(1): pp. 25–35.
100. Lv X, et al. The role of the apelin/APJ system in the regulation of liver disease. Front Pharmacol. 2017;8:221.
101. Kawamata Y, et al. Molecular properties of apelin: tissue distribution and receptor binding. Biochim et Biophys Acta (BBA)-Molecular Cell Res. 2001;1538(2–3):162–71.
102. Medhurst AD, et al. Pharmacological and immunohistochemical characterization of the APJ receptor and its endogenous ligand apelin. J Neurochem. 2003;84(5):1162–72.
103. Gandham R et al. Apelin and its receptor: an overview. J Clin Diagn Res, 2019. 13(6).
104. Kurowska P, et al. Apelin in Reproductive Physiology and Pathology of different species: a critical review. Int J Endocrinol. 2018;2018:p9170480.
105. Kidoya H, Takakura N. Biology of the apelin-APJ axis in vascular formation. J Biochem. 2012;152(2):125–31.
106. Wang X, et al. Apelin/APJ system in inflammation. Int Immunopharmacol. 2022;109:108822.
107. Wysocka MB, Pietraszek-Gremplewicz K, Nowak D. The role of apelin in cardiovascular diseases, obesity and cancer. Front Physiol. 2018;9:557.
108. Chapman FA, et al. Targeting the apelin system for the treatment of cardiovascular diseases. Cardiovasc Res. 2023;119(17):2683–96.
109. Girault-Sotias P-E, et al. Apelin and vasopressin: the Yin and Yang of water balance. Front Endocrinol. 2021;12:735515.
110. Lv SY, Chen WD, Wang YD. The Apelin/APJ system in psychosis and neuropathy. Front Pharmacol. 2020;11:320.
111. Kang Y, et al. Apelin-APJ signaling is a critical regulator of endothelial MEF2 activation in cardiovascular development. Circ Res. 2013;113(1):22–31.
112. Than A, et al. Apelin attenuates oxidative stress in human adipocytes. J Biol Chem. 2014;289(6):3763–74.
113. Lu L, et al. Apelin/APJ system: a bifunctional target for cardiac hypertrophy. Int J Cardiol. 2017;230:164–70.
114. Cheng X, Cheng XS, Pang CC. Venous dilator effect of apelin, an endogenous peptide ligand for the orphan APJ receptor, in conscious rats. Eur J Pharmacol. 2003;470(3):171–5.
115. Szokodi I, et al. Apelin, the novel endogenous ligand of the orphan receptor APJ, regulates cardiac contractility. Circul Res. 2002;91(5):434–40.
116. Farkasfalvi K, et al. Direct effects of apelin on cardiomyocyte contractility and electrophysiology. Biochem Biophys Res Commun. 2007;357(4):889–95.
117. Fernandez KX, et al. Metabolically stable apelin-analogues, incorporating cyclohexylalanine and homoarginine, as potent apelin receptor activators. RSC Med Chem. 2021;12(8):1402–13.
118. Wang W, et al. Angiotensin-converting enzyme 2 metabolizes and partially inactivates pyr-apelin-13 and apelin-17: physiological effects in the cardiovascular system. Hypertension. 2016;68(2):365–77.
119. Popov SV, et al. Apelin is a prototype of novel drugs for the treatment of acute myocardial infarction and adverse myocardial remodeling. Pharmaceutics. 2023;15(3):1029.
120. Zhang X, et al. Apelin-13 protects against myocardial infarction-induced myocardial fibrosis. Mol Med Rep. 2016;13(6):5262–8.
121. Chapman FA, et al. The therapeutic potential of apelin in kidney disease. Nat Rev Nephrol. 2021;17(12):840–53.
122. Gao R et al. *The Interaction of Apelin and FGFR1 Ameliorated the Kidney Fibrosis through Suppression of TGFβ-Induced Endothelial-to-Mesenchymal Transition.* Oxid Med Cell Longev, 2023. 2023: p. 5012474.
123. Han X, et al. Apelin-13 deteriorates hypertension in rats after damage of the vascular endothelium by ADMA. Can J Physiol Pharmacol. 2013;91(9):708–14.
124. Kong X, et al. Apelin-13-Mediated AMPK ameliorates endothelial barrier dysfunction in acute lung injury mice via improvement of mitochondrial function and autophagy. Int Immunopharmacol. 2021;101(Pt B):108230.
125. Azizi Y, et al. Post-infarct treatment with [Pyr1]-apelin-13 reduces myocardial damage through reduction of oxidative injury and nitric oxide enhancement in the rat model of myocardial infarction. Peptides. 2013;46:76–82.
126. Gou Y-Y, et al. Preventive and therapeutic effects of exogenous Apelin regulating autophagy on the formation of pulmonary artery hypertension in rats. Sichuan da xue xue bao. Yi xue ban = Journal of Sichuan University. Medical Science Edition. 2020;51(2):193–9.

127. Fraga-Silva R, et al. Apelin-13 treatment enhances the stability of atherosclerotic plaques. Cardiovasc Res. 2016;111:S107.
128. Hamza RZ, et al. Ameliorative effect of apelin-13 against renal complications in L-NAME-induced preeclampsia in rats. PeerJ. 2021;9:e11110.
129. Hamza RZ, et al. Correlation between Apelin and some Angiogenic factors in the pathogenesis of Preeclampsia: Apelin-13 as novel drug for treating Preeclampsia and its physiological effects on placenta. Int J Endocrinol. 2021;2021(1):5017362.
130. Gholamzadeh R, Aboutaleb N, Nazarinia D. Intravenous injection of apelin-13 improves sensory-motor balance deficits caused by cerebral ischemic reperfusion injury in male wistar rats via restoration of nitric oxide. J Chem Neuroanat. 2021;112:101886.
131. Xin Q, et al. Neuroprotective effects of apelin-13 on experimental ischemic stroke through suppression of inflammation. Peptides. 2015;63:55–62.
132. Xia F, et al. Apelin-13 protects the lungs from ischemia-reperfusion injury by attenuating inflammatory and oxidative stress. Hum Exp Toxicol. 2021;40(4):685–94.
133. Wen R, et al. Beneficial effects of Apelin-13 on metabolic diseases and exercise. Front Endocrinol. 2023;14:1285788.
134. de Oliveira AA, et al. Apelin pathway in cardiovascular, kidney, and metabolic diseases: therapeutic role of apelin analogs and apelin receptor agonists. Peptides. 2022;147:170697.
135. Adam F, et al. Apelin: an antithrombotic factor that inhibits platelet function. Blood. 2016;127(7):908–20.
136. Strohbach A, et al. Platelet apelin receptor expression is reduced in patients with acute myocardial infarction. Vascul Pharmacol. 2021;136:106808.
137. Serfozo P, et al. Ang II (Angiotensin II) Conversion to Angiotensin-(1–7) in the circulation is POP (Prolyloligopeptidase)-Dependent and ACE2 (angiotensin-Converting enzyme 2)-Independent. Hypertension. 2020;75(1):173–82.
138. Rai R, et al. Downregulation of the Apelinergic Axis accelerates aging, whereas its systemic restoration improves the mammalian Healthspan. Cell Rep. 2017;21(6):1471–80.
139. Yao F, et al. Apelin-13 impedes foam cell formation by activating Class III PI3K/Beclin-1-mediated autophagic pathway. Biochem Biophys Res Commun. 2015;466(4):637–43.
140. Xu R, et al. Ascending aortic adventitial remodeling and fibrosis are ameliorated with Apelin-13 in rats after TAC via suppression of the miRNA-122 and LGR4-β-catenin signaling. Peptides. 2016;86:85–94.
141. Chu H, et al. Apelin-13 protects against ischemic blood-brain barrier damage through the effects of Aquaporin-4. Cerebrovasc Dis. 2017;44(1–2):10–25.
142. McLean DL, et al. Apelin/APJ signaling is a critical regulator of statin effects in vascular endothelial cells–brief report. Arterioscler Thromb Vasc Biol. 2012;32(11):2640–3.
143. Freyer L, et al. Loss of Apela peptide in mice causes low Penetrance Embryonic lethality and defects in early mesodermal derivatives. Cell Rep. 2017;20(9):2116–30.
144. Hwangbo C et al. Endothelial APLNR regulates tissue fatty acid uptake and is essential for apelin's glucose-lowering effects. Sci Transl Med, 2017. 9(407).
145. Alastalo TP, et al. Disruption of PPARγ/β-catenin-mediated regulation of apelin impairs BMP-induced mouse and human pulmonary arterial EC survival. J Clin Invest. 2011;121(9):3735–46.
146. Chandra SM, et al. Disruption of the apelin-APJ system worsens hypoxia-induced pulmonary hypertension. Arterioscler Thromb Vasc Biol. 2011;31(4):814–20.
147. Sturny M, et al. Apelin-13 protects Corpus Cavernosum against Fibrosis Induced by High-Fat Diet in an MMP-Dependent mechanism. J Sex Med. 2021;18(5):875–88.
148. Mao SZ et al. *[Effect of apelin on hypoxic pulmonary hypertension in rats: role of the NO pathway].* Sheng Li Xue Bao, 2009. 61(5): pp. 480-4.
149. Zeng XJ, et al. Apelin protects heart against ischemia/reperfusion injury in rat. Peptides. 2009;30(6):1144–52.
150. Baykal ND, et al. Evaluation of neutrophil gelatinase-associated lipocalin (NGAL), hypoxia-induced factor-1 alpha (HIF-1α) and apelin 13 levels as new potential biomarkers for pulmonary thromboembolism: a prospective clinical study. Respir Med. 2024;233:107776.
151. Karataş M, Özyurt S. Diagnostic value of serum apelin-13 in patients with pulmonary thromboembolism. 2018.
152. Selimoglu Şen H, et al. Serum apelin 13 levels in patients with pulmonary embolism. Clin Appl Thromb Hemost. 2016;22(6):543–7.
153. Janssens P, et al. On methods for the measurement of the apelin receptor Ligand Apelin. Sci Rep. 2022;12(1):7763.
154. Goidescu CM, et al. ACE2 and Apelin-13: biomarkers with a prognostic value in congestive heart failure. Dis Markers. 2021;2021(1):5569410.
155. Yu XH, et al. Apelin and its receptor APJ in cardiovascular diseases. Clin Chim Acta. 2014;428:1–8.
156. Marsault E, et al. The apelinergic system: a perspective on challenges and opportunities in cardiovascular and metabolic disorders. Ann N Y Acad Sci. 2019;1455(1):12–33.
157. Chatterjee P, et al. Interaction between the apelinergic system and ACE2 in the cardiovascular system: therapeutic implications. Clin Sci (Lond). 2020;134(17):2319–36.
158. Gourdy P, et al. Apelin administration improves insulin sensitivity in overweight men during hyperinsulinaemic-euglycaemic clamp. Diabetes Obes Metab. 2018;20(1):157–64.
159. Zhong JC, et al. Targeting the apelin pathway as a novel therapeutic approach for cardiovascular diseases. Biochim Biophys Acta Mol Basis Dis. 2017;1863(8):1942–50.
160. Wang W, et al. Apelin protects against abdominal aortic aneurysm and the therapeutic role of neutral endopeptidase resistant apelin analogs. Proc Natl Acad Sci U S A. 2019;116(26):13006–15.
161. Kearon C, et al. An evaluation of D-dimer in the diagnosis of pulmonary embolism: a randomized trial. Ann Intern Med. 2006;144(11):812–21.
162. Gao H, Liu H, Li Y. Value of D-dimer levels for the diagnosis of pulmonary embolism: an analysis of 32 cases with computed tomography pulmonary angiography. Exp Ther Med. 2018;16(2):1554–60.
163. Glober N, et al. Use of the d-dimer for detecting Pulmonary Embolism in the Emergency Department. J Emerg Med. 2018;54(5):585–92.
164. Wang X, et al. The association between serum apelin-13 and the prognosis of acute ischemic stroke. Transl Stroke Res. 2020;11:700–7.
165. Gergics M, et al. Apelin-13 as a potential biomarker in critical illness. J Clin Med. 2023;12(14):4801.
166. Japp AG, et al. Acute cardiovascular effects of apelin in humans: potential role in patients with chronic heart failure. Circulation. 2010;121(16):1818–27.
167. Japp AG, et al. Vascular effects of apelin in vivo in man. J Am Coll Cardiol. 2008;52(11):908–13.
168. Fischer C, et al. Plasma kallikrein cleaves and inactivates apelin-17: Palmitoyl- and PEG-extended apelin-17 analogs as metabolically stable blood pressure-lowering agents. Eur J Med Chem. 2019;166:119–24.
169. McKinnie SM, et al. The metalloprotease neprilysin degrades and inactivates apelin peptides. ChemBioChem. 2016;17(16):1495–8.
170. Barnes GD, et al. Sustained cardiovascular actions of APJ agonism during renin-angiotensin system activation and in patients with heart failure. Circ Heart Fail. 2013;6(3):482–91.
171. Mughal A, O'Rourke ST. Vascular effects of apelin: mechanisms and therapeutic potential. Pharmacol Ther. 2018;190:139–47.

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