Programa de Doctorado en Epidemiologia y Salud Pública

LA INSUFICIENCIA VENOSA CRÓNICA EN EL PERÍODO GESTACIONAL

Tesis Doctoral presentada por

CHAOWEN CHEN

Directores:

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MIGUEL ÁNGEL ORTEGA

Alcalá de Henares, 2022

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读博对于我来说并不是一个艰苦的过程，但是确实是我最难忘的一段经历。在人生中这一特殊的经历也让我对于活着和生活有了全新的了解。在这几年快乐的时光中，我需要感谢的人有很多，但在这其中我最需要感谢的人的人是我的导师 **Ángel Asúnsolo** 。第一次见他的时候就觉得他真的很像我的父亲。我的父亲也是一名大学教授，在他们身上我看到了一样的光。对科研的热爱，对工作的人爱，对学生的热爱和对生活的热爱。之后的这几年时光也无形中验证了我的想法，他真的是一个非常好的导师，不仅仅在学习上给予了我专业的指导，闲暇时光还会和我分享生活中点点滴滴。让一个在异国他乡求学的留学生感受到了父亲一样的关爱。更是在我读博过程中帮助我克服各种各样的困难，遇到问题他总是比我还积极的帮我解决。在整个研究的过程中，也是他教会了我如何用宏观的角度认识问题，从微观处解决问题。遇到解决不了的问题时也会教我解决问题的途径。他真的是我最棒的人生导师。

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ÍNDICE

ABSTRACT

Aims: Chronic venous disease is a persistent, progressive and frequently underestimated condition, despite being widely represented in the general population in the World. It has been demonstrated that women has a higher risk of suffer venous insufficiency problems due to the different hormonal and physiological factors they present during the perinatal period. The general objective of this dissertation is to evaluate the specific impact of venous insufficiency in perinatal women.

Methods: To achieve the objective stated above, we conducted 3 specific scientific studies. In the study I，we carried out a cross-sectional descriptive bibliographic review of all the research published on chronic venous disease since 1988. From this review, we compiled the most relevant evidence published on the topic of chronic venous disease, with special emphasis on the pathophysiology and medical cognition of disease.

Secondly, the article II includes a cross-sectional study, in which we use accurate and updated data from the administrative medical records (CMBD) corresponding to the care registered in 2015, both in the public and private health systems of the different Autonomous Communities of Spain. We used these data to study the association between the prevalence of LEPVI and different risk factors, such as smoking, obesity, asthma, hypothyroidism, and intrapartum fetal distress. We performed a conditional logistic regression to estimate the adjusted odds ratios and the 95% Confidence Intervalto assess the associations between LEPVI and the risk factors, as well as their relationship with intrapartum fetal distress.

Finally, in Article III we developed an observational, analytical and prospective study which included a sample of 114 women in the third trimester of pregnancy. A cohort study was conducted by gathering information from a database from the Carlos III Health Institute in 2017. Of these, 62 women were diagnosed with CVD according to the CEAP classification. The rest of women (n= 52) did not presented CVD diseases in their Electronic Health Record, and were included in the control group. Those women

with CVD during pregnancy were identified to investigate its relationship with ILK expression in placental villi associated with E-cadherin. The relationship between the expression level of Cadherin 17 and Cadherin 6 with CVD was also explored. For this purpose, we conducted a conditional logistic regression model to obtain 95% confidence intervals (95% CI) during pregnancy.

Results： Each study included in this dissertation provided several results which jointly contributed to achieving the objective of this thesis.

In article I, we find that CVD is a progressive and disabling condition widely represented in the world population. We compiled some of the most relevant data on such a complex topic, with special emphasis on the pathophysiological and medical perceptions of the disease. In particular for the most vulnerable groups, such as the elderly people, individuals with additional comorbidities and pregnant women.

In article II, we observed that, women with LEPVI were older and presented higher prevalence of smoking, obesity, asthma, and hypothyroidism, in comparision with those without LEPVI in the group control. In addition, women with LEPVI were more likely to have conditions associated with intrapartum fetal distress (9.35%) than women without this disease (7.41%). Women with LEPVI are 30% (OR = 1.30, 99.5% CI: 1.08-1.54) more likely to have an IFC outcome during the pregnancy, and to have placental dysfunction (OR = 1.74, 99.5% CI: 1.00-3-05), as well. After adjusting for age, smoking, obesity, asthma, hypothyroidism, coagulopathy disorders, and anemia, this association remained significant but slightly attenuated for IFC (OR=1.25, 99.5% CI, 1.05-1 .50) and placental dysfunction (OR = 1.23, 99.5% CI: 1.01-1.49). However, there was no association between LEPVI and fetal distress (OR = 1.01, 99.5% CI: 0.46-2.21).

In article III, women with CVeD during pregnancy show an increase in ILK expression in the placental villi associated with a decrease in E-cadherin. Histological analysis of protein expression by immunohistochemical techniques showed a significant increase

in ILK expression in placental villi of women with CVD during pregnancy compared to HC, ***p < 0.001 [CVeD = 2.379 ± 0.084 vs HC = 0.976 ± 0.082]. In contrast, we observed a significant decrease in E-Cad gene expression in the placental villi of women with CVeD during pregnancy compared to HC, **p = 0.0084 [CVeD = 10.726 ± 0.359 vs HC = 11.893 ± 0.461]. In addition, protein expression showed a significant decrease in E-Cad expression in the placental villi of women with CVeD during pregnancy compared to HC, ***p = 0.002 [CVeD = 0.903 ± 0.062 vs HC = 1.240 ± 0.058]. The expression level of Cadherin 17 and Cadherin 6 increases in the placental villi of women with CVD during pregnancy. Cad-17 gene expression showed a significant increase in placental villi of women with CVD during pregnancy compared to HC, *p = 0.0228 [CVeD = 7.804 ± 0.325 vs HC = 6.780 ± 0.263]. In this sense, protein expression showed a significant elevation by immunohistochemical techniques in the placental villi of women with CVD during pregnancy compared to HC, ** p = 0.0026 [CVeD = 1.403 ± 0.067 vs HC = 1.159 ± 0.085]. Similarly, our results have shown an increase in Cad-6 gene expression in the placental villi of women with CVD during pregnancy compared to HC, ** p = 0.0016 [CVeD = 7.083 ± 0.251 vs HC = 5.807 ± 0.247]. Furthermore, protein expression showed a significant increase in the placental villi of women with CVeD during pregnancy compared to HC, **p = 0.0033 [CVeD = 1.202 ± 0.065 vs HC = 0.923 ± 0.066].

Conclusions: CVD is a progressive and disabling disease with high impact in the world population health. Therefore, the management of pathophysiology and therapeutics is fundamental and was one of the objectives of our study. An increasing number of studies, focused on CVD, show the relevance of this vascular pathology, especially in the most advanced stages (CVI). In this dissertation, we collect some of the most relevant data on this important topic, with a special emphasis on pathophysiological and medical insights into the disease. An integrative perspective of this condition would bring immediate benefits for the clinical management of these patients, particularly for the most vulnerable groups, such as the elderly people, individuals with additional comorbidities and pregnant women.

The second study aimed to determine the association between varicose veins in pregnancy and placental insufficiency. This study represented a nationwide cross-sectional analysis in Spain to explore the association between the presence of varicose veins and the existence of alterations in the ischemic placental function. An extensive database gathered from hospital administrations was used for that purpose.

In the third study, the relationship between chronic venous disease in pregnant women was observed, causing an increase in ILK in the placental villi associated with a decrease in E-Cadherin, demonstrating a significant increase in the expression of ILK proteins and genes, cadherin-6 and cadherin-17 and a reduction in e-cadherin, associated with the development of CVD during pregnancy.

ABREVIATURAS

Abreviaciones de términos en español:

ANOVA	Análisis de variación
CMBD	El conjunto mínimo básico de datos
CDM14	Categorías diagnosticas mayores All-GRD. Embarazo,parto,puerperio
CIE	Clasificación Internacional de las Enfermedades
ECV	Enfermedad venosa crónica
GRD	Grupos relacionados por el diagnóstico
IC	Intervalo de confianza
IDC9-MC	Clasificación Internacional de Enfermedades, Novena. Revisión, Modificación Clínica.
IVC	Insuficiencia Venosa Crónica
LEPVI	Venas varicosas en las extremidades inferiores o la pelvis
OMS	Organización Mundial de la Salud
OR	Odds ratio
SVT	Superficial venous thrombosis
VP	Varices pelvica
VV	Varices vulvar
VVs	Venas varicosas

Abreviaciones de términos en inglés:

BMI	Body mass index
CADASIL	Cerebral autosomal-dominant arteriopathy with subcortical. infarcts and leukoencephalopathy
EDS	Ehlers-Danlos syndrome
CEAP	Clinical-etiology-anatomy-pathophysiology
CVD	Chronic Venous Disease
CVeD	Chronic Venous Disease
CVI	Chronic venous insufficiency
ECM	Extracellular matrix
ECs	Endothelial cells
EMA	European Medicines Agency
EGFL7	Epidermal growth factor like-domain 7
EVLA	Endovenous laser ablation
Factor GATA2	GATA binding protein 2
FOXC2	C2 isoform of the forkhead family transcription factor
GPCRs	G protein coupled receptors
GWAS	Genome-wide association studies
HC	Healthy controls
HFE	Homeostatic iron regulator
HIF-1α and HIF-2α	The hypoxia-inducible factor 1α and 2α
LDS	lymphedema distichiasis syndrome
IGF-1	Insulin-like growth factor 1
IL-6	Interleukin-6
ILK	Integrin-linked kinase

LOXL-4	Lysyl oxidase-like 4
MAPKs	MAP kinases
MCP-1	Monocyte chemotactic protein 1
MGP	Matrix Gla protein
MMPs	Metalloproteinases
MiRNA	Micro RNAs
NFATC2	Nuclear factor of activated T cells 2
PAI-1	Plasminogen activator inhibitor one
PDGF	Platelet-derived growth factor
PTS	Post-trombotic syndrome
PPP3R1	Protein phosphatase 3 regulatory subunit B, alpha
PUFA	Polyunsaturated fatty acids
PW	Pregnant women
PWS	Parkes Weber syndrome
QOL	Quality of life
RBCs	Red blood cells
RFA	Radiofrequency ablation
siRNA	Short-interfering RNA
SMCs	Smooth muscle cells
SNPs	Single nucleotide polymorphisms
STIM2	Stromal interaction molecule 2
TGF- β1	Transforming growth factor beta 1
TIMPs	Tissue inhibitors of metalloproteinases
VEGF	Vascular endothelial growth factor
WBCs	White blood cells

INTRODUCCION

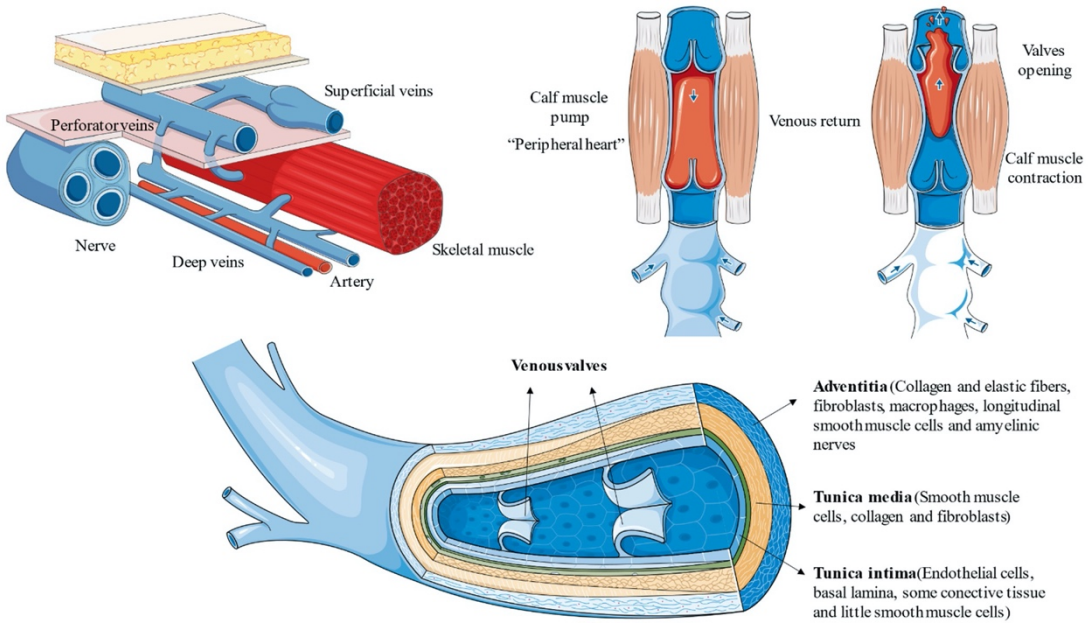
1.1 Definición y justificación del problema de estudio

La enfermedad venosa crónica (ECV) es una enfermedad persistente, progresiva y frecuentemente subestimada. La condición emparejada ampliamente representada en la población general, que tiene un enorme impacto socio-económico, físico y psicológico (1,2). ECV implica un amplio espectro de anomalías venosas en las que el retorno de la sangre está gravemente comprometido. En la fisiopatología de la ECV, la interacción entre la genética y los factores ambientales son responsables para aumentar la presión venosa ambulatoria, lo que lleva a cambios sustanciales en el conjunto estructura y funcionamiento del sistema venoso(3). Es importante destacar que el término ECV debería diferir del trastorno venoso crónico, que solo incluye la morfología completa y anomalías funcionales del sistema venoso, sin considerar manifestaciones clínicas y otras preocupaciones graves que afectan al paciente (4).

Las venas varicosas (VV) son la manifestación más común de ECV. Otros signos venosos de las ECV incluyen telangiectasias y venas reticulares. A pesar de estos, los signos clínicos pueden afectar cualquier vena del cuerpo. El sistema venoso ubicado en las extremidades inferiores suele ser la estructura más vulnerable a sufrir ECV (5). Esto se debe principalmente al aumento de la resistencia. superar por la fuerza gravitacional, que es notablemente más alta que el resto de regiones en el cuerpo. En las extremidades inferiores se pueden distinguir tres grandes sistemas: 1) venas superficiales, representadas principalmente por la gran vena safena, la pequeña vena safena, la vena safena accesoria anterior y sus afluentes; 2) venas profundas, el principal transportador del flujo sanguíneo; 3) y venas perforantes, que conectan ambos sistemas(6).

Desde una perspectiva histológica, las venas están conformadas por tres capas: 1) túnica íntima o capa interna, principalmente conformado por células endoteliales (CE); 2) la túnica media o capa media, representada por células de músculo liso vascular (CML) y

pequeñas fibras elásticas; y 3) túnica adventicia o capa externa, que está compuesta por tejido conectivo, con un desarrollo importante de fibras elásticas, dando al vaso un importante soporte y elasticidad(7). En el caso de VV, sin embargo, la estructura normal de la vena está alterada, con cambios prominentes en el espesor y composición de la pared venosa (8). Además, las venas presentan válvulas venosas, es decir, prolongaciones bicúspides del tejido venoso esenciales para mantener el flujo sanguíneo en la dirección correcta, que impiden el reflujo venoso (9). También, diferentes bombas musculares actuan coordinadamente con las válvulas venosas para asegurar el flujo sanguíneo unidireccional. El músculo, a menudo llamado "corazón periférico", se considera especialmente el potenciador más prominente del retorno venoso de las extremidades inferiores al corazón (10). Es importante destacar que las fallas en ambos sistemas también son cruciales para comprender la patogenia y la progresión de las enfermedades cardiovasculares que llevan al reflujo venoso a la estasis venosa (11-14). La anatomía del sistema venoso en los miembros inferiores, su fisiología e histología se resumen brevemente en la figura.



El término insuficiencia venosa crónica (IVC) incluye las manifestaciones más graves, tales como edema, cambios en la piel o úlceras en las piernas (15), frecuentemente

asociadas con una peor calidad de vida de estos pacientes (16). Actualmente se establecen diferentes criterios para identificar las diferentes manifestaciones de CVD. La clasificación CEAP (clínico-etiología-anatomía-fisiopatología) es un método aceptado a nivel mundial para estudiar los trastornos venosos. Se basa en diferentes médicos características que permiten analizar con precisión los aspectos clínicos, etiológicos, anatómicos y patogénicos sobre el estado lógico de las venas (17). En este contexto, es frecuente utilizar manifestaciones clínicas (C) para presentaciones específicas de ECV. Por ejemplo, los VV podrían clasificarse como C2 mientras que CVI es comprendido entre las etapas C3 a C6 (18).

Dado lo expuesto anteriormente, las ECV, y en particular las IVC, constituyen graves problemas de salud y son una preocupación creciente en las sociedades occidentales, al tiempo que representa un desafío para el individuo o el sistema de salud. En la presente tesis doctoral incluimos una revisión de la literatura que pretende recopilar toda el conocimiento actual sobre las ECV desde una perspectiva integradora. Para esta labor, es necesario prestar atención a las ECV desde diferentes enfoques que incluyen su diagnóstico, etiología, fisiopatología, clínica y tratamiento. Tras esta revisión, también se realizara una discusión sobre futuras orientaciones y consideraciones importantes que deben tenerse en cuenta en el campo de estudio de las ECV.

1.2. Epidemiología y factores de riesgo de la enfermedad venosa crónica (ECV)

La ECV es un trastorno común que afecta a un porcentaje importante de personas en el mundo. En términos generales, la prevalencia estimada de ECV oscila entre el 60 y el 80% (19-21), aunque estos datos pueden ser muy heterogéneos en la literatura dependiendo de las características de las poblaciones estudiadas y los métodos de estudio utilizados, incluyendo las propias definiciones de las enfermedades (22). En la mayoría de los casos se definen como Clases C0 y C1, mientras que aproximadamente el 25% de los pacientes son diagnosticados con VV (C2).

Las IVC (C3-C6) afectan a un pequeño porcentaje de pacientes con ECV (hasta un 5% de pacientes) (23). La incidencia de VV fue aproximadamente del 2,6% para las mujeres y del 1,9% para los hombres según el estudio de Framingham (24). Así, se reconoce que el riesgo de sufrir de VV y CVI es mayor en mujeres que en hombres, y aumenta con la edad, lo que explicaría su progresivo aumento en la prevalencia e incidencia (25). Además, esta condición puede conllevar importantes consecuencias para la calidad de vida (CV) de las personas afectadas. De hecho, incluso los pacientes con VV sin complicaciones notan una reducción significativa en su calidad de vida y si no se tratan, seguramente progresarán a CVI (26).

Solo en Estados Unidos, más de 25 millones de personas se ven afectadas por ECV y aproximadamente 6 millones presentan etapas y CVI, con un costo estimado de USD 3 mil millones por año (27). Por tanto, es innegable que la ECV representa una de las enfermedades vasculares más frecuentes en el mundo, lo que conlleva una carga importante no solo para el individuo, sino también para los sistemas de salud.

Como se mencionó anteriormente, la edad es uno de los principales factores de riesgo de ECV. De acuerdo con el Estudio de la vena de Edimburgo, aproximadamente 1 de cada 3 pacientes desarrollaron IVC a partir del diagnóstico inicial de VV después de un seguimiento de 13 años, y la tasa aumenta constantemente con la edad (28). Vuylstekeet (29) al informó que las personas mayores (> 65 años) eran más vulnerables a los diferentes factores de riesgo asociados a la ECV, con efectos diferenciales según la geografía regiones. Así, las personas mayores son notablemente susceptibles de padecer ECV y progresar a CVI, particularmente cuando se combina con otros factores de riesgo, como el índice de masa corporal (IMC) (30) y el género femenino (31). Las razones por las que las mujeres tienen más probabilidades de sufrir de ECV, residen en diferentes factores hormonales y fisiológicos que los hacen más sensible a desarrollar esta condición (32). En este grupo, las mujeres embarazadas (PC) son más propensas a desarrollar esta condición, y aproximadamente el 40% de ellos padecen ECV (33).

Además, el número de embarazos también puede ser una variable a destacar para adquirir esta condición. Así, se estima que el 20% de las mujeres nulíparas mayores de 40 años desarrollan VV. Sin embargo, este porcentaje aumenta a un 40% en el caso de las mujeres que fueron embarazadas de 1 a 4 veces y hasta el 65% en aquellas con cinco o más gestaciones (34).

Aparte de sexo femenino y envejecimiento, se han identificado una plétora de factores de riesgo en el desarrollo de ECV. El sobrepeso y la obesidad son uno de los factores mejor establecidos, directamente relacionados a las categorías CEAP C, independientemente del resto de factores de riesgo (35). Esto podría deberse a muchos efectos plausibles y sinérgicos, incluido el estado proinflamatorio en general asociado con el aumento de la adiposidad, y otros factores, como un aumento intra-presión abdominal, que puede provocar un mayor reflujo, un aumento del diámetro de la vena y presión (36). El sedentarismo es otro factor de riesgo importante para desarrollar y agravar las manifestaciones de la ECV (37). Por el contrario, la actividad física no se relacionó con un mayor riesgo de ECV, pero se considera un método esencial para prevenir su progresión (38). Sentado prolongado y, de manera más prominente, la bipedestación prolongada también se reconoce como factores de riesgo para el sufrimiento de CVD (39). Con frecuencia, ambas situaciones están relacionadas con puestos ocupacionales. Los antecedentes familiares de ECV también se reconocen como un factor de riesgo para padecer esta afección (40). Es más, eventos previos de coágulos de sangre en las venas, como la trombosis venosa predominantemente profunda (TVP), también se han descrito como un factor de riesgo potencial para padecer ECV (41), así como fumar (42). Por otro lado, se hace necesario profundizar en la investigación sobre la posible asociación entre altura y CVD. Aunque algunos estudios han descrito una relación causal (43), parece que la altura no influye en la progresión de la enfermedad (44). En general, existen muchos factores de riesgo que podrían estar relacionados con la aparición y desarrollo de ECV como pueden influir

en diferentes mecanismos fisiopatológicos implicados en el inicio y progresión de la enfermedad.

1.3. Diagnóstico

Como se mencionó anteriormente, la clasificación CEAP es la más precisa y utilizada a nivel mundial como método para establecer un diagnóstico preciso de ECV. Se basa en aspectos clínicos, etiológicos, anatómicos y los criterios físicos y fisiopatológicos. Se resumen los principales valores asignados en CEAP en la Tabla 1(ver en página 115). En esta parte resumiremos las manifestaciones clínicas (C) de la ECV mientras que La etiología (E) y la fisiopatología (P) se discutirán posteriormente. El uso de este sistema permite una mejor clasificación de los pacientes con el objetivo de estudiar sus síntomas iniciales. La principal limitación de este método es, sin embargo, la variabilidad Inter observador que puede depender de la experiencia del médico (45). Según CEAP, consideramos C0 cuando no hay signos clínicos de ECV, C1 con telangiectasias / venas reticulares, C2 corresponde a la presencia de VV, y desde C3-C6 hasta CVI, se consideran un rango de manifestaciones que van desde edemas y alteraciones dermatológicas (C3 / C4 respectivamente) hasta cicatrización y ulceraciones activas (C5 / C6) (18).

Para la realizacion del diagnóstico, deben tenerse en cuenta una serie de factores. Primero, se debe realizar cuidadosamente una historia clínica del paciente considerando las alergias, prescripciones médicas previas (anticonceptivos hormonales, anticoagulantes, etc.), familiares antecedentes de VV o ECV y antecedentes personales de tromboembolismo, cardiovascular ootra enfermedad relevante (46). Asimismo, la presencia de sintomatología específica de ECV debe también ser evaluado. Luego, los pacientes serán sometidos a una exploración física con el objetivo de encontrar signos clínicos de ECV, como VV, edema, cambios en la piel o ulceraciones venosas (47).

En cuanto a los métodos de diagnóstico utilizados en la ECV, la ecografía dúplex a color (CDU) es el examen de investigación más utilizado (48). De hecho, la CDU ha reemplazado al resto de técnicas de diagnóstico debido a que no son invasivas, sino que son reproducibles y fáciles de usar al importar datos sobre cambios morfo-hemodinámicos de las venas en la extremidad afectada (49). Por lo tanto, el uso de CDU es fundamental para realizar un correcto manejo del paciente (50).

1.4. Etiología, patogenia y fisiopatologia de las ECV

La ECV es un trastorno vascular en el que se compromete el retorno venoso. De acuerdo con la clasificación CEAP, la etiología de la ECV podría ser la siguiente: 1) ECV primaria; 2) ECV secundaria, que a su vez se dividen en (i) causas secundarias intravenosas; (ii)causas secundarias extravenosas; y 3) ECV congénita (51). La ECV es una enfermedad multifactorial que implica complejos mecanismos fisiopatológicos. El aumento de la presión venosa ambulatoria y la dilatación de las venas en las extremidades inferiores promueve una intrincada respuesta vascular, que conduce a la estasis y la consiguiente inflamación secundaria como respuesta a un esfuerzo cortante alterado. La obstrucción y / o el reflujo son los dos patomecánicos principales, que conducen a cambios significativos en la pared venosa y reflujo venoso patológico, contribuyendo así a la progresión de la ECV (52).

Además, estos cambios proporcionan la creación de un ambiente hipóxico, que se cree que es un contribuyente importante en la fisiopatología de la enfermedad. Además, el papel de ciertas mutaciones o variantes genéticas también están implicados en la etiología o fisiopatología de la enfermedad. También se han encontrado alteraciones epigenéticas en pacientes con ECV, así como evidencia de marcadores sistémicos de daño relacionados con la progresión de la enfermedad (37,53-55).

Dentro de los trastornos vasculares, el término trastorno venoso crónico (CVeD) se utiliza para describir el espectro de morfología y anomalías funcionales del sistema

venoso (56). Uno de las manifestaciones de ECV son el desarrollo de venas varicosas en las extremidades inferiores y / o pélvicas. La insuficiencia venosa (LEPVI) durante el embarazo es una complicación (57,58). Sin embargo, su relevancia clínica no ha sido investigada en profundidad.

Recientemente, se observó que LEPVI se asoció con el desarrollo de lesiones estructurales de patogénesis hipoxémica de las vellosidades placentarias (59,60). Esta asociación entre varices infradiafragmática y el de daño placentario podría explicarse por el impacto de la hipertensión venosa gestacional y estasis sanguínea en el área placentaria y / o anormal del desarrollo placentario. Cualquiera de estas condiciones produce una circulación de flujo bajo y alta resistencia que predispone a hipoperfusión, hipoxia, lesión por reperfusión y estrés oxidativo dentro de la placenta. Si el insulto hipóxico es lo suficientemente severo y de larga duración en condiciones intensivas, como una vagina parto, la función trofoblástica podría alterarse lo suficiente como para afectar bienestar fetal.

Las contracciones uterinas en el trabajo de parto dan como resultado una reducción del 60% de perfusión uteroplacentaria, que provoca transitorios fetales y placentarios. Un feto a término sano con un desarrollo normal la placenta es capaz de adaptarse a esta hipoxia transitoria. Sin embargo, cuando existe una disfunción placentaria preexistente, esta disfunción predispone al feto al "compromiso intraparto fetal" (IFC) (61). Por ejemplo, infarto placentario y fetal. El compromiso puede observarse de diversas formas, como la fetal, angustia, paso de meconio al útero, equilibrio ácido-base anormal equilibrio, e incluso la muerte en casos extremos. En mujeres con preparto, la disfunción placentaria es más probable que desarrolle CFI. Sin embargo, especialmente en fetos adultos normales y poblaciones de bajo riesgo, no hay herramientas para identificar estas condiciones durante la labor. Por tanto, la presencia de varices podría ser un factor externo, síntoma y marcador de esta disfunción.

1.5. Las enfermedades venosas crónicas (ECV) durante el embarazo: análisis y problemas asociados

La aparición de varices durante la gestación supone una complicación frecuente, sobre todo a partir de la segunda mitad del embarazo (62). Esta complicación aumenta con la edad, los antecedentes familiares (63,64), el sobrepeso y con el número de embarazos y de fetos.

Estudios previos han demostrado que la placenta es el órgano más afectado por esta enfermedad, definiendo un conjunto de procesos anormales y marcadores de daño en esta estructura (65-67). En esta línea, hemos demostrado que la hipertensión venosa materna también induce múltiples anomalías y daño en la placenta, evidenciando un aumento de marcadores hipóxicos y apoptosis mejorada (68), marcadores de estrés oxidativo (69) y angiogénesis y linfangiogénesis alterada (70). Además, también encontramos que la ECV se asocia con cambios en la composición de la placenta (56,71) y la señalización (72), por lo que respalda que esta condición conduce a modificaciones perjudiciales en la estructura y el funcionamiento de la placenta, lo que probablemente representa una característica fisiopatológica única en respuesta a la hipertensión venosa.

En este contexto, el estudio de la transducción celular de las señales externas puede ser de gran ayuda para comprender los mecanismos fisiopatológicos de la ECV en la placenta. La cinasa ligada a integrina (ILK) es una molécula intracelular que se une al dominio citoplásmico de las integrinas β1 y β3, y se considera un mediador crucial de las interacciones célula-ECM (73). La relevancia de este componente ha sido ampliamente establecida en el sistema cardiovascular, especialmente en el corazón y los vasos sanguíneos, modulando una amplia variedad de procesos fisiológicos y participando en condiciones de enfermedad (74). En la placenta, la expresión de ILK es fundamental durante las primeras etapas del embarazo, ya que regula comportamientos celulares particulares (75). Además, la ILK parece desempeñar un papel importante en el desarrollo de la preeclampsia, surgiendo como un importante

objetivo terapéutico (76). Las cadherinas son proteínas transmembrana implicadas en la adhesión de célula a célula y son determinantes centrales de la citoarquitectura tisular. La cadherina epitelial (e-cadherina) es una de las cadherinas mejor caracterizadas estudiadas, que regula el desarrollo celular y la morfogénesis desde etapas tempranas (77) y con consecuencias adversas en la placenta cuando se desregula (78). Otros miembros de la familia de las cadherinas, como las cadherinas 7 y 9, también están surgiendo como indicadores prometedores del estado de salud y enfermedad (79).

Parece probable que la patología venosa se desarrolle por la interacción de diferentes factores. La compresión y los cambios hormonales desarrollados durante el embarazo podrían poner de manifiesto la susceptibilidad subyacente en la pared vascular de algunas mujeres. De esta manera, los estrógenos placentarios provocan un incremento de la retención de sodio y agua, aumentando la volemia, mientras que la progesterona sería responsable de una disminución del tono de la musculatura lisa vascular (80). Al mismo tiempo, durante el segundo y tercer trimestre y ligado al desarrollo fetal, aparecería el factor mecánico. El crecimiento del útero comprime la vena cava inferior, la vena iliaca y las venas uterinas, dificultando el retorno venoso (81). Finalmente, algunos estudios sugieren una susceptibilidad individual genéticamente determinada.

No obstante, a pesar de la alta frecuencia, se desconoce si debido a esta susceptibilidad o esta compresión, las varices pudieran ser un signo visible y precoz de otras alteraciones vasculares en el territorio placentario. El intercambio de nutrientes y oxígeno entre la madre y el feto se produce en la cámara intervellosa por una diferencia de presiones entre la circulación arterial y venosa. La aparición durante la gestación de venas varicosas en el territorio infradiafragmático, son un síntoma de hipertensión venosa. Esta situación podría provocar cierto grado de éxtasis sanguíneo, lo que incrementaría la dificultad del intercambio y difusión de sustancias entre la madre y el feto. Cualquier factor que afecte negativamente a la función trofoblástica, restringirá el

aporte fetal de oxígeno y nutrientes; lo que provocaría, en función de la severidad, desde un cuadro de hipoxia y acidosis, hasta sufrimiento fetal o, en casos extremos, la muerte.

En un trabajo reciente (human pathology, HISTOLOGY 8-9), se investigó la asociación entre la insuficiencia venosa en extremidades inferiores (diagnosticada ecográficamente y categorizadas según su gravedad clínica) y los cambios estructurales de la vellosidad placentaria, así como la expresión génica de algunos marcadores de hipoxia. Este trabajo fue pionero en mostrar la existencia de una relación entre la insuficiencia venosa e hipoxia placentaria.

El daño hipóxico produce un cambio severo del metabolismo celular, pasando este de ser aeróbico a anaeróbico a nivel placentario. Esto podría reflejarse, clínicamente, en la presencia de infartos o alteraciones placentarias. Asimismo, se podría generar una respuesta metabólica similar en un feto con déficit de oxigenación y de nutrientes. Lo que podría relacionarse con algunas alteraciones reflejadas en el momento del parto como la presencia de meconio o alteraciones del pH fetal.

1.5.1 Prevalencia e incidencia de las venas varicosas y problemas de insuficiencia placentaria en el embarazo

Como se ha expuesto en el epigrafe 1.2. la enfermedad venosa crónica es un trastorno común que afecta a un porcentaje importante de personas en el mundo (con una prevalencia estimada de EVC oscila entre el 60 y el 80% según las poblaciones estudiadas y métodos utilizados) (82-84)(85).

Basándose en diferentes características médicas, la clasificación CEAP (Clinical-Cause-Anatomy-Pathophysiology)(tabla 1)actualmente reconocida internacionalmente puede analizar con precisión el estado clínico, etiológico, anatómico y patológico de las venas (86).

Las varicosidades primarias, es decir, venas dilatadas con insuficiencia valvular, tienden a ser familiares y ocurren con frecuencia sin otros eventos causales (87). Las venas varicosas pueden aparecer en varios sitios del cuerpo, p. Ej. en el escroto, vulva, esófago, pero con mayor frecuencia en las extremidades inferiores. La definición de venas varicosas de las extremidades inferiores (VVLE) es dilatación y formación anormales de vena safena magna, y raramente vena safena parva en las extremidades inferiores con síntomas de ardor, estallido, hematomas o dolor en los pacientes (87) . Estas alteraciones son, aproximadamente, dos veces más frecuente en mujeres que en hombres. Además, el embarazo induce la dilatación y proliferación de los vasos sanguíneos, por lo que la congestión venosa y el aumento de la permeabilidad vascular durante el embarazo suelen causar edema de la piel y el tejido subcutáneo, en particular la vulva y la parte inferior de las piernas (88). Un historial de embarazo en el desarrollo de venas varicosas aumenta significativamente en un 82% en comparación con las mujeres sin antecedentes de embarazo (89).

Depende de los datos de la EMA, una afección debe tener una prevalencia alrededor de cinco casos por cada 10 000 habitantes en la UE (90). En el caso de afecciones que duran menos de un año, por ejemplo, las que ocurren solo durante el embarazo, la incidencia anual debe ser inferior a cinco casos por 10 000 habitantes de la UE. Al estimar la incidencia anual de enfermedades obstétricas es necesario considerar la proporción de embarazos que se ven afectados, la proporción de embarazos que no terminan en nacidos vivos y las tasas de natalidad nacionales y de la UE.

1.6. Enfoques terapéuticos en la ECV

La atención médica de las ECV conlleva diferentes estrategias que se pueden utilizar solas o en combinación, para maximizar el manejo clínico de la sintomatología, pronóstico y, por tanto, la calidad de vida de los pacientes. En este sentido, tres enfoques principales deben mencionarse en este punto: (a) terapias de compresión, dirigidas a la hemodinámica venosa; (b) intervenciones médicas dirigidas a controlar la insuficiencia venosa, y (c) terapias farmacológicas dirigidas a mecanismos fisiopatológicos específicos de la enfermedad (91).

Debemos considerar que las personas mayores y los pacientes con comorbilidades adicionales, a pesar de requerir más intervenciones, responden bien a las diferentes terapias recibidas al igual que pacientes más jóvenes o sin ningún tipo de afección (92). Por tanto, toda la población afectada por ECV podría ser beneficiario de las terapias disponibles.

La terapia de compresión actúa aumentando la presión intersticial, disminuyendo así tanto el calibre de las venas superficiales y profundas, reduciendo la presión venosa y el edema mientras promover la actividad contráctil de los músculos de la pantorrilla (93). Las medias de compresión son fáciles utilizar, y con frecuencia, son la primera medida conservadora a tomar, logrando importantes mejorías en la mayoría de los pacientes sin causar muchas molestias (94).

Las medias proporcionan la mayor eficacia en cuanto a reducción de volumen y hemodinámica venosa. Además, las medias de compresión favorecen la curación de úlceras asociadas a CVI; por lo tanto, disminuyendo la recurrencia de ulceraciones en enfermedades venosas después de una intervención quirúrgica (95). Sin embargo, la utilidad de la compresión la terapia después del tratamiento con VV tiene un grado limitado de evidencia (96,97).

Aun así, debe ser considerado que los pacientes con enfermedad arterial periférica requieren una aplicación cuidadosa de este tratamiento en particular, ya que podría interferir con la circulación sanguínea en las extremidades inferiores y empeorar la enfermedad subyacente.

HIPÓTESIS Y OBJETIVOS

La enfermedad venosa crónica es una patología con una incidencia y prevalencia relevante en la población general. Los diferentes estudios a nivel mundial han mostrado como esta condición aparece de manera especialmente frecuente en mujeres embarazadas. Sin embargo, se desconoce las posibles implicaciones que la aparición o desarrollo de la enfermedad venosa durante la gestación pudiera tener en la salud de la madre o del niño. Por todo ello, se plantea la siguiente hipótesis：

La enfermedad venosa crónica es una patología sistémica y su aparición durante la gestación constituye un signo de alteraciones placentarias con repercusiones en el desarrollo o funcionamiento de la misma.

Objetivo

1. Revisar los conocimientos sobre la enfermedad venosa crónica, incluida su epidemiología, etiología y factores de riesgo. Así como las repercusiones en la salud de la madre o del feto durante el periodo gestacional.

2. Estudiar si existe una relación entre la frecuencia de aparición de insuficiencia venosa en el embarazo y la presencia de insuficiencia placentaria durante el parto.

3. Conocer si existe una alteración en la estructura y función celular de la placenta que sea el sustrato de los resultados gestacionales. En concreto, la implicación de la ILK y un conjunto de cadherinas (e-adherina, cadherina-6, y cadherina 7) en la placenta de mujeres con enfermedad venosa .

METODOLOGÍA

Para alcanzar los objetivos de la investigación se proponen tres estudios, cada uno de ellos con diferente enfoque metodológico. El primero, una revisión sistemática y extensa de la literatura, con objeto a identificar los estudios realizados y el conocimiento de las implicaciones de la enfermedad venosa en la gestación. En segundo lugar, un estudio transversal de una muestra poblacional, con objeto a identificar si existe relación entre esta enfermedad venosa y la aparición de manifestaciones clínicas que representen un mal funcionamiento placentario. Finalmente, un tercer estudio, de carácter longitudinal, que analice las alteraciones que son el origen y sustrato de las manifestaciones clínicas.

Articulo I

En el artículo I，Se realizó un estudio descriptivo mediante el método de revisión bibliográfica sobre la enfermedad venosa crónica. Recolectamos algunos datos más relevantes sobre el tema de dicha enfermedad, enfatizándose en la fisiopatología y la cognición médica. Las unidades de análisis fueron artículos científicos de tipo primario, localizados mediante los bases de datos reconocidas por su rigor científico.

La ecuación de búsqueda utilizada en el campo de palabras clave (KW) fue: “Enfermedad venosa crónica (EVC)” “varices” “fisiopatología venosa” “terapias vasculares” “hipertensión venosa”. Estas palabras clave sirven para asegurar el número más amplio posible de artículos publicados sobre el tema. La búsqueda de documentos incluyó el idioma español e inglés, fijándose en el período de los años 1988 a 2021, publicados y/o realizados en países de todo el mundo.

Articulo II

En el artículo II, se realizó un estudio transversal nacional que incluyó todos los partos vaginales (n=256.531) ocurridos en los hospitales de España durante 2015. En el diagrama de flujo (Gráfico 2) se detalla el tamaño de la muestra y las exclusiones. Se utilizaron los datos precisos de los registros médicos administrativos (CMBD) correspondientes a las atenciones registradas en el año 2015, tanto en el sistema de salud público como privado de las diferentes Comunidades Autónomas de España. A partir de estos datos, la exposición se especificó como mujeres embarazadas que presentaban varices en las piernas (IDC9-CM: 671.0), varices en la vulva y el periné (IDC9-CM: 671.1) o hemorroides (IDC9-CM: 671.8). La principal variable de los resultados fue los signos de compromiso fetal intraparto (CFI) debidos a una función placentaria inadecuada: presencia de sufrimiento fetal (CIE9-CM: 656.3), equilibrio ácido-base anormal, acidosis intrauterina o meconio en el líquido (CIE9-CM: 656.8) e infarto placentario o placenta anormal (CIE9-CM: 656.7). Se consideraron las siguientes variables como factores de confusión: la edad, los hábitos tóxicos y la presencia de comorbilidades. En concreto, se consideraron los diagnósticos de hipertensión, patología cardiaca, enfermedad respiratoria, cáncer, enfermedades renales o hepáticas, patología tiroidea, diabetes mellitus, obesidad y alteraciones de la coagulación, así como la presencia de depresión o demencia para calcular el índice de comorbilidad de Charlson para cada mujer. La lista completa de los códigos ICD9-CM utilizados para clasificar las variables se proporciona en el material suplementario (Tabla 1S).

Se realizó un análisis descriptivo de la población presentando medias y proporciones. Se utilizó un modelo de regresión logística para cuantificar la asociación entre el LEPVI y el compromiso fetal intraparto antes y después del ajuste. Dado el número de comparaciones, se ajustó el nivel de significación, y se consideró significativo un $\alpha <$ 0,005. Debido al mayor tamaño de la muestra, se realizaron dos análisis de sensibilidad diferentes. En primer lugar, se ajustó un modelo de regresión logística que incluía solo los hospitales con más de 500 nacimientos en 2015 y la presencia de al menos un caso

de LEVPI en mujeres. En segundo lugar, se realizó un análisis de puntuación de propensión utilizando las mismas covariables de comorbilidad en el análisis de regresión logística. Se analizó la propensión (es decir, la probabilidad condicional) de que una mujer tenga varices en función de sus características clínicas. Utilizamos la puntuación de propensión para unir, sin reemplazo, a las mujeres con varices y a las mujeres sin varices en una proporción de 1:1. Las OR se calcularon mediante una regresión logística condicional univariante. Se utilizó STATA/IC (versión 14.2) para todos los análisis estadísticos.

Artículo III

En el artículo III hemos realizado un estudio observacional, analítico y prospectivo que incluyó a 114 mujeres en el tercer trimestre del embarazo. De ellas, había 62 mujeres diagnosticadas de ECV según la clasificación CEAP y 52 mujeres sin antecedentes de ECV, denominadas controles sanos (CS). Durante la consulta del tercer trimestre, se revisó la historia clínica y una exploración física general de la mujer. Además, se realizaron ecografías de las extremidades inferiores utilizando un Eco-Doppler (Portable M- Turbo Eco-Doppler; SonoSite, Inc., Bothell, WA, USA) a 7,5 MHz.

Se tomaron biopsias de la placenta después del parto en las 114 pacientes. En todos los casos, se obtuvieron 5 fragmentos de placenta mediante el uso de un bisturí para incluir varios cotyle- dones mixtos. Posteriormente, las muestras se procesaron en una campana de flujo laminar de clase II en un entorno estéril. Las muestras conservadas se almacenaron en 1 mL de RNAlater® a -80 ◦C hasta su posterior procesamiento para el análisis de la expresión génica. Las placentas MEM conservadas se emplearon para estudios histológicos e inmunohistoquímicos.

Al mismo tiempo, se realizaron estudios de expresión génica mediante la transcripción inversa-cuantitativa PCR (RT-qPCR).El ARN se extrajo siguiendo el método de tiocianato de guanidinio y cloroformo de fenol, lo que permitió analizar los niveles de expresión del ARNm de los genes seleccionados. Se realizaron estudios de inmunohistoquímica para el análisis de la expresión proteica. Las reacciones antígeno/anticuerpo se detectaron mediante el método del complejo avidina-biotina, con avidina-peroxidasa.

El análisis estadístico se realizó con el programa GraphPad Prism® v6.0 (GraphPad, Inc., San Diego, CA, USA). Se utilizó la prueba U de Mann-Whitney para comparar los 2 grupos, y los datos se expresaron como mediana ± SEM.

RESULTADOS y DISCUSIÓN

Articulo I

Understanding Chronic Venous Disease: A Critical Overview of Its Pathophysiology and Medical Management

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Resumen artículo I:

La enfermedad venosa crónica (ECV) es una condición multifactorial que afecta a un porcentaje importante de la población mundial. Va desde signos clínicos leves, como telangiectasias o venas reticulares; hasta manifestaciones graves, como ulceraciones venosas. Sin embargo, las venas varicosas (VV) son la manifestación más común de ECV.

Los mecanismos explícitos de la enfermedad no se conocen bien. Parece que la genética y una plétora de agentes ambientales juegan un papel importante en el desarrollo y progresión de las ECV. La exposición a estos factores conduce a la alteración de la hemodinámica del sistema venoso, descrita como hipertensión venosa ambulatoria, por lo tanto, promueve cambios microcirculatorios, respuestas inflamatorias, hipoxia, remodelación de la pared venosa y variaciones epigenéticas, incluso con importantes implicaciones sistémicas. Por lo tanto, un manejo clínico adecuado de los pacientes con ECV es fundamental para prevenir los daños potenciales de la enfermedad, que también implica una pérdida significativa de la calidad de vida de estos individuos.

En esta primera publicación, realizamos una revisión bibliográfica para recoger el conocimiento actual de la ECV, incluyendo su epidemiología, etiología y factores de riesgo, pero haciendo énfasis en la fisiopatología y atención médica de estos pacientes, incluyendo manifestaciones clínicas, diagnóstico y tratamientos. Además, este estudio incluye una discusión sobre los futuros retos que deben abordarse en este campo de estudio para entender mejor el contexto de las ECV.

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Review

Understanding Chronic Venous Disease: A Critical Overview of Its Pathophysiology and Medical Management

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Abstract: Chronic venous disease (CVD) is a multifactorial condition affecting an important percentage of the global population. It ranges from mild clinical signs, such as telangiectasias or reticular veins, to severe manifestations, such as venous ulcerations. However, varicose veins (VVs) are the most common manifestation of CVD. The explicit mechanisms of the disease are not well-understood. It seems that genetics and a plethora of environmental agents play an important role in the development and progression of CVD. The exposure to these factors leads to altered hemodynamics of the venous system, described as ambulatory venous hypertension, therefore promoting microcirculatory changes, inflammatory responses, hypoxia, venous wall remodeling, and epigenetic variations, even with important systemic implications. Thus, a proper clinical management of patients with CVD is essential to prevent potential harms of the disease, which also entails a significant loss of the quality of life in these individuals. Hence, the aim of the present review is to collect the current knowledge of CVD, including its epidemiology, etiology, and risk factors, but emphasizing the pathophysiology and medical care of these patients, including clinical manifestations, diagnosis, and treatments. Furthermore, future directions will also be covered in this work in order to provide potential fields to explore in the context of CVD.

Keywords: chronic venous disease (CVD); varicose veins; venous pathophysiology; vascular therapies; venous hypertension

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1. Introduction

Chronic venous disease (CVD) is a persistent, progressive, and frequently underestimated condition widely represented in the general population, having a huge socioeconomic, physical, and psychological impact associated [1,2]. CVD entails a broad spectrum of venous abnormalities in which blood return is seriously compromised. In the pathophysiology of CVD, the interplay between genetics and environmental factors are responsible

for increasing the ambulatory venous pressure, leading to substantial changes in the whole structure and functioning of the venous system [3]. Importantly, the term CVD should be differed from chronic venous disorder, which only includes the full morphological and functional abnormalities of the venous system, but without considering the clinical manifestations and other serious concerns affecting the patient [4].

Varicose veins (VVs) are the most common manifestation of CVD. Other venous signs of CVD include telangiectasias and reticular veins. Despite these, clinical signs may affect any vein in the body, the venous system located in the lower limbs is often the most vulnerable structure to suffer CVD [5]. This is mainly due to the increased resistance to overcome by the gravitational force, which is notably higher than the rest of regions in the body. In the lower limbs, three great systems may be distinguished: superficial veins, represented mainly by the great saphenous vein, the small saphenous vein, the anterior accessory saphenous vein and their tributaries; deep veins, the major transporter of blood flow; and perforating veins, connecting both systems [6]. From a histological perspective, veins are conformed by three layers: (1) tunica intima or inner layer, mainly conformed by endothelial cells (ECs); (2) the tunica media or media layer, represented by vascular smooth muscle cells (SMCs) and little elastic fibers; and (3) tunica adventitia or outer layer, which is composed by connective tissue, with an important development of elastic fibers, giving the vessel an important support and elasticity [7]. In the case of VV however, the normal structure of the vein is altered, with prominent changes in the thickness and composition of the venous wall [8]. In addition, veins present venous valves, bicuspid prolongations from the venous tissue essential to maintain the blood flow in the proper direction, impeding venous reflux [9]. Furthermore, different muscle pumps act coordinately with the venous valves to assure the unidirectional blood flow. The calf muscle, often named the "peripheral heart", is especially considered the most prominent enhancer of venous return from lower limbs to heart [10]. Importantly, failures in both systems are also crucial to understand the pathogenesis and progression of CVD leading to a venous reflux and to venous stasis [11–14]. The anatomy of the venous system in the lower limbs, its physiology, and histology are briefly summarized in Figure 1.

The term chronic venous insufficiency (CVI) includes the most severe manifestations, such as edema, skin changes, or leg ulcers [15], frequently, associated with a poorer quality of life of these patients [16]. Different criteria are currently established to distinguish CVD presentations. CEAP (clinical–etiology–anatomy–pathophysiology) classification is a globally accepted method to study venous disorders. It is based on different medical features that permits to accurately analyze the clinical, etiological, anatomical, and pathological status of veins [17]. In this context, it is frequent to use clinical (C) manifestations to refer specific CVD presentations. For instance, VVs could be classified as C2 while CVI is comprised between C3 to C6 stages [18]. As CVD, and particularly CVI, are a worrisome and a growing concern in westernized societies while representing a challenge either for the individual or the healthcare system, the purpose of this review is to collect the current knowledge of CVD from an integrative perspective. Therefore, different approaches including epidemiology and risk factors, diagnosis, etiology, pathophysiology, and utilized treatments will be tackled. Moreover, future directions and important considerations will also be addressed.

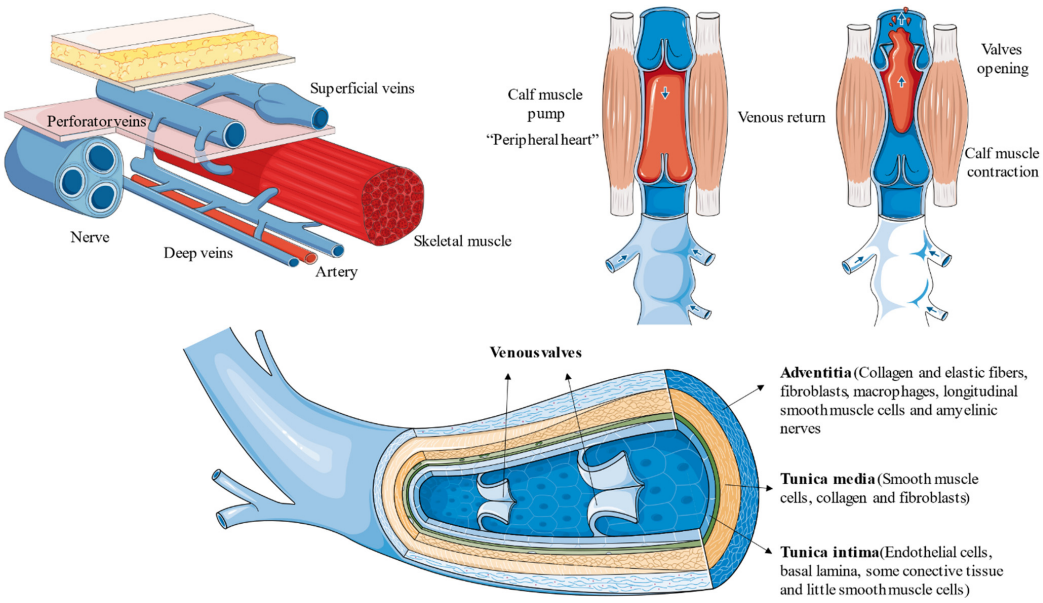

Figure 1. A general overview of the anatomy, physiology, and histology of venous system in the lower limbs. Superficial veins, mainly represented by the great and small saphenous veins, carry blood from the skin and subcutaneous tissues. Superficial veins might transport the blood through the saphenous junctions and the perforator veins to the deep venous system, the major contributors of the venous return. Deep veins are accompanied by an artery, nerves, and skeletal muscle at both sides, surrounded by a fascial compartment. Calf muscle pump is the most important source to assure an appropriated blood return from the lower limbs and it is frequently designed as the "peripheral heart". Venous return is also permitted by the presence of venous valves, which are essential to prevent blood reflux. The cytoarchitecture of the vein comprises three main layers, intima, media, and adventitia, with unique properties.

2. Epidemiology and Risk Factors

CVD is a common disorder that affects an important percentage of people in the world. In general terms, the estimated prevalence of CVD ranges from 60 to 80% [19–21], although these data may be highly heterogeneous in the literature depending on the populations studied, the methods followed and disease definitions [22]. Most cases are defined as C0 and C1 classes, while approximately 25% of patients are diagnosed with VVs (C2). CVI (C3–C6) concern small percentage of patients with CVD, representing up to a 5% of patients [23]. The incidence of VVs was about 2.6% for females and 1.9% for males, according to the Framingham study [24]. Thus, it is recognized that the risk for suffering from VV and CVI is higher in women than men, and it also increases with age, therefore explaining its great prevalence and growing incidence [25]. Furthermore, this condition may entail important consequences for the quality of life (QOL) of the affected individuals. In fact, even patients with uncomplicated VVs note a significant reduction on their QOL and if untreated they will surely progress to CVI [26]. Only in the United States, more than 25 million people are affected by CVD and approximately 6 million present advanced stages and CVI, with an estimated cost of USD 3 billion per year [27]. Thus, it is undeniable that CVD represents one of the most frequent vascular diseases in the world, entailing an important burden not only for the individual, but also for the healthcare systems.

As above mentioned, age is one of the major risk factors of CVD. According to the Edinburgh Vein Study, ~1 in 3 patients developed CVI from the initial VV diagnosis after a 13-year follow-up, with the rate consistently augmenting with age [28]. Vuylsteke et al. [29] reported that elder individuals (>65 years) were more vulnerable to the different

risk factors associated to CVD, with differential effects according to the geographical regions. Thus, elder people are notably susceptible of suffering from CVD and progress to CVI, particularly when combined with other risk factors, such as body mass index (BMI) [30] and the female gender [31]. The reasons why women are more likely to suffer from CVD, reside on different hormonal and physiological factors that make them more sensitive to develop this condition [32]. In this group, pregnant women (PW) are more prone to develop this condition, with approximately 40% of them suffering from CVD [33]. In addition, the number of pregnancies might also be a variable of note to acquire this condition. Thus, it is estimated that 20% of nulliparous women over 40 years old will undergo VVs. However, this percentage increases to 40% in the case of women who were pregnant 1 to 4 times and up to a 65% in those with five or more gestations [34]. Apart from female sex and aging, a plethora of risk factors have been identified in the development of CVD. Overweight and obesity are one of the best-established factors directly related to CEAP C categories, independently from the rest of risk factors [35]. This could be due to many plausible and synergic effects, including the pro-inflammatory status generally associated to the increased adiposity, and other factors, such as an augmented intra-abdominal pressure, which may lead to greater reflux, increased vein diameter, and venous pressure [36]. Sedentarism is another important risk factor to develop and aggravate CVD manifestations [37]. Conversely, physical activity was not related with higher risk of CVD, but it is seen as an essential method to prevent its progression [38]. Prolonged sitting and, more prominently, prolonged standing are also recognized as risk factors for suffering from CVD [39]. Frequently, both situations are related to occupational positions. Family history of CVD is also recognized as a risk factor for suffering this condition [40]. Moreover, previous events of blood clots in the veins -prominently deep venous thrombosis (DVT) has also been described as a potential risk factor for suffering from CVD [41], as well as regular smoking [42]. On the other hand, more doubts remain regarding the possible association between height and CVD. Although some studies have described a causal relationship [43], it seems that height does not influence on the progression of the disease [44]. Overall, there are many risk factors that could be related to the onset and development of CVD as they may influence different pathophysiological mechanisms involved in the onset and progression of the disease.

3. Clinical Manifestations

As above mentioned, CEAP classification is the most accurate and globally used method to establish a precise CVD diagnosis. It is based on clinical, etiological, anatomical, and pathophysiological criteria. Main values assigned in CEAP are summarized in Table 1. In this part we will summarize clinical manifestations (C) of CVD whereas etiology (E) and pathophysiology (P) will be subsequently discussed. The use of this system allows a better classification for patients with the aim to study their initial symptoms and progression of the disease, [45]. The main limitation of this method is, however, the interobserver variability that may depend on the experience of the physician [46]. According to CEAP, we consider C0 when there are no clinical signs of CVD, C1 with telangiectasias/reticular veins, C2 correspond to the presence of VVs, and C3–C6 as CVI, ranging from edema and dermatological alterations (C3/C4 respectively) to healed and active ulcerations (C5/C6) [18].

Table 1. Last revision of CEAP classification on CVD.

Clinical (C) Classification	Etiologic (E) Classification	Anatomic (A) Classification	Pathophysiologic (P) Classification
C0 No visible or palpable signs of venous disease C1 Telangiectasias or reticular veins C2 Varicose veins C2r Recurrent varicose veins C3 Edema C4 Changes in skin and subcutaneous tissue secondary to CVD C4a Pigmentation or eczema C4b Lipodermatosclerosis or atrophie blanche C4c Corona phlebectatica C5 Healed C6 Active venous ulcer C6r Recurrent active venous ulcer	Ep Primary Es Secondary Esi Secondary—intravenous Ese Secondary—extravenous Ec Congenital En No cause identified	As Superficial Old New Description 1. Tel Telangiectasia 1. Ret Reticular veins 2. GSVa Great saphenous vein above knee 3. GSVb Great saphenous vein below knee 4. SSV Small saphenous vein AASV Anterior accessory saphenous vein 5. NSV Nonsaphenous vein Ad Deep 6. IVC Inferior vena cava 7. CIV Common iliac vein 8. IIV Internal iliac vein 9. EIV External iliac vein 10. PELV Pelvic veins 11. CFV Common femoral vein 12. DFV Deep femoral vein 13. FV Femoral vein 14. POPV Popliteal vein 15. TIBV Crural (tibial) vein 15. PRV Peroneal vein 15. ATV Anterior tibial vein 15. PTV Posterior tibial vein 16. MUSV Muscular veins 16. GAV Gastrocnemius vein 16. SOV Soleal vein Ap Perforator 17. TPV Thigh perforator vein 18. CPV Calf perforator vein An No venous anatomic location identified	Pr Reflux Po Obstruction Pr,o Reflux and obstruction Pn No pathophysiology identified

CEAP: clinical–etiology–anatomy–pathophysiology; CVD: Chronic venous disease.

Clinical manifestations in the initial stages of the disease are often scarce, and the more CVD progress, the more symptomatology will appear [47]. VVs are the most representative manifestation in the venous system, although reticular veins and telangiectasias might also be recognized as important clinical signs [48]. In advanced stages, the most important expression of CVI consist of the lower limb pain, usually defined as heaviness, discomfort, or pressure exacerbated at the end of the day. Patients frequently show a malleolar edema with fovea getting worse under situations in which venous pressure increases and the lymphatic drainage is impaired [49]. Differential diagnosis should exclude other causes of the edema, such as cardiac insufficiency, hypoalbuminemia, or hypothyroidism, among others [50]. On the contrary, patients usually refer to the presence of dilated superficial tortuous veins clearly identified as an esthetic alteration. Additionally, patients with CVI are also more susceptible to suffer from DVT mainly in the femoropopliteal segment, with the added risk of pulmonary thromboembolism, a potentially mortal condition [51]. Superficial venous thrombosis (SVT) could also be distinguished as a potential clinical sign of CVD. Although for a long time this manifestation was considered as a harmless condition, recent evidence warn about the dangerous of suffering from SVT, as it is also significantly associated to an increased risk to develop DVT and pulmonary thromboembolism [52]. Moreover, in advanced stages of valvular incompetence, a superficial traumatism on large VVs could result in a hemorrhage, which could be fatal in some cases [53].

Furthermore, during advanced CVI stages (CVI $\geq$ 4), the inflammatory environment and edema affect skin and subcutaneous tissues, finally leading to epidermis breaking and the development of cutaneous ulcerations [54]. Prior changes in the skin includes eczema [55] and pigmentation, which appears to be caused mainly by a hemosiderin deposit occurred in early stages of CVI [56], lipodermatosclerosis, a specific chronic fibrosing panniculitis related to CVI [57] and corona phlebectatica, identified as an abnormal visible cutaneous blood vessels at the ankle with four components: "venous cups," blue and red telangiectasias, and capillary "stasis spots" [58]. Furthermore, a rare malignant skin degeneration could also be observed in patients with CVI [59,60]. These skin changes and their clinical manifestations are strongly associated with a worse QOL of these patients [61]. Moreover, these cosmetic defects may have important consequences not only on physical but also on the psychosocial well-being of the individual [62]. It is of note that many patients with CVI may suffer from depression and anxiety, mainly due to the esthetical concerns, long-term complications and the submission to the different therapeutic regimens [63]. In this sense, Blättler et al. [64] have recently conducted a study aiming to inquire the neuropsychological impact of CVD. They observed that some individuals with mild venous pathology may refer venous symptoms that are not be correlated with their clinical state. Collectively, it can be concluded that CVD should be handled from an integrative perspective, utilizing different tools and questionnaires to accurately evaluate the QOL of these patients [65]. Particularly, Villalta-Prandoni scale (SF-36) and EuroQol 5D scale, which may be repeated over time while demonstrating its efficacy on validating this aspect of the disease [66,67].

4. Diagnosis

First, a clinical history of the patient must be carefully conducted considering allergies, previous medical prescriptions (hormonal contraceptives, anticoagulants, etc.), family history of VVs or CVD and personal antecedents of thromboembolism, cardiovascular or other relevant disease [68]. Likewise, the presence of specific symptomatology of CVD must also be assessed. Then, patients will be subjected to a physical exploration with the aim to find clinical signs of CVD, such as VVs, edema, skin changes, or venous ulcerations [69].

Regarding diagnostic methods used in CVD, color duplex ultrasound (CDU) is the most used investigational exam [70]. In fact, CDU has replaced the rest of the diagnostic techniques because of being non-invasive, reproducible, and easy to use while importing data about vein morpho-hemodynamic changes in the affected limb/s. [71]. Thus, the use of CDU is essential to conduct a proper management of the patient [72]. Similarly, the

use of CDU could also be useful to assess the presence of the venous pathology in the abdominal or pelvic areas [73,74].

Likewise, the use of other procedures, such as plethysmography, might be useful to identify pathophysiological mechanisms occurring in the patient by distinguishing among venous reflux, obstruction, or both, or conversely, muscle pump dysfunction. However, it does not offer the possibility of studying anatomical features [75]. Computed tomography venography or magnetic resonance are also valuable techniques in the CVD diagnosis, although it is normally reserved to complex cases with prominent venous alterations, malformations, or abdominal affections [76].

5. Etiology and Pathogenesis of CVD

CVD is a vascular disorder in which venous return is compromised. According to the CEAP classification, the etiology of CVD could be as follows: (1) primary CVD; (2) secondary CVD, which in turn are divided into (i) intravenous secondary causes; (ii) extravenous secondary causes; and (3) congenital CVD [77].

5.1. Primary CVD

Primary CVD could be defined as a progressive process occurred in the venous wall or venous valve that leads to an abnormal dilatation and weakness in the vein, eventually resulting in pathological and demonstrated reflux. Primary CVD is frequently manifested in the saphenous veins, which is known as truncal insufficiency [78], although it could also be presented in the superficial non-truncal tributaries' veins [79,80]. The use of animal models has shed light on the pathogenesis of primary CVD, recognizing that the sustained induction of ambulatory venous hypertension injured the vein wall and venous valves; hence, promoting the development and progression of CVD [81]. However, unlike experimental models where the increased venous pressure is induced by the researcher, the etiological agents of venous hypertension in human beings are much more complex.

Primary CVD is the result of different genetic and environmental factors. The vast majority of people with CVD are carriers of certain polymorphisms or genetic variants that are in part responsible for the development of the disease. In fact, it is estimated that the genetic component of CVD is approximately a 17% [82]. The remaining proportion resides on a wide variety of environmental components generally associated to the previous mentioned risk factors [83]. As an example, quiet standing for 30 min was enough to trigger an inflammatory response in the venous wall [84], as the venous pressure while standing is higher in comparison to the pressure while sitting and even higher than the pressure when walking. Permanent standing in the workplace starts a persistent and harming venous stasis that progressively makes subjects more vulnerable to suffer from venous reflux and CVI, particularly if combined with other factors like aging [85]. Progesterone has also been identified as an important etiological agent of CVD, as it is associated with SMC relaxation, vasodilation, and valvular incompetence [86]. Similarly, a redistribution of progesterone receptors has been shown to participate in the progression of the disease [87]. In the case of PW, significant changes occur, mainly due to hormonal, hemodynamic and mechanical factors, which induce an important remodeling in the venous system that may be associated with the onset and progression of CVD [88–90].

5.2. Secondary CVD

Secondary CVD are those cases in which the manifestation of the disease is caused by a previous event that, as mentioned below, could be subdivided into secondary intravenous—when the vein wall and valves are adversely affected—and secondary extravenous, in which there is no evidence of vein damage, but the local or systemic venous hemodynamic is impaired (e.g., external causes, such as central venous hypertension, extrinsic compression caused by a tumoral mass, by arteries such as in May-Thurner syndrome, or in case of diaphragmatic or limb muscle pump dysfunction) [77].

The most common cause of secondary intravenous CVD is a previous episode of acute DVT. DVT is a major cause of morbidity and mortality, affecting approximately 1 in 1000 of individuals per year [91]. Primary CVI is identified as a risk factor for suffering DVT [51]. In turn, clinical or subclinical DVT is identified as a potential cause of CVI [92]. A central link in this bidirectional relationship may reside on different genes shared in both pathologies, such as the THBD (thrombomodulin) and MTHFR (methylenetetrahydrofolate reductase) [43]. Apart from genetics, the increased venous pressure and damage in the venous wall may conduct to the development of both CVD and DVT. In contrast, DVT is characterized by the formation of a thrombus, caused by the interaction of the coagulation components with the endothelium and hemodynamics (stasis), generally affecting deep veins located in the lower limbs [93]. This could provoke a plethora of morphological changes in the venous wall and result in a combination of partial obstruction and reflux, leading to severe presentations, such as pain, venous claudication, edema, skin changes, and ulceration; these clinical manifestations of CVI typically characterize post-thrombotic syndrome (PTS) [94]. Moreover, secondary CVD progresses more rapid than primary CVD [95], similarly being associated with a poorer QOL of these patients [96]. It is estimated that around a 20 to 50% of patients who suffered an episode of DVT will develop PTS, while a 5 to 10% will progress to venous ulceration [97]. Thus, an adequate management of patients after being diagnosed of DVT will be essential to prevent the appearance of PTS and severe forms of CVI.

Likewise, post-traumatic CVD should also be mentioned here. The cause of vascular trauma is blunt trauma (fractures or dislocations), penetrating trauma (caused by bullets, knives, etc.), or a combination of both, and they could affect either upper or lower extremities [98]. There are five types of vascular traumas: intimal injuries, complete wall defects with pseudoaneurysms or hemorrhage; complete transections with hemorrhage or occlusion; arteriovenous fistulas; and spasms [99]. Epidemiologically, the occurrence of CVD secondary to a traumatic event in the general population is very rare (1% to 2% of the total cases), as penetrating traumas are more frequently related with this condition, with higher exposure of the military setting [98]. The clinical management of vascular traumas represent an important challenge, as they might correlate with a high morbidity and mortality. In the case of venous traumas, vascular repair and ligation are the most frequent approaches used [100]. However, previous studies have demonstrated an increased risk for developing CVI even after either ligation or repair [101]. In this line, Bhatti et al. [102] found that traumatic arteriovenous fistula was the most common reason of unusual secondary VVs; therefore, showing the impact of venous trauma on CVD etiopathogenesis. Similarly, a prior trauma is also related with a greater risk of DVT and pulmonary thromboembolism [103]. Therefore, venous trauma may represent a relevant secondary cause of CVD, being equally associated with DVT and other vascular disorders.

5.3. Congenital CVD

The influence of genetics in the CVD pathogenesis is extraordinarily complex [104]. As previously defined, family history of CVD is an important risk factor from suffering this condition. Notwithstanding, the genetic factors usually interact with environmental agents, the role of certain single gene mutations might be enough to cause venous disorders [105]. In this section, we summarize the association of particular genetic alterations in the development of CVD.

Venous angiodysplasias, such as Klippel-Trenaunay syndrome (KTS), are a major example of congenital CVD. KTS is a vascular disorder generally caused by somatic mutations in the PI3KCA gene [106]. Inheritance of KTS is quite complex. Some authors have considered it to be a paradominant inheritance [107], while in other cases it appears to provide a dominant inheritance [108]. Finally, inherited gene translocations have also been reported [109,110]. Howsoever, KTS is associated to an overgrowth in the vascular cells, leading to the development of different signs, such as cutaneous hemangiomas (Port-wine stains), lymphatic, or venous malformations [111]. Thus, patients with KTS exhibit a

wide variety of venous complications, such as severe venous hypertension, complex reflux patterns, advanced valvular incompetence, calf muscle pump impairment and DVT, and venous ulcer development [112,113].

Parkes Weber syndrome (PWS) is another congenital vascular disorder affecting capillary, lymphatic, venous and arteriovenous malformations. PWS is frequently misdiagnosed with KTS, although the latter does not present arteriovenous fistulas [114]. In addition, the gene involved in PWS appears to be RASA1, which is mutated up to a 50% of the patients. Nonetheless, 10% of the cases might be attributed to alterations in the EPHB4 gene [115]. The remaining 40% of pathogenic gene variants are still undiscovered. Similar to KTS, PWS is characterized by limb overgrowth and vascular malformations, leading to a persistent venous hypertension, dilation, and varicogenesis [116]. Moreover, approximately 1 in 4 patients with PWS show chronic venous ulcerations [117]. Thus, it would be crucial to identify and elaborate proper management of patients with either PWS or KTS, as they are more likely to suffer severe CVI and venous complications. Other syndromes related to the development of CVD include lymphedema distichiasis syndrome (LDS), characterized by FOXC2 mutations, cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), affecting the Notch3, Chuvash Polycythemia (VHL mutations), severe congenital neutropenia type 4 (alterations in the G6PC3), and Ehlers-Danlos syndrome (EDS), with defective COL3A1 functioning [118]. Overall, these genetic mutations result, irretrievably, in the development of CVI, due to vascular and congenital malformations. Fortunately, this only represents a little group of patients with CVD. The different etiopathogenic mechanisms of CVD are presented in Figure 2.

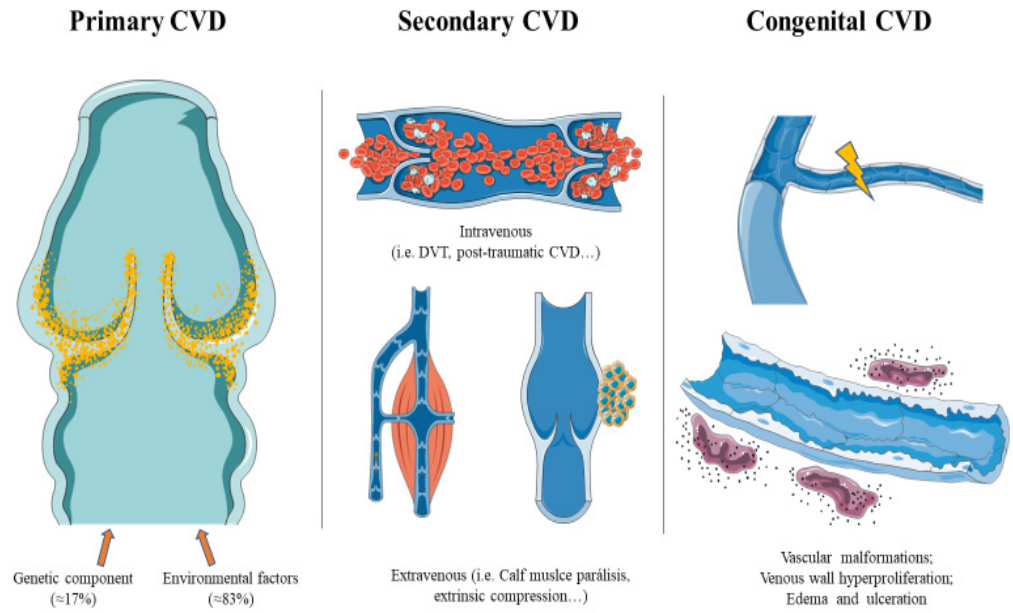

Figure 2. A general view on the different etiological mechanisms of CVD. Primary CVD (left) is caused by different genetic and environmental factors and it is characterized by a persistent venous hypertension responsible for the onset and progression of the disease. Secondary CVD (middle) is preceded by a pathological event, which could be intravenous (DVT) or extravenous (e.g., calf muscle dysfunction, extrinsic tumoral mass or arterial compression). Congenital CVD (right) represent a small percentage of the problem, and it is caused by single gene mutations or chromosomic aberrations.

6. Pathophysiology

CVD is a multifactorial disease involving complex pathophysiological mechanisms. The increased ambulatory venous pressure and vein dilation in the lower limbs promotes an intricate vascular response, leading to stasis and consequent secondary inflammatory

response, and altered shear stress. Obstruction and/or reflux are the two main pathomechanisms leading to significant changes in the venous wall and pathological venous reflux, thereby contributing to the progression of CVD [119]. Moreover, these changes provide the creation of a hypoxic environment, which is thought to be an important contributor in the pathophysiology of the disease. Moreover, the role of certain gene mutations or variants are also implicated either in the etiology or pathophysiology of the disease. Similarly, epigenetic alterations have also been found in patients with CVD, as well as evidence of systemic markers of damage related to the progression of the disease [37,120–122]. In this section we will collect the current knowledge of these pathophysiological mechanisms occurring in CVD in order to understand the most important properties of the disease.

6.1. Hemodynamic and Microcirculatory Alterations

Venous return from the lower limbs entails more difficulties than arterial circulation due to the defiance of gravity. Several morphofunctional diagnostic tests are employed to investigate CVD; together with ultrasound-based instruments, plethysmograph methods are used in combination, although they have not been standardized yet. The measurements are based on the changes in volume within veins evaluating venous filling index [123,124].

In the event of CVD, venous hypertension and dilation induces a decrease in shear stress, the frictional force within ECs. ECs sense these changes and transduce the physical signals from fluid shear stress into the altered biomolecular signaling; this prompts the typical vicious cycle in CVD, inducing hypertension, venous wall remodeling, and inflammation [3,125].

In the lower leg, ankle venous ambulatory pressure normally decreases to about 35 mm Hg [126]. In patients with CVD, this value tends to increase proportionally to the CEAP stratification [127]. The different pressure gradient may be an activator of neovascularization and reflux recurrence in the event of varicose vein treatment [128]. On the other hand, continuous changes in venous pressure with edema and hypoxemia as clinical manifestations, promote microcirculatory adverse reaction and eventually leg ulcerations [129]. In the course of CVD towards CVI, the microangiopathic changes involve a decreased number of capillaries and increased permeability of these vessels and an impairment of the lymphatic microcirculation, allowing fluid, proteins, and blood cells extravasation. These changes also explain a worse skin perfusion by nutritive capillaries and the tendency of developing venous ulcers [130]. Likewise, the increased permeability triggers inflammation due to the infiltration of white blood cells (WBCs), with cytokine release, and activation of proteolytic enzymes and platelet activating factor [131]. Hence, shear stress modulating EC behavior leads to increased permeability, controlling leukocyte performance as well.

The role of venous valves should be the prevention of backflow of blood and pooling generation; however, mechanical stress promotes their incompetence. The measurement of the reflux and obstruction components in CVD patients may serve to prognose the severity of their venous clinical condition [132]. Incompetence of perforators and deep veins is highly present in severe CVI, provoking hypoxia and ulceration in the skin. Some contrasting evidence exists about the role of perforating veins in CVI onset and deterioration, questioning the need for perforator treatment [37].

Furthermore, deep venous system is surrounded by muscles that, when contracting, press veins in favor of blood return. For this reason, peripheral muscle pumps in lower limbs are other elements to analyze in order to understand the hemodynamic alterations occurred in CVD, especially calf muscle pump. Physiologically, it acts as a pump for deep leg veins within mainly gastrocnemius and soleus muscles. Sedentary lifestyle, long time standing, possible muscle/tendon/nerve/joint diseases, and being overweight promote deficient calf pump activity, leading to venous reflux, swelling, CVI, and in the worst cases, DVT. The disturbance in the calf muscle pump is then a key pathophysiological factor, which also implied a deficient healing of leg ulcers and a larger size of them in patients with profound CVI and worse prognosis [133].

Therefore, valves competence, calf muscle pumps, venous wall integrity, and elasticity determine venous pressure difference, altered hemodynamics in the lower limbs, and ultimately, chronic venous disorders or diseases [134]. Under normal conditions, in comparison to arteries, veins present a thicker adventitia layer and thinner media layer, with more content of collagen and reduced quantity of smooth muscle cells. This confers to the venous tissue the properties of reduced elasticity, but higher extensibility. Hence, blood return will particularly depend more on the accompanying muscles and valves. At present, there is a lack of studies related to venous mechanical properties as there are plenty in the case of arteries. Some reviewed literature alleges that, in the case of thrombosis, the vein gains stiffness and loses extensibility [135]. Although there is not much current evidence, comparative studies have demonstrated that the thicker venous wall in VV, adapted to undergoing increased pressure and turbulences, entails a different type of collagen composition besides a lower content of elastin (as detailed below) with decreased extensibility and elasticity contributing to the reflux effect and slackness for blood return generating pooling [136,137].

As described, vein function is harmed by this impaired hemodynamic system, implying hemorheological changes as summarized in Figure 3. This will lead to the extracellular matrix (ECM) remodeling in adaptation, shaping vessel alterations, with important changes in composition that will later be discussed, demonstrating that biochemical alterations go together with biomechanical changes.

6.2. Inflammation and the Role of Endothelial Dysfunction

Inflammation is widely accepted as a hallmark of CVD, playing a pivotal role in the onset and progression of the disease [119,138,139]. Vascular inflammation is a complex process involving multiple interactions between WBCs, ECs, SMCs, and the ECM. This abnormal communication is also orchestrated by an uncontrolled release of cytokines, leading to a pathological vascular response [140]. The main causes of the exacerbated inflammatory response reside on the hemodynamic abnormalities and microcirculatory changes associated to CVD, which may initiate a persistent immune response [141]. However, fully understanding the role of inflammation in CVD is essential to highlight the role of the endothelium. Because of the hemodynamic alterations and altered shear stress during CVD, ECs might suffer important phenotypical variations. Meaningfully, these changes have been recognized as important drivers of many vascular disorders [142]. In this line, previous studies have shown a pro-inflammatory switch of ECs in people affected with CVD, therefore contributing to the entry of leukocytes in the pathological veins [143]. Herein, the role of the glycocalyx should be highlighted. During vascular diseases, the glycocalyx located in the ECs is prominently altered leading to an alteration of the endothelial mechanotransduction. This promotes the breakdown of the permeability barrier, enhancing the access of leukocytes and favoring the inflammatory reaction [144]. Thus, the proper development of CVI will lead to cumulative changes in the glycocalyx and ECs, leading to a progressive endothelial dysfunction. Accordingly, the grade of endothelial dysfunction was shown to be associated with the clinical severity of CVD [145]. Moreover, endothelial dysfunction appears to represent a central pathophysiological link between CVD and DVT [146], therefore showing the relevance of endothelial damage in the progressive inflammation and development of this condition.

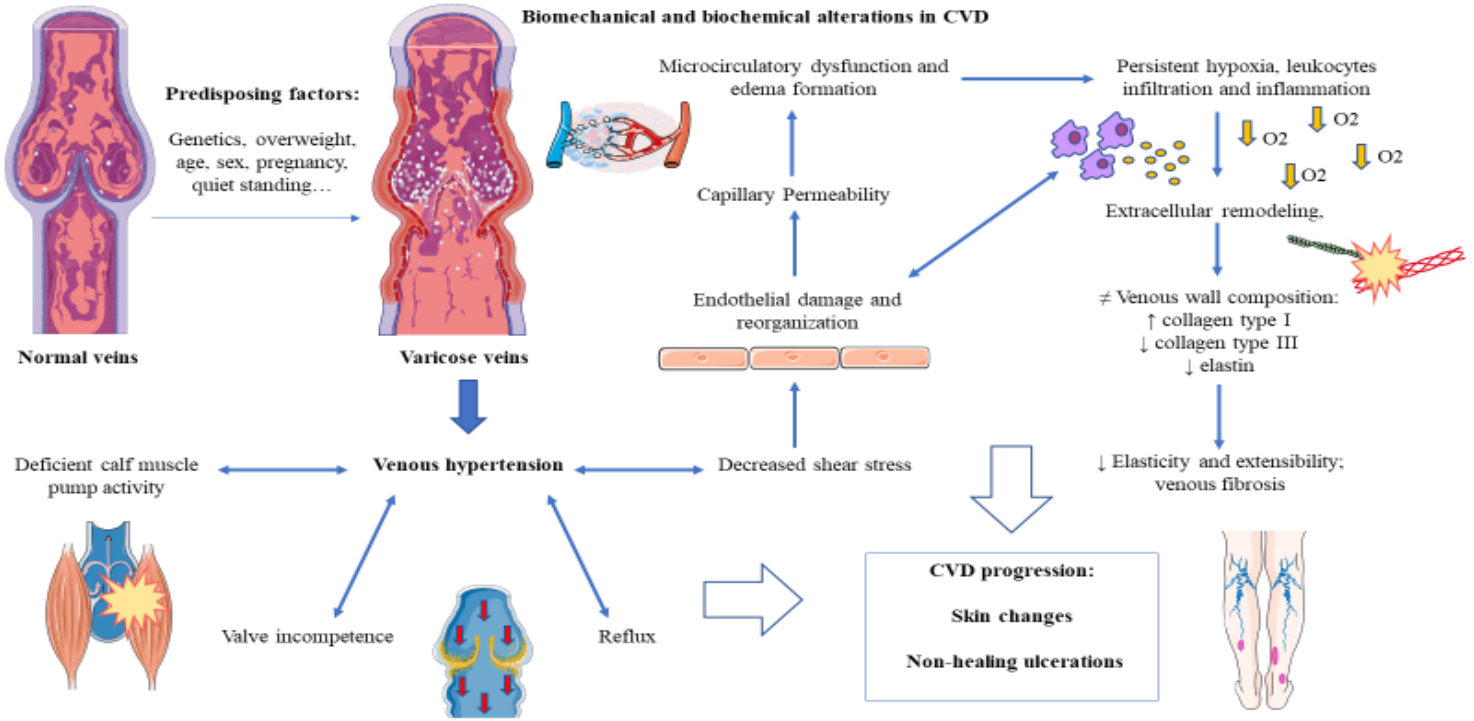

Figure 3. Biomechanical and biochemical alterations in CVD. As showed, ambulatory venous hypertension promotes a set of changes in the vascular wall, leading to a < n altered shear stress. The altered hemodynamic damage the endothelium, enhancing capillary permeability, leukocytes recruitment, lymphatic impairment, and edema formation. The microcirculatory dysfunction associated with these changes creates a hypoxic environment, and together with the inflammatory environment provide a extracellular matrix remodeling, eventually resulting in different venous wall compositions, reduced elasticity and extensibility, and venous fibrosis. This cycle is responsible for maintain and aggravate venous hypertension, getting worse with valve incompetence, reflux, and abnormal functioning of the calf muscle pump. Overall, these mechanisms are responsible for the progression of VVs to the most serious manifestations, such as skin changes and ulcerations.

In regard to immune populations, an increased infiltration of either innate or adaptative leukocytes have been evidenced in patients with CVD in comparison to healthy individuals [47,147]. Leukocytes interact with ECs in two stages: (1) a rapid phase known as I type activation characterized by EC vasoconstriction, selectin expression and von Willebrand factor release and (2) stage or "II type activation" involving adhesion molecules, cytokines, and tissue factor [148]. Monocytes/macrophages are central mediators of the inflammatory responses in CVD. Ono et al. [149] observed that incompetent venous valves were associated with an increased monocyte/macrophage infiltrate. Powell et al. [150] claimed that CVI with and without venous ulceration were related to high platelet-monocytes activation and aggregation. Furthermore, the role of macrophages appears to be particularly important in the onset and progression of CVD. Venous stasis may lead to red blood cells (RBCs) extravasation in the surrounding tissues. Then, they are degraded by interstitial macrophages and released iron from RBCs are stored in form of ferritin to later produce haemosiderin, which is responsible for the limb pigmentation in patients with CVD [151]. This altered iron deposition in the tissue might have important consequences for individuals with venous disease, inducing and sustaining the pathological oxidative stress and inflammation, while leading to the progression of CVI and leg ulcers [152–154]. Thus, targeting pro-inflammatory M1 macrophages induced by iron is proposed as a potential therapy in the management of CVI and venous ulcerations [155]. On the other hand, neutrophils and mast cells appear to be the first cells to interact with the affected endothelium, being important to initiate the inflammatory response in patients with CVD [156]. In the context of CVD, the abnormal activity of neutrophils appears to be related to increased adhesion molecules, lysosomal enzymes, and superoxide production, playing a key role in the development of CVI [157–159]. Previous studies have noticed a reduced detection of activated neutrophils in the blood of patients with VV, in comparison to healthy subjects [160]. This was explained because of a phenomenon of "leukocyte trap", which consists of the infiltration in the tissue of leukocytes and prominently, neutrophils through tiny vessels due to venous hypertension, hypoxia, and stasis [161]. Despite this fact, an increased neutrophil/lymphocyte ratio is currently used as a marker of severity in CVD [162], thereby concluding the importance of this immune population in the pathophysiology of CVD. Conversely, the role of mast cells in the pathophysiology of CVD is unclear. Although some studies described a significant increase of mast cells in the VV wall [163], other studies did not find significant differences in mast cells populations in the wall of VV versus non-varicose [164]. Additionally, Kakkos et al. [165] reported an augmented infiltrate of mast cells in patients with family history of CVD compared to those without family history; therefore, concluding that mast cells infiltration might not be a consequence of venous hypertension. In this line, another recent study detected an infiltration of mast cells in thrombotic VV [166]. Although the role of mast cells in the pathophysiology of CVD have been evidenced, their precise implications are still undiscovered.

T lymphocytes are also crucial players of the inflammatory reaction occurring in the venous wall and valves of patients with CVD [167]. The role of T cells in vascular inflammation has been widely explored [168]. T cells could be divided into T helper (Th/T CD4+) and cytotoxic T lymphocytes (CTLs/T CD8+). Th lymphocytes orchestrate immune responses according to the threat faced. They could be differentiated into various specific subsets, such as Th1, Th2, Th17 or T regulatory (Treg) cells, among others [169]. On the other side, CTLs are involved in effector cytotoxic functions [170]. The role of these T cell subsets has been extensively studied in arterial diseases, such as atherosclerosis [171]. However, to our knowledge, there are no studies regarding particular T cells subpopulations in the pathophysiology of CVD. Nonetheless, Ojdana et al. [172] demonstrated the participation of memory Th and CTL in the pathogenesis of CVD. Furthermore, B lymphocytes were also detected in the VV in comparison to healthy veins, although its precise role remains to be elucidated [173]. Grudzińska et al. [174] defined an increase of inflammatory cytokines released by lymphocytes in incompetent great saphenous veins with reflux in comparison to non-pathological blood flow. In this line, multiple studies have

demonstrated the usefulness of studying cytokine signatures of patients with CVD. For instance, Lattimer et al. [175] showed increased levels of some proinflammatory cytokines like interleukin-6 (IL-6), IL-8 and monocyte chemotactic protein 1 (MCP-1) in blood samples from patients with VV. Although an exacerbated inflammation is found in patients with CVI, Guss et al. [176] reported that, in severe cases, the level of inflammatory cytokines are diminished, thereby hypothesizing that inflammatory cytokines might be involved in tissue repair rather than damage. Similarly, Howlader and Smith [177] did not find any correlation between inflammatory markers with patient symptoms, therefore indicating the intricate role of the cytokines in CVD pathophysiology. For instance, transforming growth factor beta 1 (TGF-β1) is a central molecule involved in multiple processes of CVD. The inflammatory environment associated to CVD, induce the activation and overproduction of TGF-β1. This factor acts as a critical modulator of different targets implicated in the ECM remodeling, eventually causing vascular wall fibrosis. In turn, this exacerbates the venous wall hypoxia, ECs damage, and inflammatory reactions, leading to a greater TGF-β1 activity [178]. However, TGF-β1 signaling appears to be blocked in later stages of CVD, as previous studies reported a significant decrease in the TGF-β receptors [179,180]. Conversely, Kowalewski et al. [181] reported an increase expression of TGF-β receptor II in VVs in comparison to normal veins. Thus, TGF-β and its receptors might be relevant during the early pathogenesis of CVD, although in advanced stages of the disease, its activity might be reduced. This signaling could have important implications for future therapies, evidencing the link between inflammation and ECM remodeling in CVD.

On the whole, the role of the immune system in the pathophysiology of CVD is quite complex and it results from the pathological venous pressure and hemodynamic changes, causing and responding to valve and venous wall damage. Similarly, it reacts over the affected endothelium, which initiates the recruitment and infiltration of immune cells. In turn, the proinflammatory environment created by immune cells appears to participate in the pathogenesis of the disease, conducting to endothelial dysfunction. However, it is also unclear the possible causative role of the immunological changes, which can conversely be the outcome (and not the cause) of venous stasis in CVD patients.

6.3. The Hypoxic Environment

Prolonged hypoxia is crucial to understand the development and progression of vascular diseases [182]. The cause of hypoxia in CVD is a reduced oxygen supply to the vascular wall. In the case of lower limbs veins, this event relies directly on special vessels designed as vasa vasorum, mainly located in the adventitia and tunica media layers [183]. In the context of CVD, venous hypertension and blood stasis may lead to two different mechanisms of hypoxia: (1) endoluminal hypoxia, mainly due to blood stagnation that results in reduced oxygen flow detected by the endothelium and inner layers of the vein wall and (2) medial hypoxia because of the compression of vasa vasorum as a reaction of venous dilation and increased pressure, affecting the medial and outer layer [184]. At molecular levels, hypoxia leads to the activation of the hypoxia-inducible factor 1α and 2α (HIF-1α and HIF-2α). An increased activation of both transcriptional factors and their related genes in VV compared to non-VV was demonstrated; hence, demonstrating the central role of hypoxia signaling in the pathophysiology of CVD [185].

Endoluminal hypoxia may bring two distinguished responses. Firstly, acute hypoxia triggers the release of inflammatory mediators and growth factors, initiating the recruitment of immune cells. Indeed, more leukocytes can adhere to ECs when incubated under hypoxic conditions [160]. Then long exposure to hypoxia leads to the activation of HIF, upregulating the expression of key genes, such as proinflammatory cytokines, platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF), thereby increasing immune adhesion and recruitment in the VV [186]. Hypoxic signaling in this layer leads to intimal hyperplasia and degenerative changes in the inner wall, with a remarkable increase of the vasa vasorum as a compensatory mechanism to ensure an appropriate oxygen supply to the tunica media [187]. Nevertheless, progression of CVD

entails an augmented hypoxic environment negatively affecting to SMCs. Xu et al. [188] demonstrated important phenotypical changes in SMCs from VV versus non-VV. They concluded that these cells showed increased proliferative and synthetic capacity in comparison to SMCs derived from normal veins, also being more dedifferentiated. Furthermore, these cells present a marked degeneration, losing its morphology, while increasing its phagocytic activity of either collagen or elastic fibers and even other SMCs [189]. In the same manner, due to endothelial activation, SMC migration from the medial to the inner layer has been demonstrated [190,191]. A strong cause of this abnormal behavior is the hypoxic environment. In this sense, we detected significant changes in hypoxic markers (HIF-1α, VEGF, TGF-β1, and eNOS) in cultured SMCs obtained from patients with VV under hypoxic conditions, observing a genetic and epigenetic reprogramming of these cells during CVD [192]. Moreover, in the case of sustained and long-term hypoxia, these cells showed a reduced expression of compensatory mechanisms to hypoxia, which may provoke, among other effects, an increased cellularity and hypertrophic areas, apoptosis dysregulation, and collagenization typically observed in the venous wall of patients with CVD [193,194].

Hypoxia is a major and detrimental characteristic of CVD, partly responsible for the pathophysiology in the affected veins at early, but particularly at late stages. Thus, studying oxygen supply in VV, as well as finding specific targets related to hypoxia and its signaling, appears to be an interesting and important approach in the management of CVD [184].

6.4. Molecular Basis of CVD: The Venous Wall Remodeling

In the venous wall, vascular cells possess different mechanoreceptors responsible for the blood flow, mainly flow-sensitive ion channels, G protein coupled receptors (GPCRs) and integrins, although the role of glycocalyx and the platelet endothelial cell adhesion molecule-1/cadherin/VEGF receptor-2 complex in the mechanotransduction process should not be discarded [195]. Thus, the blood stasis and venous hypertension occurring during CVD will activate these receptors, triggering a wide variety of molecular responses in the ECs, SMCs, or vascular fibroblasts [196,197].

Firstly, shear stress will provoke important changes in the endothelium, leading to its dysfunction, as previously described. Simultaneously, a local response to the altered environment will be developed, with remarkable changes in the different layers. In this sense, significant changes in peptide growth factors and their receptors have been identified in the venous wall of patients with CVD, including acidic fibroblast growth factor (aFGF), VEGF, and insulin-like growth factor 1 (IGF-1), and its receptor (IGF-1R) [198]. The last route appears to be particularly important in the venous wall of patients with CVD, where we have recently reported an altered homeostatic IGF-1/PAPPA/STC-2 axis [199]. TGF-β1 has also been related to the pathophysiology of CVD. Pascual et al. [200] described the important role of its dysregulation in the progression of CVD, particularly in the fibrous process of the venous wall. The effect of this factor appears to be mediated by the TGFβ receptor II, whose expression has also been upregulated in VVs, but downregulated in venous ulcers [181,201]. Conversely, Serralheiro et al. [179] denoted a decrease in the gene expression of TGF-β1 receptor II and III in advanced clinical stages of CVD. The expression of TGF-β1 is associated with the dysregulation of multiple products located in the ECM, including tissue inhibitors of metalloproteinases (TIMPs), metalloproteinases (MMPs), plasminogen activator inhibitor one (PAI-1), lysyl oxidase-like 4 (LOXL-4), fibronectin, or collagen, among others, disrupting EC and SMC behavior [178]. Importantly, this molecule and its downstream signaling appears to play a critical role in the progression of CVD, linking inflammation, vascular damage, and venous wall remodeling. However, further studies are required in order to understand the precise role of TGF-β1 in CVD. The augmented detection of these growth factors and their receptors might lead to the dysregulation of various cellular routes contributing to the pathophysiology of CVD, such as PI3K/Akt. PI3K/Akt is an important route hyperactivated under pathological conditions inducing important changes in cell proliferation, survival, motility, metabolism and growth,

among other effects [202]. Ortega et al. [203] studied the role of PI3K/Akt in 110 patients with CVD according to the presence or absence of valve reflux. They found an increased activation of these components in patients with venous reflux, therefore indicating the role of this route in the progression of the disease. Similarly, the MAP kinases (MAPKs) pathway is prominently hyperactivated in the venous wall of patients with reported reflux, which may indicate the synergic effect of both pathways [204]. Interestingly, either PI3K/Akt or MAPKs were significantly higher in younger patients (<50) in comparison to elder patients, which may indicate a possible role of PI3K/Akt in the accelerated aging of the venous wall. The increased aging of the venous wall is also favored by the loss of some homeostatic mechanisms in the venous wall. In this sense, some of the changes described in the homeostatic mechanisms in CVD included an altered expression of the peroxisome proliferator-activated receptors (PPARs), associated to an abnormal lysogenesis and senescence [205] and JNK signaling, with an accelerated osteogenesis [206].

Many elements from the extensive list that conforms the pathogenesis network in CVD are Ca^{2+}-dependent signaling pathways. In SMCs from varicose saphenous vein, the observation was an impaired Ca^{2+} mobilization, implying reduced contractility [207]. For its part, the RhoA/Rho kinase signaling pathway, modulates Ca^{2+} sensitization to actin cytoskeleton and ECM fibronectin assembly in SMCs, but in VVs, both have decreased activity and deposition respectively [208]. Reduced expression of RhoA is associated to abnormal EC function and morphology, which depends on EGFL7 regulation by CASZ [209]. Mentioned variants from *Nfact* family are also Ca^{2+}-dependent signals for development and function of T cells [210]. In related literature search, NFATC3, NFATC4, and other isoforms of *Nfatc* family may be also relevant as all of them coordinate tidily the tissues-vessels relationship in vasculature design [211]. Moreover, it is known that intravascular pressure induces nuclear accumulation of NFATC3 isoform in SMCs in event of nitric oxide synthase activation [212], an occurrence observed in patients with CVI [122,213]. Overall, the shear stress and environmental strains induced by CVD provoke an important cellular reprogramming that eventually leads to an altered cell senescence, ECM remodeling, and other significant changes in the venous wall.

Matrix Gla protein (MGP) has a prominent role in mineralization, which is boosted in SMCs of VVs [214]. This protein is a central inhibitor of vascular calcification via carboxylation by vitamin K although the accurate mechanism is not clearly understood [215]. It is suggested that MGP must be a predictor for calcification status of VV whose modus operandi can be expressed in different ways: inhibition of calcium phosphate precipitation, formation of matrix vesicles, formation of apoptotic bodies, and transdifferentiation of vascular SMCs [216]. For its part, MGP induces TGFβ expression and parallel, induces VEGFs expression, which promotes angiogenesis, lymphangiogenesis, and vascular permeability. Some ex vivo studies have shown that by blocking TGFβ with antibodies, MGP does not have effect on VEGF upregulation [217]. As described, MGP, VEGF, cell–cell junctions and more elements depend on Ca^{2+} signaling, which is key for vascular tone and function to respond to hydrostatic pressure. It is known that the regulation of voltage dependent Ca^{2+} channels is altered with aging processes and hence affects vascular function [218]. Furthermore, some studies reported that primary VV show aberrant release of VEGF, which, in combination with other altered molecules, might increase the permeability of venous wall, allowing the infiltration of inflammation markers [219].

As reviewed above, rheological properties hold a relationship with venous wall now that there are histological differences between pathological and non-pathological veins, underlying the composition of ECM in venous remodeling. MMPs are secreted by fibroblasts and, when activated, they degrade collagen and elastin, which can affect other elements in vessel structure like SMCs migration. WBCs infiltration promotes an anomalous functioning of MMPs and TIMPs distinguishing zones with atrophy (little ECM) and hypertrophy (abundant ECM). The different levels of expression/activity of MMPs determine irregularity in VVs with dilated zones and tortuosity [220,221]. The dilation of vein due to hyperpolarization of K+ channels by MMP-2 was suggested by Raffetto et al.

Since MMPs degrade collagen, peptides formed may attach to SMCs integrin receptors that entail the activation of K+ channels [222]. The changes in collagen, fibronectin, elastin, and calcium deposits establish the stiffening of vessels [223]. The deposition of certain collagen subtypes makes intimal and middle layers become thicker and, in general terms, collagen fibers increase while elastics decrease, contributing to the alteration of common orientation of SMCs [224]. For instance, type I collagen is expressed in bigger amount than III, in comparison to non-varicose veins [225]. Collagen III is involved in tissue elasticity, and the high collagen I:III ratio would also explain the low extensibility of VVs [226]. Moreover, elastin degradation, a critical event associated to an accelerated aging, generates products with cytokine-like activities, exerting chemotactic activity for leukocytes, especially polarizing towards Th1 subtypes [227]. The degradation of fibronectin is also bigger than its synthesis and deposition. This would also explain the long period of healing in typical CVD leg ulcers, now that fibronectin is synthesized by fibroblasts from skin, but the deposition is impaired [228].

Proteomics studies have elucidated the role of dermatopontin, and tenascin C, showing an increased proteolysis of these two factors in patients with VVs [229]. Firstly, dermatopontin is especially found in dermal fibroblasts, on the surface of collagen fibers, indicating a role in wound healing [230], and it increases the cellular response to TGFβ [231]. Secondly, tenascin C is an extracellular glycoprotein highly expressed in wound healing, is synthesized by SMCs in response to cell proliferation or migration [232]. Changes in both proteins could also play a vital role in the pathophysiology of CVD. Moreover, another protein that could be included in the complex network of multifactorial CVD is vinculin, a mechanosensory protein that takes part in endothelial cell-cell adhesion remodeling [233]. In any case, there is still a lack of knowledge related to these proteins and their participation in CVD pathogenesis.

Furthermore, the study of the glycome, the group of glycoconjugates united to lipids and proteins, unravels different glycosylation pathways in health and disease, becoming a prominent target for new advances towards therapy [234]. Glycosylation of ECM proteins affect to folding, solubility, binding, and degradation [235], and the accumulation of its end products increases the crosslinking of collagen. The crosslinking needs hydroxyproline amino acid to form hydrogen bonding in collagen molecules and this posttranslational modification affecting proline is highly represented in VVs, contributing to the stiffness of venous wall, aging, and inflammation.

As presented throughout the text, changes in biomechanical aspects alter the biochemical signaling pathways and these lead to changes in biophysical properties of venous wall, as summarized in Figure 4.

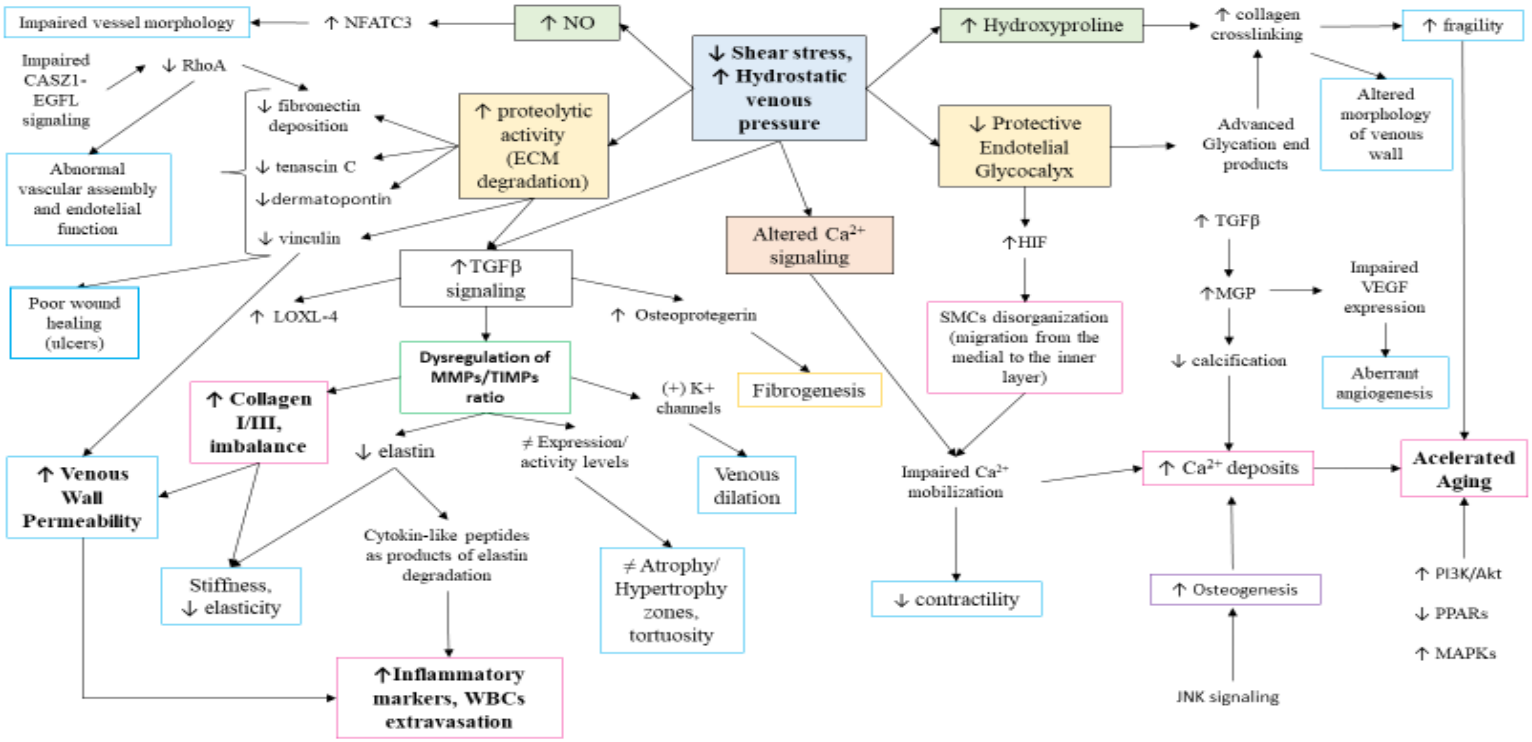

Figure 4. Schematic representation of molecular changes induced in venous structure resulting in different biophysical properties. As previously described altered (mostly decreased) shear stress and venous hypertension are responsible for the venous wall remodeling in VVs, influencing a plethora of molecular pathways. An increased level of hydroxyproline, reduced protective endothelial glycocalyx, altered calcium signaling, imbalance in matrix metalloproteinases and their inhibitors, enhance ECM degradation and augmented nitric oxide are the most important effects described. Collectively, these alterations are responsible for modifications in collagen and elastic fibers, venous wall permeability, disrupted vessel morphology, and functioning and multiple defects associated to the progression of CVD. Legend: Up black arrow= Increased expression; Down black arrow: Decreased expression; ≠: Differential detection.

6.5. Genetics and Epigenetics Mechanisms of CVD

6.5.1. Genetics

In summary, literature research shows a high number of genes related to the etiology of CVD, contributing to the pathophysiology. Many of them are intronic variants polymorphisms or single nucleotide polymorphisms (SNPs), others are mutations, and the rest are just upregulated under CVD being related to systemic alterations. A wide variety of genes are listed, being associated to alterations in blood pressure, vascular wall remodeling, and impaired wall properties (tension and elasticity), besides typical inflammation of CVD. Genome-wide association studies (GWAS) found much more variants at susceptibility loci with enough significance associated to CVD. Nevertheless, there are still few studies and peer reviewed articles related to CVD heritability and further efforts in this field are also needed.

In recent years, GWAS in VVs have allowed the identification of genetic risk factors previously unexplored. These findings showed some susceptibility SNPs for the genes of the pore-forming subunit *KCNH8* (rs727139) (the potassium voltage-gated channel subfamily H member 8) and the extracellular matrix (ECM) glycoprotein *EFEMP1* (rs17278665). *KCNH* is involved in many functions including neuronal excitability and smooth muscle contraction, which is believed to be associated to venous dilation and VV formation [236]. *EFEMP1* variants are related to MMPs and TIMPs altered expression, which leads to remodeling of ECM components and changes in vessel elasticity [237]. Another study found positive correlation between VV and polymorphisms in promoter region of *MMP-9* and *TIMP-2*, which prevent excessive ECM [238].

More genetic analyses have been related to etiology and pathophysiology of CVD in terms of hypertension and abnormal vasculogenesis, revealing as causal genes: *CASZ1*, *PIEZO1*, *PPP3R1*, *EBF1*, *STIM2*, *HFE*, *GATA2*, *NFATC2*, and *SOX9* [239]. CASZ1 (castor zinc finger 1) in its variant rs11121615 SNP has been found with strong association in severe CVD, but not in forms of this disease without VV. The transcription factor, *CASZ1*, directs the expression of EGFL7 (epidermal growth factor like-domain 7) that will lead to a signaling pathway concluding in angiogenesis stimulation and vascular assembly. A study settled that the variant rs11121615 for CASZ1 is influencing risk of developing VV [240]. For its part, *PIEZO1* is a cationic channel that functions as a sensor of shear stress by Ca^{2+} influx among ECs, which is decisive factor for vascular structure. It is also permeable to Na^{+} and K^{+}, functions as a baroreceptor and is necessary for valve morphogenesis and regulation of vascular tone [241] with consequences in circulation of lymph as well [242]. A mice model showed that *Piezo1* can mediate blood flow and is followed by reorganization of ECs [243]. Loss-of-function mutations in this gene can cause autosomal recessive lymphatic dysplasia, a congenital disease that affects lymphatic system and edema in lower limbs [244]. Moreover, some variants have been associated particularly to VV recurrence, rs2911463 [43].

According to Shadrina et al., *PPP3R1*, *EBF1*, *GATA2* and *NFATC2* were genes associated to immune response or inflammation in vascular remodeling, a noted part related to VVs [239]. PPP3R1 (protein phosphatase 3 regulatory subunit B, alpha) associated variants, rs2861819 and rs2241173, have been studied as genetic determinants for CVD development, being involved in vascular integrity [33]. EBF1 (early b cell factor transcription factor 1) has a role in cell adhesion and migration during early B lymphopoiesis, being crucial for managing B cell immunity, and provides susceptibility to VV by its variant rs11135046 [245,246]. Next, the endothelial transcription factor GATA2 (GATA binding protein 2) can activate the transcription of genes involved in lymphatic vessel valve development, an impairment function correlated with lymphedema [247]. In this case, the associated variant of CVD risk found in GWAS was rs9880192 [239].

The role of NFATC2 (nuclear factor of activated T cells 2) in VV development has not been elucidated yet, but some variants have been found as well; the risk SNPs are rs3787184 and rs12625547 [246,248]. *Nfatc* signaling is calcineurin-dependent and acts in consonance with other transcription factor, FOXC2 (C2 isoform of the forkhead family transcription

factor), in the regulation of GJC2, gap junction proteins of endothelial tissue. Mutations in genes FOXC2 and GJC2 are cause of disruption in venous valve function. Ulceration and hypertension will expound in absence of a proper valve control in which these genes are involved [249]. Moreover, animal models have shown that FOXC2 inactivation is correlated with abnormal shear stress sensing and junction disassembly due to cell-cell junction defects and valves impairment [250]. This problem has been studied in lymphedema-distichiasis syndrome that, as previously narrated, was associated to FOXC2 mutations.

Continuing with other possible targets, STIM2 (stromal interaction molecule 2) is an endoplasmic reticulum protein that controls Ca^{2+} concentration in cytosol and seems to be implicated in typical morbidity in aging, like autoimmune disorders [251]. GWAS studied proposed rs28558138 as risk SNPs [248]. HFE (homeostatic iron regulator) regulates iron absorption by regulating hepcidin expression. Recessive mutations confer hemochromatosis, a disorder of iron storage [252]. Iron overload is correlated with impaired endothelial function in patients with hereditary hemochromatosis [253]. On the other hand, in CVD, the RBCs extravasation and deposits of iron could highly activate MMPs or block their inhibitors, besides generating free radicals. The fact that iron could be an elicitor of venous leg ulcers has been hypothesized. In an observational study, patients with variant H63D (rs1799945) developed ulcers at an earlier age [254]. Another variant found in GWAS was rs7773004.

Next, *SOX9* or SRY-box transcription factor 9, can modulate extracellular matrix, associated with high calcium deposition and its overexpression is influenced by TGF-β1 [255]. GWAS found variant rs2241173 for varicosity susceptibility. Gene *COL2A1* (variant: rs73107980), which codes for collagen type II alpha 1 chain, component of extracellular matrix, is a target of *SOX9* whose ectopic expression had previously been linked to abnormal modeling of extracellular matrix [239,256]. Presumably, the isotype *COL1A2* has been studied in more depth, in collagen dysregulation. Genetic variations (insertion/deletion rs3917 polymorphism) seem to provide susceptibility to CVI [257].

In this extensive list, there are, finally, two genes (that were previously mentioned) that are an important link between thromboembolic disease and CVD: *THBD* and *MTHFR*. In this line, previous studies found that both conditions shared familial susceptibility [258]; hence, indicating the implication of some genetic mechanisms in the overlap of these diseases. Indeed, mutations in the two associated genes has been related to the development of either DVT or VV [43]. Blood samples obtained from VV exhibited higher levels of prothrombotic and inflammatory markers along with evidence of endothelial damage in comparison to non-VV blood [259]. These two genes could be taken part in the aberrant environment affecting the venous wall and the local response in the vein, connecting, to some extent, DVT and CVD pathophysiology. The main genes involved in the various pathophysiological mechanisms of CVD are summarized in Table 2.

Table 2. Principal causal genes associated to CVD. ECM = Extracellular matrix, VV = Varicose veins.

Gene	Whole Name	Chromosome and Location	Variant(s) (rs ID)	Risk Allele	Mutation	Highlighted Associated Function	(Possible) Implication in CVD Etiology/ Pathogenesis	References
KCNH8	Potassium voltage-gated channel subfamily H member 8	chr3:19293203 (3p24.3)	rs727139	A > G	-	Smooth muscle contraction	Venous dilation and VV formation	[237]
EFEMP1	EGF containing fibulin extracellular matrix protein 1	chr2:55868859	rs17278665	C > G	-	Cell adhesion and migration	Remodeling of ECM components and changes in vessel elasticity by altering the expression of MMPs and TIMPs	[238]
MMP-9	Matrix Metallopeptidase 9	chr20q11.2-q13.1	-	-	✓	ECM degradation	Collagen type I degradation entailing stiffness of vein	[239]
TIMP-2	Tissue inhibitor of metalloproteinases 2	chr17q25.3.	-	-	✓	Inhibition of MMPs	Lower expression implies higher collagen degradation by MMPs	[239]
CASZ1	Castor zinc finger 1	chr1:10765520 Intron 1	rs11121615	C > T	-	Transcription factor for EGFLD7	Angiogenesis stimulation and aberrant vascular assembly	[241]
PIEZO1	Piezo type mechanosensitive ion channel component 1	chr16:88769137 (16q24.3)	rs2911463	G > A/G > C/G > T	✓	Shear stress sensing by Ca^{2+}	Impaired function implies aberrant vascular structure, ECs reorganization and edema	[242–245]
PPP3R1	Protein phosphatase 3 regulatory subunit B, alpha	chr 2:68262089 (2p14) Intergenic	rs2861819	G > A/G > C	-	Ca^{2+} sensitivity	Abnormal vascular integrity	[43]
EBF1	Early B Cell Factor Transcription Factor 1	chr5:158803005	rs11135046	G > A/G > T	-	Adhesion and migration in early B lymphopoiesis	Possible epigenetic reprogramming and B cells activation	[247,248]
GATA2	GATA Binding Protein 2	chr3:128578726	rs9880192	G > A/G > C	-	Lymphatic vessel valve development	Impaired function implies lymphedema	[240,249]
NFATC2	Nuclear Factor of Activated T cells 2	chr20:51541298 chr20:51538108	rs3787184 and rs12625547	A > G T > G	-	Induces immune response or inflammation in vascular remodeling	Not well understood; Acting in consonance with FOXC2 and GJC2.	[248,250]

Table 2. *Cont.*

Gene	Whole Name	Chromosome and Location	Variant(s) (rs ID)	Risk Allele	Mutation	Highlighted Associated Function	(Possible) Implication in CVD Etiology/ Pathogenesis	References
FOXC2	Fork-head box protein C2	16q24	-	-	✓	Critical product in developmental processes	Inactivation implies abnormal shear stress sensing and valve incompetence	[251,252]
GJC2	Gap junction gamma-2	1q41-q42	-	-	✓	Implicated in the gap junctions between cells	Cell-cell junction defects and valve incompetence	[251]
STIM2	Stromal interaction molecule 2	4:26857601-27025381 (4p15.2) Intergenic variant Mapped gene(s): STIM2, TBC1D19	rs28558138	G > C	-	Controls Ca^{2+} concentration in cytosol	Higher Ca^{2+} deposition	[250,253]
HFE	Homeostatic iron regulator	chr6:26090951 chr6:26267527	rs1799945 and rs7773004	C > G C > T A > C/A > G/A > T	✓	Regulates hepcidin expression, involved in iron storage	Iron overload implies endothelial dysfunction. Moreover, activation of MMPs and inhibition of TIMPs, deposits of iron, RBCs extravasation	[254–256]
SOX9	SRY-Box Transcription Factor 9	17:72032304 17q24.3	rs2241173	A > C A > G	-	ECM remodeling	Influenced by TGF-β1may involve higher Ca^{2+} deposition	[24,257,258]
COL2A1	Collagen type II alpha 1 chain	12:47793818 (12q13.11)	rs73107980	C > G C > T	-	Coding collagen type II alpha 1	Abnormal modelling of ECM	[239]
COL1A2	Collagen type I alpha 2 chain	chr7:94431047-94431048	rs3917	(indels)	-	Coding collagen type I alpha 2	Collagen dysregulation, higher susceptibility to CVI	[259]
THBD	Thrombomodulin	20p11.21	-	-	✓	Related with thromboembolic diseases	Prothrombotic markers	[43]
MTHFR	Methylenetetrahydrofolate reductase	1p36.22	-	-	✓	Related with thromboembolic diseases	Prothrombotic markers	[43]

6.5.2. Epigenetics

The term epigenetics refers to the different molecular mechanisms involved in the modulation of the genotype into a particular phenotype. Importantly, these changes do not usually affect directly DNA sequence, although they could be mitotically and meiotically hereditable [260]. In this sense, the epigenetic mechanisms are the following: (1) DNA methylation; (2) histone modifications, including acetylation/deacetylation, methylation, phosphorylation, ribosylation, ubiquitylation, SUMOylation, and citrullination; and (3) non-coding RNA, with a central role of micro RNAs (miRNA), short-interfering RNA (siRNA), and long non-coding RNA (lncRNA) [261,262]. The epigenetic mechanisms are orchestrated by several external factors including aging, nutrition, behavior, psychological stress, physical activity, working habits, circadian rhythms, smoking, and alcohol consumption [37,263–265]. During cardiovascular diseases, all these components might promote an unfavorable environment that, along with the proper pathophysiological mechanisms, may lead to an epigenetic switch, thus contributing to the onset and progression of the disease [266]. In this line, shear stress represents one of the most important modulatory factors in ECs, initiating a wide variety of epigenetic responses [267]. Thus, an altered shear stress has been associated with the development of atherosclerosis [268]. In the same line, other mechanisms, such as hypoxia or inflammation, might induce the epigenetic reprogramming of the vascular cells, which in turn enhance the inflammatory and hypoxic responses [269,270].

Regarding genes regulated in CVD by epigenetic mechanisms, we will remark the role of two main pathways: DNA methylations and non-coding RNAs. DNA methylation consist of the adding of a CH3 group in the CpG sites in a process mediated by the methyltransferases enzymes [271]. DNA could be hypermethylated or hypomethylated, depending on the activity of the methyltransferases. Hypermethylation cause the silencing of the gene while hypomethylation leads to its activation [272]. Importantly, either hypermethylated or hypomethylated patterns could be detected in different loci under pathological conditions [273]. In the context of CVD, the above mentioned EBF1 and MTHFR act as critical gene modulators. EBF1 may be considered as an epigenetic regulator now that it can induce DNA demethylation, nucleosome modeling and modulate active chromatin of B cell gene networks [274]. In the case of MTHFR, previous research observed that certain polymorphic variants appear to induce DNA hypomethylation, thus leading to an abnormal expression of matrix and structural proteins, affecting DNA integrity and accelerated aging of the venous tissue [275]. Importantly, MTHFR mutations were associated with inherited hypercoagulability, as well as an increased risk of suffering from CVD and DVT [276].

In this line, transcriptomic and DNA-methylomic studies have identified microfibril associated protein 5 (MFAP5) as a gene hyperactivated in patients with VVs due to an increased hypomethylation [277]. In addition, other genes, such as ADCY3, DPEP2, HRC, PLXNB1, osteopontin, integrin β3 and CCN5 (WISP2) are importantly dysregulated in VVs, due to an altered methylation patterns also being linked to the signaling network of CVD [247,278]. However, further research is required in this field to establish the causes of these methylation disturbances.

Likewise, the role of non-coding RNAs in the pathophysiology of CVD has received more attention. Non-coding RNAs are RNAs without coding potential but playing multiple functions in biological and pathological processes [279]. In this context, miRNAs represent one of the most important non-coding RNAs, preventing protein expression acting at post-transcriptional levels. miRNAs could be upregulated or downregulated, thereby exerting their action or not in the corresponding product [280]. A single miRNA may regulate, on average, 200 transcripts and a single transcript could be regulated by different miRNAs [281]. In addition, miRNAs could be used as valuable biomarkers of disease, with important implications for diagnosis and prognosis for the patient [282]. Available studies of miRNAs in CVD are conducted mainly in the VV tissue, with important pathophysiological implications. In this sense, Jiang et al. [280] detected 14 miRNAs abnormally

expressed in the VV tissue. Particularly, miR-34a, miR-155 and miR-202 appears to be the most significative components in these patients. Among the most important targets of these miRNAs were MAPKs, hyperproliferative targets, apoptotic genes, cell cycle, and p53 signaling pathways. In the same manner, Anwar et al. [283] reported altered levels of miR-642a-3p, miR-4459, miR-135a-3p, and miR-216a-5p, in the vein tissue of patients with CVD, being correlated with an altered metabolomic profile, increased proliferation and inflammation. MiR-382 appears to be a key regulator of the gene COL1A2. However, in patients with the polymorphic variant rs3917 the affinity of this miRNA with this gene product decreases, hence leading to the upregulation of COL1A2 [264]. More detailed, Zalewski et al. [284] described 31 miRNAs and 62 genes recognized as potential biomarkers of CVD. These miRNAs established a complex network leading to the upregulation of certain genes like WNK1, PRRC2B, CDS2, and the histone deacetylase HDAC5, while downregulating other targets (e.g., PABPC3). All of these interactions enhance the pathophysiological mechanisms of CVD, therefore showing the promising role of miRNAs in this condition. Respecting other non-coding RNAs like lncRNA, previous research has demonstrated the important role of these components both at physiological and pathological conditions [285]. LncRNAs similar to miRNAs are able to regulate protein expression, acting through different mechanisms either in the nucleus or cytoplasm. In the case of CVD, lncRNAs also play a vital function in the pathophysiology of the disease, influencing a plethora of genes involved in the development of CVD, mainly through metabolic, structural, and inflammatory mechanisms, among others [286,287]. Despite the relevance of non-coding RNAs in the pathogenesis of CVD further studies are needed to deepen on the pathological role of these elements. Moreover, to our knowledge there are no studies regarding the use of non-coding miRNAs as diagnostic or prognostic factors of CVD, and future efforts could be placed on this direction.

Finally, it is important to highlight that epigenetic mechanisms are reversible, as well as contribute to the development and progression of CVD, it might act favorably by modulating certain factors, such as diet, physical activity, emotional management, and rest while limiting alcohol consumption and tobacco smoking.

6.6. Systemic Affections

There is a growing body of evidence indicating that CVD is not only a local disease, but also a systemic malady, with detrimental implications for the whole organism. Contrary to popular beliefs and as reviewed throughout the study, the presence of VV is much more than an esthetic concern, but also a worrisome condition with many symptoms and signs, entailing devastating consequences in the QOL of the patients [2]. In this sense, it is known that individuals with CVD present a disrupted serum levels of many compounds including proinflammatory cytokines [176], circulating parameters (prominently estradiol, homocysteine, and VEGF) [288], oxidative stress indicators [122] and epigenetic markers [285]. In addition, much of these agents increase at advanced stages of CVD, therefore suggesting the systemic implications of the disease.

Moreover, these effects could be even greater in the most vulnerable populations, like PW. As above mentioned, this group is particularly susceptible to suffer from CVD. In this sense, we conducted multiple studies regarding the impact of this condition during gestation in comparison to healthy pregnancies. The placenta is a crucial organ during pregnancy, involved in multiple functions, providing oxygen and blood supply to the fetus, among other functions [289]. Thus, a proper working of the placenta is key to determine pregnancy success and fetal well-being. Conversely, impairment in the placental function and abnormalities on its structure during pregnancy could enhance fetal reprogramming, which may negatively impact, even in adulthood [290,291]. In this context, we have reported significant changes in the placentas of women with CVD, showing increased damage and hypoxia [292], villous calcifications [293], altered lipidomic profile [294], augmented angiogenesis and lymphangiogenesis [295], ECM remodeling [296], and evidence of oxidative damage, in either the mother or fetus [297]. Importantly, many of these changes have

also been reported in the placentas of patients with pre-eclampsia, a hypertensive disorder affecting the arterial system [298]. Although contrary to gestational venous hypertension, pre-eclampsia could be a severe and life-threatening condition, our results show the CVD course, with significant changes in the placenta of PW affected with this condition, and the necessity of further studies to assess the impact of the disease, in both fetal and maternal well-being.

7. Therapeutical Approaches in CVD

The medical care of CVD entails different strategies that can be used either alone or in combination, in order to maximize the clinical management of the symptomatology, prognosis and therefore the QOL of patients. In this sense, three main approaches are worth mentioning here: (a) compression therapies, targeting venous hemodynamics; (b) medical interventions directed to control venous insufficiency, and (c) pharmacological therapies directed to specific pathophysiological mechanisms of the disease [299]. It is of note that elder people and patients with additional comorbidities, despite requiring more interventions, are good responders to the different therapies received as younger patients or patients without any affection [300]. Thus, the entire population affected by CVD might be beneficiary from available therapies.

Compression therapy act by increasing the interstitial pressure, thus decreasing both the superficial and the deep veins caliber, reducing venous pressure and edema while promoting the contractile activity of the calf muscles [301]. Compression stockings are easy to use, and frequently, they are the first conservative measure to take, achieving significant improvements in most patients without causing much discomfort [302]. Thigh length stockings provide the greatest efficacy regarding volume reduction and venous hemodynamic, without affecting patient comfort [303]. In addition, compression stockings favor the healing of ulcers associated to CVI; hence, decreasing the recurrence of ulcerations in venous diseases after surgical intervention [304]. However, the usefulness of compression therapy after VV treatment has a limited degree of evidence [305,306]. Even so, it must be considered that patients with peripheral arterial disease require a careful application of this particular treatment, as it might interfere with blood circulation in the lower limb and worsen the underlying disease. Therefore, the ankle-brachial index should be calculated to discard arterial involvement, which would contraindicate the use of compression stockings in specific patients [307].

Similarly, CVD can be treated pharmacologically through various venoactive agents. These compounds aim to decrease vascular permeability, ameliorating the inflammatory response and, to some extent, they may increase vascular tone and operate on platelet aggregation [47]. Pharmacological treatments range from saponins, flavonoids, pentoxifylline, micronized purified fractions of flavonoids, and acetylsalicylic acid [308]. The main applications of these pharmacological agents are guided to treat non-serious symptoms like pain or edema at initial stages. In addition, a moderate level of evidence also supports their use in some clinical signs of CVI including trophic disorders, cramps, restless legs, swelling, and paresthesia, but its efficacy in venous ulcerations is inconsistent [309]. Nevertheless, certain drugs, such as sulodexide or purified flavonoids, appears to play a promising role in the management of CVI and venous ulcers, particularly when combined with compression therapy [310,311]. In the event of patients with refractory ulcers, acetylsalicylic acid could be utilized under some cautions [14,312]. However, some authors question the usefulness of the pharmacological therapy in the management of patients with CVD, claiming for further studies to unravel the real position of these drugs [313]. Anticoagulants play a specific role in a few venous diseases, such as DVT, SVT, PE, and they have been proposed in other cases of CVD, such as PTS and venous ulcerations [37].

It should be emphasized that different technologies and surgical approaches have been validated as interventional treatments in the management of CVD. The rationale of this type of treatment relies on the effective control, either of the localized progression at

early stages of the disease, or on the prevention of long-term complications, also targeting optimal cosmetic results [314].

Varicose vein treatment is the most practiced interventional approach in CVD patients. Ultrasound-guided sclerotherapy is one of the best-known procedures in the management of VV of the lower limbs. Sclerotherapy is based on the use of different chemical agents (polidocanol, sodium tetradecyl sulfate, glycerin, etc.) to ablate veins, venules, and in general, any type of varicose manifestation in patients with CVD [315]. This therapy is indicated especially, but not only, in patients with baseline comorbidities, where more aggressive techniques could be contraindicated [316]. Moreover, sclerotherapy can be an alternative to other procedures in patients with advanced CVI and saphenous vein incompetence who are not candidates for surgery or endovenous techniques [317]. The results of sclerotherapy are similar to other interventional techniques, properly managing the associated symptoms of CVD with a low rate of adverse effects [318]. Moreover, sclerotherapy has provided substantial benefits for patients with superficial venous reflux, enhancing ulcer healing and preventing recurrences in similar rates than other procedures [319]. More recently, sclerosant foam-based and catheter-based procedures have proven to achieve outcomes in the range of thermal ablation techniques at mid-term [37]. However, most trials show similar results for all VV therapies, in regard to patient-reported outcomes.

As to open surgery (saphenous junction ligation and stripping) in VV disease, conflicting data exist [37] in terms of mid-long term recurrence; some literature data report on a superior efficacy to medical and conservative approaches to resolve the symptoms associated with VV [320,321]. Before the introduction of the endovenous thermal or chemical ablation interventions, surgery was considered the gold standard for the treatment of CVI for a long time [322]. However, because of its invasiveness, the higher rate of potential complications, including the appearance of hematomas, infections on the surgical wound or nerve injury, the surgical approach is usually reserved to patients with large and tortuous dilations in superficial VVs or patients with some vascular malformations [323].Because of that, nowadays, ultrasound-guided endovenous ablation has replaced previous techniques used in the field of CVD. Two different types of endovenous thermal ablation can be distinguished: endovenous laser ablation (EVLA) and radiofrequency ablation (RFA). Both techniques are based on the induction of a controlled thermal injury at the VV level, leading to a thrombotic occlusion eventually causing fibrosis of the saphenous vein [324,325]. This technique has now replaced surgical intervention because it offers similar results with less convalescence and a very low rate of complications [326,327]. Serious complications are rare and include DVT and SVT, pigmentary changes, nerve damage.

More recently cyanoacrylate-based options have been validated as alternative techniques in VV treatment, though the cost/effectiveness ratio may be unfavorable in many cases [37].

Finally, there are certain therapeutic alternatives reserved for deep vein occlusions/obstructions in acute DVT or in PTS, as it is the case of the endovenous PTA + stent. The use of stent in patients with CVD is indicated in presence of chronic iliac vein occlusions which are non-responders to medical interventions [328]. Conclusively, as presented in Table 3, we can observe that CVD has an amalgam of possible therapeutic approaches, which may be used to improve or alleviate the clinical manifestations of this debilitating disease.

Table 3. Medical therapies, uses, and level of evidence of current available treatments in patients with CVD.

Treatment	Uses	Level of Evidence and Recommendation
Compression therapy (stockings, bandages, adjustable compression wraps)	Initial therapeutical method of CVD A powerful tool in CVI ulceration Increase the efficacy after interventional procedures	IB [303] IB [305] IA [307,308]
Pharmacological therapies	Venotonics to ameliorate early symptoms (Pain, edema) Flavonoids and derivates as complement therapy with compression stockings in venous ulcers	IIaA [310] IIaA [311,316]
Sclerotherapy	Second-line treatment for patients who are not candidates for endovenous ablation or surgery First-line therapy in patients with recurrent CVD and fragile patients with venous ulceration	IA [319] IIaB [320]
Endovenous thermal ablation	First line therapy for patients with CVD and great saphenous vein reflux	IA [318,328]

8. Future Directions in CVD—Towards Clinical and Translational Improvements

CVD is a multifactorial disease that generates a huge economic burden, besides the impact on patient QOL, demanding extensive treatment and sometimes hospitalization. Given the rising prevalence, these days CVD is still an underestimated and underdiagnosed malady. For this reason, scientific evidence and clinical management must fulfill the duty of achieving enough knowledge to engender practical guidelines, in order to restore normalcy for these patients and diminish healthcare costs.

Medical technologies are supporting the optimization of current treatments. One of the most used, foam sclerotherapy, may benefit patients for some 5 years [329], though a higher success rate is reported with complementary techniques, such as catheter foam sclerotherapy with tumescence and irrigation [37]. For its part, tissue engineering is facing approaches in valve development for replacement, finding adequate materials to reach desired outcomes [330]. In the diagnosis area, new ultrasound technologies are more sophisticated and effective, enabling to reach better visualizations of blood flow, and diameter and compressibility in veins, which could provide an earlier diagnostic [331]. Moreover, further molecular research is required for a better understanding of CVD pathogenesis, especially in the field of epigenetics, which would allow to treat each case individually. Precision medicine based on miRNAs and DNA changes could be used as prognostic biomarkers and for earlier diagnosis too.

Nevertheless, we ought to considerate that prevention is better than cure. The burden of inheritance in the CVD represents only ~17%, which means that the remaining 83% can be modulated in order to avoid its manifestation. In this line, nutrition intervention and physical exercise programs are the two most cost-effective feasible lifestyle factors to promote health and independence. Both hold enough evidence to verify their ability to reduce oxidative stress and pro-inflammatory markers.

It is undeniable that the rising results of interventional studies related to adherence to a Mediterranean diet demonstrate the cardioprotective effects and anti-inflammatory properties on individual's health. A well-balanced low-carb diet with plenty of fiber, vitamins, polyphenols, and polyunsaturated fatty acids (PUFA) satisfy the well-functioning of the immune system. On the contrary, Westernized diets denote a lack of micronutrients that lead to malnourishment and impaired immune competence associated to the origin of chronic diseases, such as CVD, obesity, cardiovascular, type 2 diabetes, or some types of cancer [332]. Indeed, previous studies have demonstrated that old adults with chronic venous leg ulcers present some worrisome markers of malnutrition, with high plasma levels of n-6/n-3 PUFA ratio, and low consumption of vitamins and fiber, accompanied by high saturated fat and sugar consumption [333]. There is a need to include dietary

and lifestyle interventions in medical guidelines, which would improve the success of pharmacological treatment as well [37].

Furthermore, in order to reduce symptoms of CVD, some physical exercise trials have already been performed. Physical conditioning of CVD patients has defied the impaired calf muscle pump to handle patient disability and decreased exercise capacity [334], showing improved muscle hemodynamics and function [335]. A six-month program of structured exercise in patients with CEAP 4, 5, or 6 was demonstrated to improve calf muscle pump strength [336]. With the aid of compression therapy, exercise has also shown promising results in microvascular changes in the process of venous leg ulcer healing in CVI patients [337]. In any case, a more comprehensive and integrated approach should be advocated to target a series of epigenetic factors, which undoubtedly affect CVD onset and course.

Additionally, age represents one of the main risk factors to suffer from CVD. Life expectancy has significantly increased; however, quantity does not seem to imply QOL now that the rising morbidities are a threat to society and the economy as well [338]. Longevity and physical status, conditioned by obesity and sedentariness, are the main risk factors for the increasing incidence of CVI in elderly [37,339]. Malnutrition is also a common condition in the elderly, associated with sarcopenia and frailty, and these correlate with higher rates of mortality and longer hospital stays. Evidence alleges that strategies, such as routine screening of nutritional status, would provide, again, earlier diagnostics [340]. Nevertheless, sooner nutrition care, routinely, and lifestyle modifications would ameliorate prognosis in cases of age-related diseases [341]. This is one of the hallmarks in science targeting healthy aging.

In the same manner, due to overweight conditions and inflammatory status—pregnant women are another group to carefully watch. The existing possibility of developing CVI during pregnancy implies placental damage induced by oxidative stress [297] and the impact of this stress on epigenetic patterns can be determinant for the occurrence of vascular diseases in the offspring, even in adulthood [342]. Nutritional intervention during this period, together with tailored physical exercise may help to reprogram epigenetic mechanisms for the health status of the mother and future newborn.

We should also note that chronic diseases, such as CVD, implying overweight, pain, and morbidities impeding self-independence, are causes of high prevalence of depression and other mood disorders [63]. These reasons, together with psychological affections and disturbed QOL, entail a high number of medical sick leaves. It would be fruitful to remind the population about the importance of self-application of good habits, encouraging them from childhood. In this context, investment in prevention would play a prominent role, for instance, incorporating programs of adequate nutrition, psychological support, and physical exercise in education, and in medical assessment. These future directions, herein discussed, are summarized in Figure 5.

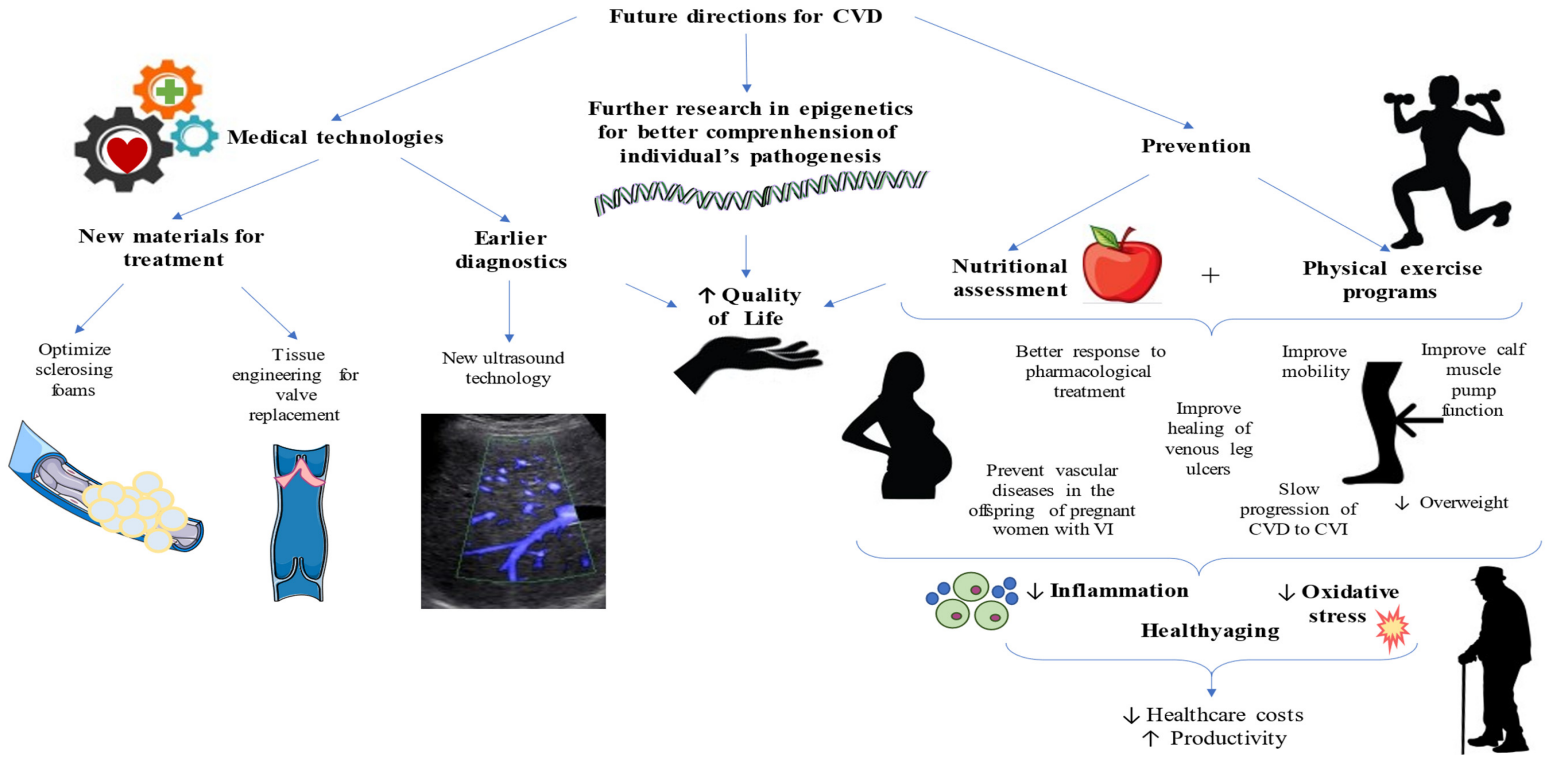

Figure 5. Future directions for CVD. The emergence of novel therapeutic or diagnostic methods are playing a key role in the clinical management of the patients with CVD. However, it is equally important to "deep" on the epigenetic and individual mechanisms, to allow for better comprehension of the disease, opening the possibilities of novel translational approaches. Perhaps one of the most disregarded and important factors are encompassed around prevention. In this sense, nutritional assessment and physical exercise could be powerful tools in the handle of patients affected by CVD, as they might favorably regulate clinical signs, pathophysiological mechanisms, such as inflammation or oxidative stress, and even psychological variables. Overall, these points will be crucial to improve the QOL of the patients, which might be particularly vulnerable by other variables (e.g., pregnant women or elder people).

9. Conclusions

CVD is a progressive and disabling condition widely represented in the global population. Prolonged exposure to genetic and environmental risk factors may lead to important biophysical and biochemical changes in the venous system, entailing a complex vascular response. A growing number of studies, focusing on CVD, evidence the relevance of this vascular pathology, prominently in most advanced stages (CVI) [1–3]. Herein, we collected some of the most relevant data on such an intricate topic, with special emphasis on the pathophysiological and medical perceptions of the disease. Additional studies are needed in order to obtain further understanding of CVD, exploring novel translational and medical approaches to improve the QOL of these patients. Finally, society should be aware of the real impact of the disease, and attempt to adopt measures to prevent the development and/or progression of CVD to CVI (e.g., by increasing physical activity or through nutrition and lifestyle interventions). An integrative perspective of this condition would bring immediate benefits to the clinical management of these patients, particularly for the most vulnerable groups, such as elder people, individuals with extra comorbidities, and pregnant women.

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Articulo II

Association Between Lower Extremity Venous Insufficiency and Intrapartum Fetal Compromise: A Nationwide Cross-Sectional Study

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Resumen articulo II:

La enfermedad venosa crónica (EVC) tiene una alta prevalencia, siendo comúnmente diagnosticado por la presencia de venas varicosas. De hecho, el desarrollo de varices en extremidades inferiores y/o insuficiencia venosa pélvica (LEPVI) es bastante frecuente en la gestación (artículo 1). No obstante, su impacto potencial en la salud fetal no ha sido aún investigado en la literatura existente hasta la fecha.

El segundo trabajo tuvo como objetivo examinar si la presencia de venas varicosas en mujeres gestantes está relacionada con un evento de compromiso fetal intraparto. Para ello, realizamos un estudio transversal utilizando registros médicos administrativos (CMBD) de todos los partos vaginales (n = 256.531) registrados en 2015 en España. La variable independiente se definió como la presencia de varices en piernas, vulva y perineo o hemorroides. Se utilizó un modelo de regresión logística para evaluar la asociación de interés, ajustándose a su vez por medio de un propensity score.

Las mujeres con LEPVI presentaron una probabilidad significativamente mayor de compromiso fetal intraparto (OR = 1,30, IC 99,55% = 1,08–1,54) que aquellas mujeres que no padecían venas varicosas. Después del ajuste, esta asociación siguió siendo significativa (OR = 1,25, IC del 99,5 % = 1,05–1,50).

Los hallazgos de este estudio muestran una asociación entre las venas varicosas en las extremidades inferiores y/o la pelvis de las mujeres y el compromiso fetal intraparto. Este hallazgo sugiere que las venas varicosas pueden ser un factor de riesgo clínico, que no se había explorado demasiado hasta la fecha, y su incorporación al estudio de los casos puede suponer importantes avances para el bienestar y la salud de las mujeres embarazadas y sus fetos.

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Association Between Lower Extremity Venous Insufficiency and Intrapartum Fetal Compromise: A Nationwide Cross-Sectional Study

Ángel Asúnsolo[1,2,3], Chen Chaowen[1], Miguel A. Ortega[2,4], Santiago Coca[2,4], Luisa N. Borrell[1,3], Juan De León-Luis[5], Natalio García-Honduvilla[2,4], Melchor Álvarez-Mon[2,4,6†] and Julia Buján[2,4†]*

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Introduction: Chronic venous disorder (CVeD) has a high prevalence, being commonly diagnosed by the presence of varicose veins. In fact, the development of varicose veins in lower extremities and/or pelvic venous insufficiency (LEPVI) is frequent. However, its potential impact on fetal health has not been investigated. This study aimed to examine whether the presence of varicose veins in women's LEPVI is related to an intrapartum fetal compromise event.

Materials: A cross-sectional, national study was conducted using medical administrative records (CMBD) of all vaginal births (n = 256,531) recorded in 2015 in Spain. The independent variable was defined as the presence of varicose veins in the legs, vulva, and perineum or hemorrhoids. A logistic regression model was used to assess the association of interest.

Results: Among women with vaginal deliveries, those with varicose veins in their LEPVI have a significantly greater odds of intrapartum fetal compromise (OR = 1.30, 99.55%CI = 1.08–1.54) than their counterparts without varicose veins. After adjustment, this association remained significant (OR = 1.25, 99.5%CI = 1.05–1.50).

Conclusions: Our findings of an association between varicose veins in women's lower extremities and/or pelvis and intrapartum fetal compromise suggest that varicose veins may be a novel and important clinical risk factor for fetal well-being and health.

Keywords: placental insufficiency, varicose veins, intrapartum fetal compromise, Spain, fetal

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INTRODUCTION

Within the vascular disorders, the term chronic venous disorder (CVeD) is used to describe the spectrum of morphological and functional abnormalities of the venous system (1). One of the manifestations of CVeD is the development of varicose veins. The development of varicose veins in lower extremities and/or pelvic venous insufficiency (LEPVI) during pregnancy is a common complication (2, 3). However, its clinical relevance has not been investigated.

Recently, it was observed that LEPVI was associated with the development of structural lesions of hypoxemic pathogenesis of the placental villi (4, 5). This association between varicose veins in the infra-diaphragmatic area and placental damage could be explained by the impact of gestational venous hypertension and blood stasis in the placental area and/or abnormal placental development. Either one of these conditions could produce a high-resistance, low-flow circulation predisposing to hypoperfusion, hypoxia, reperfusion injury, and oxidative stress within the placenta. If the hypoxic insult is severe enough and long lasting under intensifying conditions such as a vaginal delivery, trophoblastic function could be altered enough to affect fetal well-being.

Uterine contractions in labor result in a 60% reduction of uteroplacental perfusion, causing transient fetal and placental hypoxia (6). A healthy term fetus with a normally developed placenta is able to accommodate this transient hypoxia. However, when there is a preexisting placental dysfunction, this dysfunction predisposes the fetus to intrapartum fetal compromise (IFC) (6). For example, placental infarct and fetal compromise could be observed in a variety of ways, such as fetal distress, passage of meconium *in utero*, or abnormal acid–base

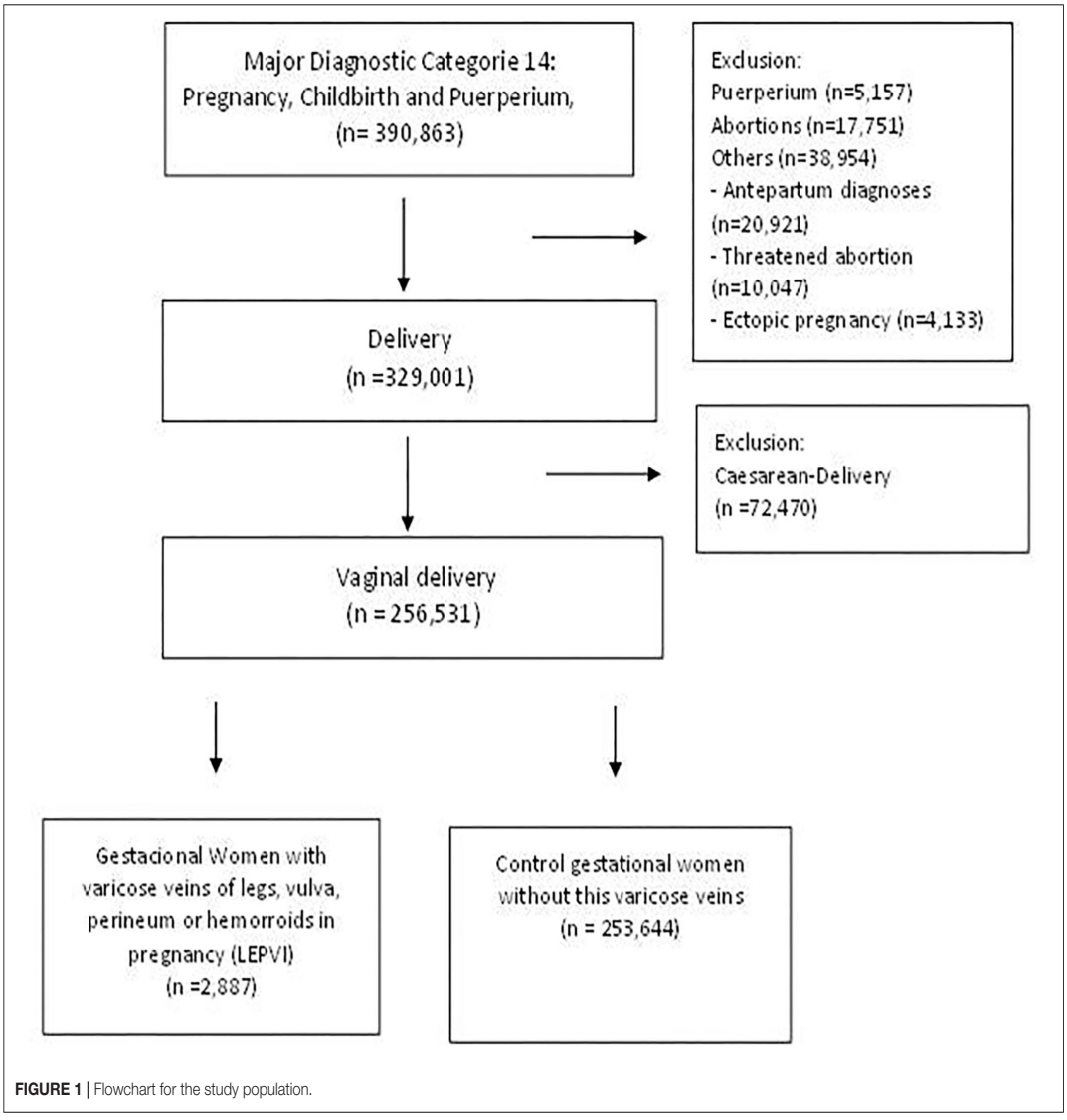

FIGURE 1 | Flowchart for the study population.

balance, and even death in extreme cases. Women with pre-labor placental dysfunction are more likely to develop IFC. However, especially in normal grown fetuses and low-risk populations, there are no tools to identify these conditions during pre-labor. Thus, the presence of varicose veins could be an external symptom and marker for this dysfunction.

This study examines whether there is an association between the presence of LEPVI and IFC in women who had vaginal deliveries in Spain in 2015.

METHODS

Design

A national cross-sectional study was conducted including all vaginal births ($n = 256{,}531$) occurring in hospitals in Spain in 2015. We selected our population using the diagnosis-related groups (DRGs), codes 372-372 and 652 (all patient refined DRGs, version 32). Please see flowchart in **Figure 1** for further details on the sample size and exclusions. The Health Information Institute of the Spanish Ministry of Health provided data from the Minimal Basic Dataset (CMBD), Hospital Discharge Records in the National Health System (7). The CMBD is a mandatory and common medical administrative registry for all hospitals in Spain containing the administrative, demographic, and clinical information for users, centers, and units attending the patients and their treatments. Specifically, it contains 19 compulsory variables, with the most important being age, sex, main diagnosis, secondary diagnoses (diagnoses that coexist with the main diagnosis at the time of admission or have developed during the hospital stay), procedures, and circumstances regarding hospital discharge. The International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9 CM) codes were used for the diagnosis.

Variables

The exposure was specified as pregnant women who presented varicose veins in the legs (ICD-9-CM: 671.0), varicose veins in the vulva and perineum (ICD-9-CM: 671.1), or hemorrhoids (ICD-9-CM: 671.8). The main outcome variable was signs of IFC due to inadequate placental function, specified as a composite variable of the presence of any of the following: fetal distress (ICD-9-CM: 656.3), abnormal acid–base balance, intrauterine acidosis or meconium in liquor (ICD-9-CM: 656.8), and placental infarct or abnormal placenta (ICD9-CM: 656.7). We considered the following variables as confounders: age, toxic habits, and the presence of comorbidities. Specifically, diagnosis of hypertension, cardiac pathology, respiratory disease, cancer, kidney or liver diseases, thyroid pathology, diabetes mellitus, obesity, and alterations in coagulation as well as the presence of depression or dementia were considered to calculate the Charlson index of comorbidity for each woman (8). A complete list of the ICD-9-CM codes used to classify the variables is provided in the **Supplementary Material**.

Statistical Analysis

Descriptive analysis for the population was performed presenting means and proportions according to whether the variable was continuous or categorical, respectively. A logistic regression model was used to quantify the association between the presence of LEPVI and IFC event before and after adjustment. Firstly, we fitted an explanatory logistic regression model with IFC as the dependent outcome and LEPVI as the main exposure before and after controlling for the potential confounding variables included in the database. The covariates selected had to have a plausible biological relation with the exposure and/or the outcome. Because we noted important differences in the baseline clinical characteristics between women with and without LEVPI, we also conducted a second analysis using propensity scores to mitigate confounding bias caused by the imbalance between the characteristics of the groups under comparison (**Figure 2**). We developed a score representing the propensity (i.e., the conditional probability) of a woman who has varicose veins in light of their clinical characteristics. We used the propensity score to join, without replacement, women with varicose veins and women without varicose veins at a 1:1 ratio. Odds ratios (ORs) were calculated using univariate conditional logistic regression. Given the number of covariates and the comparisons made, an $\alpha < 0.005$ was considered as significant. Finally, we conducted a sensitivity analysis. We fitted a logistic regression model including only hospitals with more than 500 births and the presence of at least one case of LEVPI in women. STATA/IC (version 14.2) was used for all the statistical analyses.

We followed the RECORD recommendations (reporting of studies conducted using observational routinely collected data) as an extension of the existing STROBE guidelines in this paper.

This study was approved by the Clinical Research Ethics Committee of the Gómez-Ulla-UAH Defense Hospital (37/17).

RESULTS

The prevalence of LEPVI among pregnant women was 1.13% ($n = 2{,}887$; **Table 1**). When compared with women without LEPVI, those with LEPVI were older and more likely to smoke, to be obese, and have asthma, hypothyroidism, coagulation disorders, and anemia (all $p < 0.05$). The high prevalence for these conditions yielded a high prevalence for the Charlson Index score for women with LEPVI. There were no differences observed for hypertension and diabetes between pregnant women with and without LEVPI.

Women with LEPVI were more likely to have conditions associated with IFC (9.4%) than women without LEPVI (7.4%; **Table 2**). Women with LEPVI have a 30% (OR = 1.30, 99.5%CI = 1.08–1.54) greater odds of having a birth outcome with IFC relative to women without LEPVI during pregnancy. Women with LEPVI also have a greater odds of placental dysfunction (OR = 1.74, 99.5% CI = 1.00–3.05) than their counterparts without LEPVI. After adjusting for age, smoking, obesity, asthma, hypothyroidism, coagulopathy disorders, and anemia, these associations remained significant but slightly attenuated for IFC [adjusted OR (aOR) = 1.25, 99.5% CI = 1.05–1.50] and placental dysfunction (aOR = 1.23, 99.5%CI = 1.01–1.49; **Table 2**). However, there was no association between LEPVI and fetal distress (aOR = 1.01, 99.5% CI = 0.46–2.21) after adjustment.

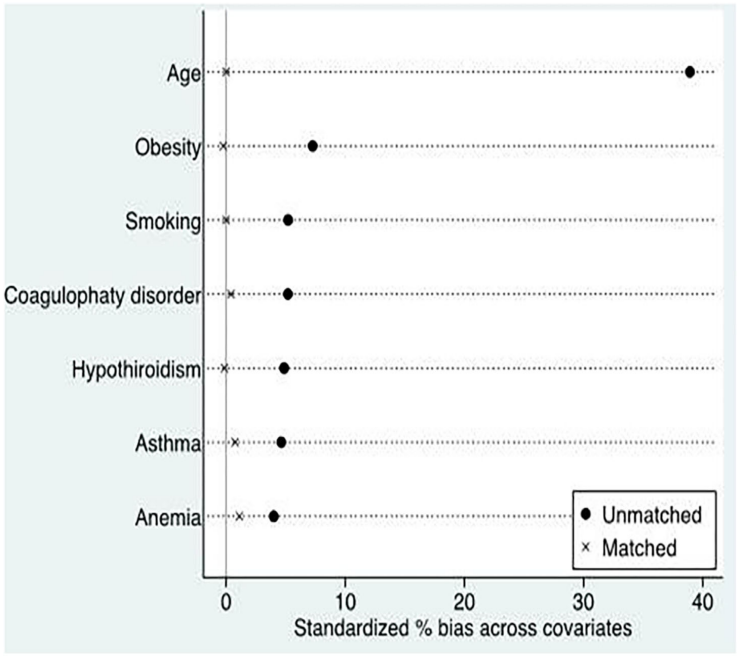

FIGURE 2 | Propensity score matching of women with and without varicose veins (LEPVI).

TABLE 1 | Descriptive statistics for selected characteristics for pregnant women with and without LEPVI, Spain 2015.

Characteristics	LEPVI % (N)	No LEPVI % (N)	*P*-value
Total	1.1 (2,887)	98.9% (253,644)	
Age, mean (SD)	33.55 (4.97)	31.49 (5.59)	< 0.001
Smoking (%)	7.9 (229)	6.6 (16,812)	0.006
Obesity (%)	2,8 (80)	1.7 (4,287)	< 0.001
Hypertension (%)	2.7 (77)	2.7 (6,725)	0.95
Diabetes (5)	5.7 (164)	5.3 (13,491)	0.38
Asthma (%)	2.3 (67)	1.7 (4,392)	0.015
Hypothyroidism (%)	7.7 (227)	6.5 (16,610)	0.022
Coagulopathy Disorder (%)	1.0 (29)	0.5 (1,383)	0.002
Anemia	1.9 (54)	1.4 (3,564)	0.039
Charlson index score (%)			
0	96.8 (2,795)	97.4 (247,163)	0.090
1	3.1 (89)	2.4 (6,207)	
2	0.1 (3)	0.1 (274)	

Myocardial infarction, congestive heart failure, cerebrovascular disease, peripheral arteriovascular disorders, paralysis, renal disease, liver disease, rheumatic disease, AIDS/HIV, peptic ulcer disease excluding bleeding, depression, dementia, alcohol and drugs consumption are <1% in both groups. None of them were statistically significant. P-values were obtained from chi-squared statistics with the exception of the one for age (t-test). LEPVI, varicose veins in lower extremities and/or pelvis; N, number of patients; %, percentage; SD, standard deviation.

TABLE 2 | Prevalence estimates and OR with their 99.5% confidence intervals for loss of fetal well-being among pregnant women, Spain 2015.

	LEPVI % (*N*)	No LEPVI % (*N*)	Unadjusted OR [99.5% CI]	Adjusted[a] OR [99.5% CI]
Intrapartum fetal compromise	9.4 (270)	7.4 (18,792)	1.30 [1.08–1.54]	1.25 [1.05–1.50]
Fetal distress	0.5% (13)	0.4% (1,110)	1.03 [0.47–2.26]	1.01 [0.46–2.21]
Placental infarct or abnormal placenta	0.9% (26)	0.5% (1,313)	1.74 [1.00–3-05]	1.72 [0.98–3.00]
Abnormal acid-base balance, intrauterine acidosis or meconium in liquor	8.2% (236)	6.6% (16,633)	1.27 [1.05–1.54]	1.23 [1.01–1.49]

[a]*Adjusted for age, smoking, obesity, asthma, hypothyroidism, coagulopathy disorders, and anemia. LEPVI, varicose veins in lower extremities and/or pelvis; N, number of patients; %, percentage; CI, confidence interval.*

The ORs between LEVPI and IFC showed the same direction and remained significant regardless of the method used for the analysis. The OR based on selected hospitals with more than 500 births in 2015 and the presence of at least one case of LEPVI in women was similar to the one obtained using the entire database (aOR = 1.21, 99.5% CI = 1.01–1.46). However, the propensity score analysis yielded a greater odds of IFC for women with LEVPI (aOR = 1.84, 99.5% IC = 1.37–2.47) relative to those without LEPVI.

DISCUSSION

Our findings show that LEPVI may explain IFC due to a placental hypoxic dysfunction in women who give birth

by vaginal delivery after controlling for age, smoking, obesity, asthma, hypothyroidism, coagulopathy disorders, and anemia. Fetoplacental circulation depends on a complex balance between arterial, placental, and maternal venous circulation. Previous studies have shown that vascular diseases (high blood pressure, heart failure, etc.) and blood disorders (anemia, clotting disorders, etc.) in the mother can cause placental insufficiency (also known as uteroplacental vascular insufficiency) and placental and fetal hypoxia (9–11). Our findings suggest that a common pathological condition such as LEPVI may be an independent risk factor for the onset of this IFC not yet identified. Therefore, varicose veins could be a novel risk factor for placental vascular alterations.

While the pathogenic mechanisms underlying the association between gestation and LEVPI with the development of IFC have not been established, it is worth noting that the development of LEPVI during pregnancy causes structural alterations in the placenta of hypoxic pathogenesis. Previous studies (4, 5) have shown a relationship between maternal venous insufficiency and placental hypoxia. In addition, histopathological Tenny–Parker changes in the villi or placental damaging alterations, increase of apoptosis, as well as changes in the gene and protein expressions of HIF-1α were observed (4, 5). Therefore, there is a biological basis for our findings. In this sense, it has been demonstrated how, in the placental villi of patients with CVeD, there is an increase in oxidative stress with an increase in lipid peroxidation levels with angiogenic and lymphangiogenesis processes (12, 13). Furthermore, these patients have presented a systematic increase in malondialdehyde levels, correlated with a decrease in fetal pH in newborns (12). Despite this potential mechanism, we found no association of LEPVI with fetal distress. There could be two potential explanations for such finding: firstly, placental dysfunction due to chronic hypoxia tends to be corrected by increasing the number of villi (4). In addition, the fetus develops in an environment of hypoxia. Therefore, it is only in a greater need for physiological requirements for tissue homeostasis, when the inability to provide oxygen and nutrients could occur, with a risk to the well-being of the fetus. Secondly, when fetal distress is detected, a cesarean delivery is frequently performed. Therefore, we only expected a small number of cases in our database.

Because of the cross-sectional nature of the study, we cannot establish temporality. Moreover, it is possible that the presence of LEPVI in pregnant women is underreported. Therefore, our findings could be affected by non-differential misclassification bias, leading to an underestimation of the association observed. In addition, this database lacked information on prescriptions and treatments that may confound the results. However, we adjusted for comorbidities and for the presence of other risk factors related to lifestyle. Despite these adjustments, the lack of information in prescriptions or antenatal care may have biased our results depending on the relationship with the exposure and/or outcome. Despite these limitations, the use of the CMBD data shows numerous strengths, such as a large sample size, consistency in the coding of the variables, and potential for generalization of the findings to the entire Spanish population of women.

CONCLUSIONS

Our findings suggest that varicose veins may be a novel and important clinical risk factor for fetal well-being and health. The association between the presence of varicose veins and the existence of placental dysfunction could be especially relevant in cases with high risk of hypo-oxygenation of the fetus, such as the presence of anemias, coagulopathy, heart failure, and smoking. Thus, the inclusion of the diagnosis of venous insufficiency should be considered as part of the monitoring protocol for pregnant women, as an external and easily detectable indicator of risk for placental dysfunction and IFC. Moreover, these findings call attention to the need for a prospective clinical study to establish the relevance of including LEVPI among the risk factors for IFC and its diagnosis, especially in low-risk populations.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

ETHICS STATEMENT

This study was approved by the Clinical Research Ethics Committee of the Gómez-Ulla-UAH defense Hospital (37/17).

AUTHOR CONTRIBUTIONS

ÁA, CC, MO, NG-H, MÁ-M, LB, and JB contributed to data curation and formal analysis. ÁA, CC, MO, MÁ-M, JB, and LB wrote the manuscript. ÁA, CC, SC, LB, JD, MÁ-M, and LB did the investigation. ÁA, CC, MO, SC, MÁ-M, and JB helped with project administration and funding acquisition. All authors discussed the results and contributed to the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.577096/full#supplementary-material

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

Articulo III

Chronic venous disease in pregnant women causes an increase in ILK in the placental villi associated with a decrease in E-Cadherin.

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Resumen Articulo III:

Las mujeres son una población vulnerable a sufrir la enfermedad venosa crónica (EVC) que se manifiesta frecuentemente en los miembros inferiores en forma de venas varicosas, especialmente durante el embarazo (artículo 1). Esta manifestación clínica, como se ha visto en estudios previos, podría tener una asociación con la función de la placenta y el resultado perinatal (artículo 2). De ser cierta esta asociación, debe existir una alteración en la estructura y función celular de la placenta que sea el sustrato de los resultados gestacionales. La quinasa ligada a integrina (ILK) es una molécula crítica involucrada en múltiples condiciones fisiológicas y patológicas, y junto con las cadherinas, es esencial para mediar la interacción de célula a MEC y de célula a célula, respectivamente.

En este tercer trabajo, nuestro objetivo fue evaluar la implicación de ILK y un conjunto de cadherinas (e-cadherina, cadherina-6 y cadherina-17) en placentas de mujeres con ECV para desentrañar el posible papel fisiopatológico de estos componentes.

Se realizaron estudios de expresión génica (RT-qPCR) y expresión de proteínas (inmunohistoquímica). Nuestros resultados muestran un aumento significativo en la expresión génica y proteica de ILK, cadherina-6 y cadherina-17 y una disminución de e-cadherina en la placenta de mujeres con ECV. En general, este trabajo muestra que una expresión anormal de ILK, e-cadherina, cadherina-6 y cadherina-17 puede estar implicada en los cambios patológicos que ocurren en el tejido placentario. Se necesitan más estudios para determinar las posibles asociaciones de estos cambios con el bienestar materno y fetal.

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Article

Chronic Venous Disease in Pregnant Women Causes an Increase in ILK in the Placental Villi Associated with a Decrease in E-Cadherin

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Abstract: Chronic venous disease (CVD) is a multifactorial vascular disorder frequently manifested in lower limbs in the form of varicose veins (VVs). Women are a vulnerable population for suffering from CVD, especially during pregnancy, when a plethora of changes occur in their cardiovascular system. Previous studies have indicated a worrisome association between CVD in pregnancy with the placental structure and function. Findings include an altered cellular behavior and extracellular matrix (ECM) composition. Integrin-linked kinase (ILK) is a critical molecule involved in multiple physiological and pathological conditions, and together with cadherins, is essential to mediate cell to ECM and cell to cell interplay, respectively. Thus, the aim of this study was to evaluate the implication of ILK and a set of cadherins (e-cadherin, cadherin-6 and cadherin-17) in placentas of women with CVD in order to unravel the possible pathophysiological role of these components. Gene expression (RT-qPCR) and protein expression (immunohistochemistry) studies were performed. Our results show a significant increase in the gene and protein expression of ILK, cadherin-6 and cadherin-17 and a decrease of e-cadherin in the placenta of women with CVD. Overall, this work shows that an abnormal expression of ILK, e-cadherin, cadherin-6 and cadherin-17 may be implicated in the pathological changes occurring in the placental tissue. Further studies should be conducted to determine the possible associations of these changes with maternal and fetal well-being.

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1. Introduction

Chronic Venous Disease (CVD) is a vascular disorder frequently manifested by the appearance of varicose veins (VVs), generally in the lower limbs [1]. Women seems to be more prone to develop CVD, especially during pregnancy, as the whole organism undergoes a plethora of physiological, anatomical and adaptative changes which are critical for the fetus [2]. In the cardiovascular system, these alterations include notable hemodynamic modifications, variations in the oxygen transport and a profound vascular remodeling [3,4]. Occasionally, this may lead to the development of CVD, which is associated with the detection of different local and systemic markers of damage that could entail negative consequences for the maternofetal well-being [5]. Indeed, different studies indicate that approximately 1 in 3 women during pregnancy may suffer from VVs [6,7] and these data could increase to 50–70% when considering additional manifestations of CVD [8]. Thus, further studies are needed to understand the complex implications of pregnancy-related CVD for both women and fetus in order to achieve a better management of such a common condition.

CVD is also recognized as venous dysfunction. Its analogue in the arterial system, pre-eclampsia, is one of the most worrisome complications of pregnancy [9]. Previous studies have demonstrated that the placenta is the hardest-hit organ affected by this malady, defining a set of abnormal processes and markers of damage in this structure [10–12]. In this line, we have demonstrated that maternal venous dysfunction also induces multiple abnormalities and damage in the placenta, evidencing an increase of hypoxic markers and enhanced apoptosis [13], markers of oxidative stress [14] and altered angiogenesis and lymphangiogenesis [15]. Moreover, we also found that CVD is associated with changes in the placental composition [7,16] and signaling [17], suggesting that this condition drives detrimental modifications in the placental structure and functioning, probably representing a unique pathophysiological feature in response to the venous dysfunction.

In this context, the study of the cellular transduction of external signals may be of great aid to understand the pathophysiological mechanisms of CVD in the placenta. Integrin-linked kinase (ILK) is an intracellular molecule that binds to the cytoplasmic domain of β1 and β3-integrin, and it is considered a crucial mediator of the cell-ECM interactions [18]. The relevance of this component has been widely established in the cardiovascular system, especially in the heart and blood vessels, modulating a wide variety of physiological processes and participating in disease conditions [19]. In the placenta, ILK expression is critical during the first stages of pregnancy, regulating particular cellular behaviors [20].In addition, ILK seems to play a major role in the development of pre-eclampsia, arising as an important therapeutic target [21]. Cadherins are transmembrane proteins implicated in cell-to-cell adhesion and are central determinants of tissue cytoarchitecture. Epithelial cadherin (e-cadherin) is one of the best-characterized cadherins studied, regulating cell development and morphogenesis from early stages [22] and with adverse consequences in the placenta when dysregulated [23]. Other members of the cadherin family such as cadherin 6 and 17 are also arising as promising indicators of health and disease status [24].

Thus, the purpose of this study is to analyze the differential expression of ILK, e-cadherin, cadherin 6 and cadherin 17 in the placenta of women with CVD in comparison to healthy controls. Gene and protein expression will be detected by real time quantitative PCR (RT-qPCR) and immunohistochemistry, respectively.

2. Patients and Methods

2.1. Experimental Design

We have performed an observational, analytical and prospective study including 114 women in the third trimester of pregnancy. Of them, there were 62 women diagnosed with CVD according to the CEAP classification [25] and 52 women without a history of CVD, referred as healthy controls (HC). The median age of women with CVD was 33 years (interquartile range (IQR), 22–40 years) and the median gestational period was 40.5 weeks (IQR, 39–41.5 weeks), whereas HC had a median gestational age of 34 years (IQR, 27–41 years) and a median gestational period of 41 weeks (IQR, 39–42 weeks). The current work was completed following the basic ethical principles of autonomy, beneficence, non-maleficence and distributive justice. Furthermore, the regulations of Good Clinical Practice, as well as the principles set forth in the last Declaration of Helsinki (2013) and the Oviedo Convention (1997) were also followed. Patients were informed prior to enrolment, and each participant provided their corresponding written consent. The present study was approved by the Clinical Research Ethics Committee of the Central University Hospital of Defence University of Alcalá (37/17). During the third trimester consultation, the clinical history was reviewed and a general physical examination of the woman was performed. Moreover, lower limb ultrasounds were performed using an Eco-Doppler (Portable M-Turbo Eco-Doppler; SonoSite, Inc., Bothell, WA, USA) at 7.5 MHz.

The inclusion criteria of our study were defined as women > 18 years of age, with clinical evidence of lower limb venous disease during the third trimester, according to CEAP ($\geq$1). On the other hand, the exclusion criteria included women with prior diagnosis of high blood pressure; venous malformations; heart, kidney and lung insufficiency; autoimmune diseases; body mass index $\geq$ 25; diabetes mellitus, gestational diabetes mellitus or other endocrine diseases; active infectious diseases; toxicological habits (alcohol ($\geq$1 unit a day), tobacco ($\geq$1 cigarette a day), or drugs (e.g., cannabis, heroin, cocaine, amphetamines)); pre-eclampsia and/or HELLP syndrome; known causes of intrauterine growth restrictions; existence of pathological injuries, such as placental infarction, avascular villi, delayed villi maturation or chronic villitis; as well as the appearance of any exclusion criteria in the following months (until delivery); and previous evidence of CVD.

There were no significant differences between the groups regarding the number of previous pregnancies: 33 (53.2%) for women with CVD and 19 (36.5%) for women in the HC group (Table 1). There were also no significant differences in the clinical characteristics between the CVD and HC groups (gestational age, c-section delivery, previous pregnancies, previous abortions, regular menstrual cycles and type of profession-sedentary, Table 1).

Table 1. Clinical and demographic characteristics. CVD = Chronic venous disease, HC = Healthy control.

	CVD (*n* = 62)	**HC (*n* = 52)**
Median age (IQR), years	33 (22–40)	34 (27–41)
Median gestational age (IQR), weeks	40.5 (39–41.5)	41 (39–42)
C-section delivery, n (%)	12 (19.4)	9 (17.3)
Vaginal delivery, n (%)	50 (80.6)	43 (82.7)
Varicose vein (CEAP), n (%)		
CEAP 1	37 (59.7)	0 (0)
CEAP 2	21 (33.8)	0 (0)
CEAP 3	4 (6.5)	0 (0)
Previous pregnancies, n (%)	33 (53.2)	19 (36.5)
Previous abortions, n (%)	14 (22.6)	9 (17.3)
Regular menstrual cycles, n (%)	50 (80.6)	42 (80.7)
Sedentary profession, n (%)	41 (66.1)	40 (76.9)

2.2. Tissue Samples

Placental biopsies were collected after delivery for the 114 patients. In every case, 5 placental fragments were obtained by using a scalpel to include various mixed cotyledons. Then, placental pieces were added to two different sterile tubes: One containing Minimum Essential Medium (MEM; Thermo Fisher Scientific, Inc., Waltham, MA, USA) with 1% antibiotic/antimycotic (Streptomycin, Amphotericin B and Penicillin; Thermo Fisher Scientific, Inc.) and another with RNAlater® (Ambion; Thermo Fisher Scientific, Inc., Waltham, MA, USA) solution. Subsequently, the samples were processed in a class II laminar flow hood (Telstar AV 30/70 Müller 220 V 50 MHz; Telstar; Azbil Corporation) in a sterile environment. Conserved samples were stored in 1 mL RNAlater® at −80 °C until further processing for gene expression analysis. Preserved MEM placentas were employed for histological and immunohistochemical studies.

MEM samples were washed and rehydrated five times in MEM without antibiotics to remove the erythrocytes. After, they were cut into 2 cm fragments and fixed in F13 (60% ethanol, 20% methanol, 7% polyethylene glycol and 13% distilled water) following established protocols [26]. Then, samples were embedded in paraffin, using molds. Once the paraffin had solidified, a HM 350 S rotation microtome (Thermo Fisher Scientific, Inc., Waltham, MA, USA) was used to obtain 5-µm thick sections, which were then stretched in a hot water bath and mounted on glass slides, previously treated with 10% polylysine, in order to enhance adhesion of the sections.

2.3. Gene Expression Studies Using Reverse Transcription-Quantitative PCR (RT-qPCR)

RNA was extracted following the guanidinium thiocyanate-phenol-chloroform method [27,28] allowing the analysis of mRNA expression levels of the selected genes. RNA samples at a concentration of 50 ng/µL were used to synthesize complementary DNA (cDNA) by reverse transcription (RT). 4 µL of each sample is mixed with 4 µL of oligo-dT 0.25 µg/µL solution (Thermo Fisher Scientific, Inc., Waltham, MA, USA), and incubated at 65 °C for 10 min in a dry bath (AccuBlock, Labnet International Inc., NJ, USA) to denature the RNA [16,29]. Hereunder, samples were put on ice and 10 µL of a reverse transcription mix containing the following products was added for each sample: 2.8 µL First Strand Buffer 5X (250 mM Tris-HCl and pH 8.3; 375 mM KCl; 15 mM MgCl2) (Thermo Fisher Scientific, Inc., Waltham, MA, USA); 2 µL of 10 mM deoxyribonucleotides triphosphate; 2 µL of 0.1 M dithiothreitol; 1.7 µL of DNase- and RNase-free water; 0.5 µL of RNase inhibitor (RNase Out); 1 µL of reverse transcriptase enzyme (all from Thermo Fisher Scientific, Inc., Waltham, MA, USA).

The RT process was conducted using a G-Storm GS1 thermal cycler (G-Storm Ltd.). Then, the samples were incubated at 37 °C for 75 min in order to allow cDNA synthesis. At this point, the temperature was increased to 70 °C and maintained for 15 min, thereby causing the denaturation of the reverse transcriptase enzyme, and the temperature was gradually reduced to 4 °C. A negative reverse transcription was performed to ensure the absence of genomic DNA contamination in the total RNA samples, in which the M-MLV RT enzyme is replaced by water free of DNases and RNases. The cDNA produced in RT was diluted 1:20 in water free of DNases and RNases and stored at −20 °C until further use.

Specific primers for the selected genes (Table 2) were designed de novo through the Primer-BLAST and AutoDimer online applications [30,31]. The constitutively expressed TATA-box binding protein (TBP) gene was employed as a control to normalize the results [32]. The gene expression units are expressed as relative quantities of mRNA. RT-qPCR was performed on a StepOnePlus™ System (Applied Biosystems; Thermo Fisher Scientific, Inc.) using the relative standard curve method. The reaction was completed as follows: 5 µL sample [mixed at 1:20 with 10 µL iQ™ SYBR® Green Supermix (Bio-Rad Laboratories, Inc.)] was mixed with 1 µL each forward and reverse primers, and 3 µL of DNase and RNase-free water, which were then added to a MicroAmp® 96-well plate (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA). The following thermocycling conditions were used: Initial denaturation for 10 min at 95 °C, denaturation

for 15 s at 95 °C, annealing at variable temperatures depending on the melting temperature of each primer pair for 30 s, and elongation at 72 °C for 1 min, for 40–45 cycles. Then, a dissociation curve for 15 s at 95 °C, 1 min 60 °C, 15 s 95 °C, and 15 s 60 °C was developed. Fluorescence detection was performed at the end of every repeat cycle (amplification) and at the different steps of the dissociation curve. The data collected from the selected genes were included in a standard curve made by serial dilutions of a mixture of the samples, that were included in each plate according to the constitutive expression of TBP (in agreement with the manufacturer's protocols). This RT-qPCR was performed twice in all samples of placenta tissue.

Table 2. Primer sequences used in RT-qPCR and temperature (Tm).

GENE	SEQUENCE Fwd (5′→3′)	SEQUENCE Rev (5′→3′)	Temp
TBP	TGCACAGGAGCCAAGAGTGAA	CACATCACAGCTCCCCACCA	60 °C
ILK	TCCCAAGTAAGGAACGGAGC	CACCACCAGACATGAGCACT	59 °C
E-Cad	GTGAACACCTACAATGCCGC	CCCAGGGGACAAGGGTATGA	59 °C
Cad-17	GCTCCTGGGAGGTAAGTAGA	ACCCTCGGCAAAGCTCC	57 °C
Cad-6	AGCTATTTCCTGCTTTCAGGGT	GGTGGGAAGGAAGTGAGACG	60 °C

2.4. Immunohistochemistry Studies for Protein Expression Analysis

The antigen/antibody reactions were detected using the avidin-biotin complex method, with avidin-peroxidase, as previously described [33,34]. Immunohistochemical studies were performed on paraffin-embedded placental samples. The antibodies used in our study are described in the protocol specifications (Table 3). The samples were incubated with the primary antibody (90 min; Table 3), and then with 3% BSA Blocker (cat. no. 37,525; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and PBS overnight at 4 °C. The next day, the placental tissues were incubated with biotin-conjugated secondary antibody diluted in PBS, for 90 min at room temperature (Table 3). Afterwards, the avidin-peroxidase conjugate ExtrAvidin®-Peroxidase (Sigma-Aldrich; Merck KGaA, San Luis, MO, USA) was added for 60 min at room temperature (1:200 dilution with PBS). Eventually, the protein expression level was determined using a chromogenic diaminobenzidine (DAB) substrate kit (cat. no. SK-4100; Maravai LifeSciences, CA, USA), which was prepared just before exposure (5 mL distilled water; two drops buffer; four drops DAB; and two drops hydrogen peroxide). The signal was developed with the peroxidase chromogenic substrate for 15 min at room temperature, allowing the detection of a brown stain. For each protein, sections of the same tissue were assigned as negative controls, substituting incubation with the primary antibody for a blocking PBS solution. In all tissues, the contrast was achieved using the Carazzi hematoxylin for 15 min.

Preparations were observed using a Zeiss Axiophot optical microscope (Zeiss GmbH). Then, 5 sections and 10 fields of view were randomly examined for each patient of the defined groups. The patients were described as positive when the marked mean area in the analyzed sample was ≥5% of the total, following the immunoreactive score (IRS) as established in previous studies [35,36]. Immunostaining was evaluated by two independent histologists, and then each sample was scored using the following scale: 0–1, minimum staining (≤25%); 2, moderate staining (25–65%); and 3–4, strong staining (≥65–100%).

2.5. Statistical Analysis

The statistical analysis was performed using the the GraphPad Prism® v6.0 (GraphPad, Inc., San Diego, CA, USA) program. The Mann–Whitney U test was used to compare the 2 groups, and the data was expressed as the median ± SEM. Significance was established as $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

Table 3. Primary and secondary antibodies used in the immunohistochemical studies performed, showing the dilutions used and the protocol specifications.

Antigen	Species	Dilution	Provider	Protocol Specifications
ILK	Rabbit	1:50	Abcam (ab52,480)	10 mM Sodium citrate pH = 6 before incubation with blocking solution
E-Cad	Mouse	1:250	Vitro (MAD-000761QD-3/V)	-
Cad-17	Rabbit	1:250	Vitro (MAD-000737QD-3/V)	-
Cad-6	Mouse	1:250	Vitro (MAD-000582QD-3/V)	-
IgG (Mouse)	Goat	1:300	Sigma-Aldrich (F2012/045K6072)	-
IgG (Rabbit)	Mouse	1:1000	Sigma-Aldrich (RG-96/B5283)	-

3. Results

3.1. Women with CVD during Pregnancy Show an Increase in ILK Expression in Placental Villi Associated with a Decrease in E-Cadherin

Our results have shown a significant increase in ILK gene expression in the placental villi of women with CVD during pregnancy compared to HC, *** $p < 0.001$ [CVD = 35.027 $\pm$ 0.817 vs. HC = 28.388 $\pm$ 0.934, Figure 1A]. Histological analysis of protein expression using immunohistochemical techniques showed a significant increase in ILK expression in the placental villi of women with CVD during pregnancy compared to HC, *** $p < 0.001$ [CVD = 2.379 $\pm$ 0.084 vs. HC = 0.976 $\pm$ 0.082, Figure 1B,C].

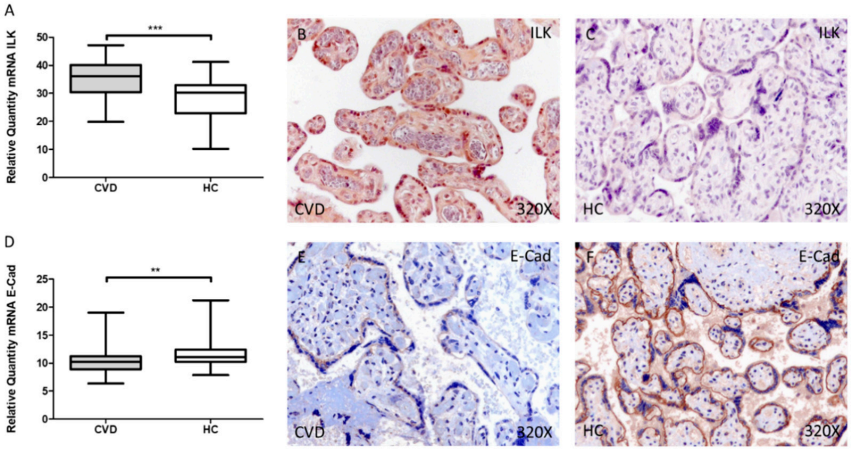

Figure 1. Levels of mRNA for ILK (**A**) and E-Cad (**D**) quantified by RT-qPCR, and histological imágenes for immunohistochemical techniques in the placental villi in of women with CVD during pregnancy and HC for ILK (**B**,**C**) and E-Cad (**E**,**F**). CVD = Chronic venous disease, HC = Healthy control. $p < 0.01$ (**), and $p < 0.001$ (***).

Conversely, we observed a significant decrease in E-Cad gene expression in the placental villi of women with CVD during pregnancy compared to HC, ** $p = 0.0084$

[CVD = 10.726 ± 0.359 vs. HC = 11.893 ± 0.461, Figure 1D]. In parallel, protein expression showed a significant decrease in E-Cad expression in the placental villi of women with CVD during pregnancy compared to HC, *** p = 0.002 [CVD = 0.903 ± 0.062 vs. HC = 1.240 ± 0.058, Figure 1E,F].

3.2. Cadherin 17 and Cadherin 6 Expression Level Is Increased in the Placental Villi of Women with CVD during Pregnancy

Cad-17 gene expression showed a significant increase in the placental villi of women with CVD during pregnancy compared to HC, * p = 0.0228 [CVD = 7.804 ± 0.325 vs. HC = 6.780 ± 0.263, Figure 2A]. In this sense, protein expression showed a significant elevation using immunohistochemical techniques in the placental villi of women with CVD during pregnancy compared to HC, ** p = 0.0026 [CVD = 1.403 ± 0.067 vs. HC = 1.159 ± 0.085, Figure 2B,C].

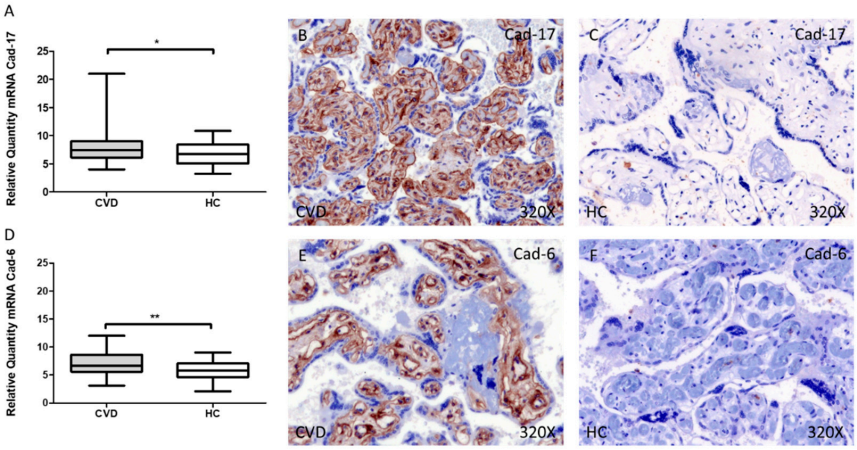

Figure 2. Levels of mRNA for Cad-17 (**A**) and Cad-6 (**D**) quantified by RT-qPCR, and histological imágenes for immunohistochemical techniques in the placental villi in of women with CVD during pregnancy and HC for Cad-17 (**B**,**C**) and Cad-6 (**E**,**F**). CVD = Chronic venous disease, HC = Healthy control. $p < 0.05$ (*), $p < 0.01$ (**).

Similarly, our results have shown an increase in the gene expression of Cad-6 in the placental villi of women with CVD during pregnancy compared to HC, ** p = 0.0016 [CVD = 7.083 ± 0.251 vs. HC = 5.807 ± 0.247, Figure 2D]. Furthermore, protein expression showed a significant increase in the placental villi of women with CVD during pregnancy compared to HC, ** p = 0.0033 [CVD = 1.202 ± 0.065 vs. HC = 0.923 ± 0.066, Figure 2E,F].

4. Discussion

For the first time, we have demonstrated significant changes in tissue expression of critical cell to ECM (ILK) and cell to cell (e-cadherin, cadherin 7 and cadherin-9) components in placentas of women with CVD. These results are in consonance with our previous studies that showed that CVD is associated with a set of changes in the placenta in the ECM [7,16] and cell behavior [13,17].

We detected that ILK is overexpressed in placentas of women with CVD. There are multiple studies reporting the same results, particularly in the field of oncology, promoting cell migration and invasion [37]. In the same manner, ILK is highly expressed in the first trimester of pregnancy, where it seems to stimulate and regulate migration and invasion of cytotrophoblast lines in vivo, which is crucial for the process of placentation [20] However, previous studies have found substantial changes in the expression of ILK under patho-

logical pregnancies such as pre-eclampsia or gestational diabetes [38,39]. Similarly, we found a possible role of ILK in the pathophysiology of CVD in the placenta of pregnant women. Within the cell, ILK is crucial for anchoring the actin filaments (microfilaments) to the integrins, also mediating signal transduction between intracellular and extracellular compartments [18]. Regarding the mediation of ILK in extracellular signaling, it is widely accepted that ILK is located in the cell-matrix focal adhesions but not in cell-to-cell adhesion sites [40]. Thus, ILK is essential for the formation of focal complexes and actine anchoring, being also involved in the assembly of fibronectin-bound fibrillar adhesions [41]. This role of ILK appears to be critical for cell cycle regulation, acting through the activation of key proteins involved in cell division like cyclin d1/d3, cyclin-dependent kinase 2/4 (CDK2/CDK4) and the downregulation of the cell cycle inhibitor p27 [42]. Conversely, ILK upregulation induces an anchorage-independent cell cycle progression, resulting in an increased expression of cyclin D1, activation of Cdk4 and an altered p27 expression, eventually leading to the stimulation of G1/S cyclin-Cdk activities, regulated by cell adhesion and integrins in normal conditions [43]. In accordance with this statement, we have previously identified an increased expression of cyclin D1 in the placenta of women with CVD [17] which may be associated with the overexpression of ILK, also indicating an abnormal regulation and progression of the cell cycle in an adhesion-independent manner. On the other hand, ILK is also related to other cell signaling routes. For instance, PI3K/Akt is one of the main signaling routes related to ILK [44]. It seems that ILK is activated in a PI3K-dependent manner and in turn, ILK activates Akt, also suppressing Glycogen synthase kinase 3β (GSK-3β), an inhibitor of c-Jun and β-catenin [45]. Similarly, the PI3K/Akt inhibitor PTEN is also involved in the downregulation of ILK [46]. In agreement with these facts, we have demonstrated that the placenta tissue of women with CVD present an increased expression of PI3K/Akt and β catenin [17] and ILK may play a key role in the activation of these components. In addition, ILK also seems to be a crucial regulator of metalloproteinases (MMPs) particularly MMP-9 [47]. MMP-9 is involved in the remodeling of the ECM and, consistently, we have previously reported an increased expression of this protein in the placenta of women with CVD [7].

Likewise, we have observed a reduced expression of e-cadherin, cadherin 17 and cadherin 6 associated to CVD. Cadherins and prominently, e-cadherin are critical components for maintaining cell attachment and the layered phenotype of the villous cytotrophoblast. On the other hand, reduced expression of cadherins is involved in the loss of cellular connectivity with a reduced apico-basal polarity [48]. Of the different cadherins analyzed in this work, e-cadherin is the most relevant and broadly studied. Prior research shows significant alterations in the expression of e-cadherin in obstetric complications, including in placenta percreta [49], placenta accrete [50], gestational trophoblastic diseases [51] and pre-eclampsia [22]. A reduced expression of e-cadherin seems to be associated with an invasive phenotype and abnormal behavior of the placental cells, hence supporting the possible pathophysiological implications of this component in CVD. A plausible explanation of the reduced expression of e-cadherin in the placenta tissue may be related to the inhibitory action of ILK through the Poly (ADP-ribose) polymerase (PARP) [52]. We have recognized that not only ILK but also PARP are upregulated in the placenta tissue of women with CVD [14] and it is possible that this mechanism explains in part the downregulation of e-cadherin.

Finally, less data are collected regarding the role of cadherin-6 and cadherin-17 in the placenta. Cadherin-6 has been identified as a pivotal molecule implicated in implantation and placentation [53,54]. On the other hand, cadherin-17 is a molecule exclusively expressed in the embryonic epithelial cells and in some parts of the adult gastrointestinal tract [55]. Our results show a significant increase of both cadherins in the placenta of women with CVD. Previous studies have described a pathological association between upregulated cadherin-6 and cadherin-17 with an increased invasiveness and cell proliferation, respectively [24]. To our knowledge, this is the first study to demonstrate a pathophysiological role of cadherin-6 and cadherin-17 in the vascular disorders affecting the placenta.

Future studies should be conducted to unravel possible causes and consequences of this dysregulation. Biochemical studies are needed that allow giving more mechanistic information, such as the Immunoblottting method and cultured placental villous explants with inhibitors of ILK or cadherin to better investigate the mechanism.

5. Conclusions

Our study demonstrates a significant increase in the protein and gene expression of ILK, cadherin-6 and cadherin-17 and a reduction of e-cadherin, associated to the development of CVD during pregnancy. Consistent with previous results, we demonstrate an abnormal functioning of the placenta in women affected by this condition, probably with negative pathophysiological implications. Future studies should be conducted in order to assess the impact of CVD in maternofetal structures and well-being.

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Data Availability Statement: The data used to support the findings of the present study are available from the corresponding author upon request.

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CONCLUSIONES

La Enfermedad Venosa Crónica es una enfermedad progresiva e invalidante presente con alta frecuencia en la población mundial. La exposición prolongada a factores de riesgo ambientales, los estilos de vida y la presencia de factores genéticos puede conducir a cambios biofísicos y bioquímicos importantes en el sistema venoso, lo que implica que la respuesta vascular es compleja. **A pesar del aumento de su aparición en las mujeres embarazadas, no se han encontrado estudios que analicen su efecto o repercusión en la gestación.** Limitándose estos estudios a la epidemiología de las misma y de sus factores de riesgo.

La presencia de venas varicosas en las mujeres que dieron a luz por vía vaginal **se asoció a un incremento del riesgo de aparición de cuadros de compromiso fetal intraparto** relacionados con la función placentaria como son: las alteraciones del equilibrio acido-base, la aparición de meconio o la presencia de infartos o malformaciones placentaria.

Finamente, **se observó que la presencia de enfermedad venosa crónica en mujeres embarazadas provoca una alteración de diversas moléculas relacionadas con procesos de remodelación tisular en las vellosidades placentarias**. Observándose un aumento de ILK, cadherina-6 y cadherina-7, asociado con una disminución de e-cadherina. Lo que revela un funcionamiento anormal de la placenta en mujeres afectadas por esta condición, probablemente con implicaciones fisiopatológicas negativas.

Por todo lo expuesto, podemos elevar nuestra hipótesis a tesis: ¨***La enfermedad venosa crónica es una patología sistémica y su aparición durante la gestación constituye un***

signo de alteraciones placentarias con repercusiones en el desarrollo o funcionamiento de la misma.¨

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ANEXOS

Discusión de las enfermedades comunes en mujeres perinatales durante el embarazo y posparto

Fundamentos：Comprender los cambios físicos de la mujer en las diferentes etapas, las diferentes enfermedades que pueden presentarse y los diversos tipos de complicaciones en el proyecto de embarazo. Nos permite prevenir mejor los diversos peligros que pueden ocurrir, para comprender mejor la situación de todo el proceso del embarazo y cuidar mejor a la madre y al recién nacido.

Objetivo：1，Cuáles son las enfermedades más frecuentes dentro la población EPP en la etapa embarazo y la etapa postparto.

2. Cuáles son las enfermedades más frecuentes dentro la población ECP en la etapa embarazo y la etapa postparto.

3. Cuáles son las comorbilidades en la población EPP más frecuentes y algunos datos básicos (coste, estancia…)

4. Cuáles son las comorbilidades en la población EVP más frecuentes y algunos datos básicos.

Métodos: se realizó un estudio transversal nacional que incluyó todas las mujeres que ingresaron al hospital en las tres etapas embarazo, parto (parto vaginal o parto cesárea) y puerperio (postparto). Luego se divide este grupo en dos categorías principales de acuerdo con diferentes métodos de parto, parto vaginal y parto cesárea, ocurridos en hospitales en España durante 2015, consulte el diagrama de flujo (grafico 1) para obtener más detalles sobre el tamaño de la muestra y las exclusiones.

Los datos de la Base de Datos de Alta Hospitalaria Centralizada (Conjunto Mínimo Básico de Datos CMBD) fueron proporcionados por el Instituto de Información Sanitaria del Ministerio de Sanidad de España. El CMBD es un registro médico administrativo obligatorio y común para todos los hospitales de España y contiene datos demográficos, información clínica y administrativa de los usuarios, los centros y unidades que atienden al paciente y su proceso asistencial, en concreto, contiene 19 variables obligatorias, siendo las más importantes la edad, el sexo, el diagnóstico principal, los diagnósticos secundarios (los diagnósticos que coexisten con diagnóstico principal en el momento del ingreso o desarrollado durante la estancia hospitalaria), procedimientos y circunstancias del alta hospitalaria. Se utilizaron códigos de la Clasificación Internacional de Enfermedades, Novena Revisión, Modificación Clínica (CIE -9 -CM) para el diagnóstico.

Población

Se seleccionaron todos los ingresos que tuvieron un CDM=14 de Embarazo-Parto-Puerperio. Posteriormente seleccionamos mujeres que fueron hospitalizadas durante las tres diferentes etapas del embarazo (EPCPost) (etapa embarazo, etapa parto y etapa postparto). Se excluyeron los ingresos por solo una de las etapas. O cualquiera de las dos etapas así como los ingresos por abortos. Luego, divídalos en dos grupos principales de acuerdo con diferentes métodos de parto. Los llamamos población parto vaginal (EPPost) y parto cesárea (ECPost). Sacar diferentes conclusiones al estudiar y comparar estos tres grupos diferentes.

Variables

Se consideraron como factores de confusión las siguientes variables: edad, hábitos tóxicos y presencia de comorbilidades. En concreto, el diagnóstico de hipertensión, patología cardíaca, enfermedad respiratoria, cáncer, enfermedades renales o hepáticas, patología tiroidea, diabetes mellitus, obesidad y alteraciones en la coagulación, así

como la presencia de depresión o demencia se consideraron para calcular el índice de comorbilidad de Charlson cada mujer. En el material complementario se proporciona una lista completa de los códigos ICD9-MC utilizados para clasificar las variables.

Se recogió información sobre la edad, hábitos tóxicos como el tabaquismo, consumo de alcohol o drogas, o la presencia de comorbilidad debido a hipertensión arterial, patología cardiaca, tiroidea, respiratoria, cáncer, enfermedades renales o hepáticas, presencia depresión o demencia. En el anexo I se proporciona una lista completa de los códigos utilizados para clasificar exposiciones, resultados, factores de confusión y modificadores de efectos. Así mismo, se calculó el índice de Charlson de comorbilidad para cada mujer (cita-sobre códigos Charlson).

Describimos las variables cualitativas mediante frecuencia absoluta y porcentajes y las cuantitativas mediante media y desviación estándar. Usamos STATA / IC (versión 16.0) para todos los análisis estadísticos restantes.

Disponibilidad de datos

La base de datos es pública, pero de acceso restringido. Pertenece al Instituto de Información Sanitaria del Ministerio de Sanidad, Asuntos Sociales e Igualdad, en donde se puede solicitar. En todo momento, los autores se hacen responsables del grado de exactitud o fiabilidad de la información derivada del fichero.

Complicaciones del embarazo

Las complicaciones del embarazo son problemas de salud que ocurren durante el embarazo. Pueden involucrar la salud de la madre, la salud del bebé o ambas. Algunas mujeres tienen problemas de salud que surgen durante el embarazo y otras mujeres tienen problemas de salud antes de quedar embarazadas que podrían provocar complicaciones. Es muy importante que las mujeres reciban atención médica antes y durante el embarazo para disminuir el riesgo de complicaciones del embarazo.

La salud materna se ha convertido en un tópico fundamental para evaluar tanto los servicios de salud como el desarrollo de una población, siendo este un indicador que hace parte de los Objetivos de Desarrollo del milenio(1). Para este tema es importante conocer tanto los diagnósticos y las atenciones realizadas durante el embarazo, el parto y el puerperio (2), así como las características principales de la población que las presenta. Estos conocimientos llevan una constante retroalimentación de los diferentes programas de salud materna, que buscan no solo reducir la mortalidad sino también las complicaciones, así como preparar y entrenar al personal que brinda las atenciones gineco-obstétricas (2).

El postparto, el de especial interés para este escrito, es de gran importancia pues diversos estudios lo referencian como un periodo crítico, donde se presenta una mayor mortalidad (3-5) razón por la cual, conocer los diagnósticos más frecuentemente presentados resulta relevante para los diferentes servicios sanitarios y de emergencias y a su vez poder hacer énfasis en la importancia de la vigilancia médica rutinaria durante las primeras 24 horas, tanto a mujeres sanas como a aquellas con comorbilidades. Los reingresos hospitalarios, también deben ser tenidos en cuenta, pues estas consultas presentan una relación con la morbi-mortalidad materna (6,7).

Los cuidados médicos en el postparto son importantes para disminuir los índices de mortalidad materna e infantil, pues las complicaciones en las primeras 24 horas pueden tornarse graves y requerir de unidades de cuidados intensivos, lo que también se asocia a un aumento de la mortalidad (8,9).

Muchas son las causas de hospitalización en mujeres embarazadas, llevando a más de 4 millones de ingresos hospitalarios solo en Estados Unidos con una mayor probabilidad de este cuando el embarazo es adolescente o en mujeres mayores por más

riesgo de presentar complicaciones ya sea en el periodo de embarazo, en el parto o por los cuidados postparto incluido cuando hay pérdida del feto (10-12), sin embargo para la población de este estudio se encontró la media de edad de 31 años para toda la muestra y de 32 años para la población Posparto, siendo rango de edad similares a los de estos estudios, pese a esto los estudios no son comparables pues entre otras razones la cobertura de seguridad social difiere en los países de estudio (12).

Dentro de las comorbilidades más severas se encuentran la hemorragia, el embolismo, falla renal aguda, ataque cerebral, IAM, entre otras siendo causas directas del aumento de los costos en la hospitalización y de la estancia hospitalaria, esto de la mano del incremento en el número de cesáreas practicadas (11,13). En este sentido la hemorragia posparto no solo es una causa de morbilidad sino también de mortalidad de forma directa e indirecta, pues es un factor de riesgo para desarrollar sepsis y complicaciones cardiacas (14,15). Fumar durante el embarazo también se asocia con el riesgo de presentar hemorragia, bajo peso al nacer, complicaciones respiratorias y fallo orgánico de la madre y el recién nacido (16) .

La anemia es una enfermedad frecuente durante el embarazo y aunque se puede tratar muestra una fuerte asociación con las posibles complicaciones durante el parto y el posparto, favoreciendo incluso la presencia de hemorragia (17).

Dentro de la literatura se encuentra documentada la depresión durante el embarazo y los primeros seis meses de postparto como altamente prevalente llegando hasta el 15% pese a esto en la selección de este estudio no se han encontrado casos lo que supondría un impacto positivo puesto que esta depresión se asocia con problemas en la salud mental de los menores recién nacidos(18). Se ha encontrado asociación entre el dolor post cesárea, la lactancia materna exclusiva y la depresión postparto, además de un aumento en el coste hospitalario(19).

En este estudio la principal atención estuvo relacionada con la hemorragia en el postparto, independiente de la edad de las participantes, lo que se relaciona con la literatura actual, que referencia esta complicación como una de las principales causas tanto de complicaciones obstétricas como de mortalidad materna.

A pesar de no contar con más variables sociodemográficas, que permitan caracterizar a la población, la literatura ha mostrado que tanto en países desarrollados como aquellos en desarrollo, la hemorragia bien sea en parto vaginal o en cesárea, continúa siendo de las principales complicaciones(20,21); sin embargo, en los países desarrollados se cuenta con servicios obstétricos más oportunos que permiten en muchos casos prevenir las complicaciones del embarazo y realizar un parto programado, disminuyendo las complicaciones de la cesárea de emergencia(22). Otro aspecto a tener en cuenta, es el número de controles en el embarazo, dato que se sugiere correlacionar en futuras revisiones.

Las heridas por cirugía obstétrica y sus posibles complicaciones, tales como infección, disrupción y sangrado, también son de interés, ya sea las causadas por la cesárea o de origen perineal, pues se documentan lesiones de este tipo en alrededor del 50% de las mujeres(23,24). Este diagnóstico y su severidad pueden acarrear disrupciones funcionales futuras para la mujer, generando incontinencia, dificultades sexuales y afectaciones emocionales (25).

Estas principales causas de consulta postparto también representan un porcentaje importante como motivo de atención hospitalaria. En este estudio la hemorragia representa el 22.17% y 14.47% de las consultas en parto vaginal y parto cesárea respectivamente. Además, la infección puerperal grave representa el 14.93% y 15.79% de los diagnósticos en los partos vaginal y de cesárea respectivamente. La infección

también puede presentarse como Fiebre de origen desconocido durante el puerperio y Endometritis puerperal, diagnósticos de origen infeccioso que requiere tratamiento farmacéutico, asociadas en su mayoría al procedimiento quirúrgico de cesárea, cuando las bacterias de la flora vaginal contaminan o se diseminan a diferentes áreas(26,27), pero también pueden estar asociadas a otras causas de más difícil diagnóstico relacionado con el propio estado de salud de la mujer pero enmascarado en el mismo proceso de embarazo y puerperio(28,29). Al descartar estas causas de fiebre se encuentran las infecciones de la mama y el pezón que se presentaron durante el posparto vaginal y por cesárea en 4.52% y 6.58% de las mujeres respectivamente, teniendo repercusiones para el bebé pues la lactancia materna exclusiva les protege de presentar infecciones respiratorias, gastroenteritis, obesidad en la niñez, entre otras, estando aun en niveles subóptimos, siendo la mastitis una de las causas para cesar la lactancia, por lo que conocer sus causas y tratarla es prioritario(30-32).

En conclusión, los resultados de este trabajo muestran la importancia que siguen representando las complicaciones postparto, tales como hemorragias e infecciones principalmente. Para formular una relación causal existe la necesidad de estudiar otros factores que pueden afectar y modificar los efectos, teniendo en consideración factores ambientales, fisiológicos y los relacionados con la atención recibida, así como antecedentes gineco-obstétricos.

Tabla 1					
	(ECP)	**(EVP)**	**(EVCP)**	**Población general**	**P**
N participantes (%)	474 (40.93)	684 (59.07)	1158	382169	
Coste -€ (SD)	3121.44 (988.02)	2432.46 (399.99)	2714.48 (779.99)	2613.26 (828.77)	<0.001
Coste (total)	1479564	1663802	3143365		
Edad –años (SD)	32.29 (6.07)	30.12 (6.83)	31.00 (6.61）	31.82 (5.71)	<0.001
Estancia –días (SD)	5.31 (6.11)	3.66 (5.50)	4.34 (5.81）	3.05 (2.61)	<0.001
N con IVC	0	0		3,234 (0.85)	
N con varices pélvicas	0	0		34 (0.01)	
N con varices vulvares	0	0		304 (0.08)	
N con hemorroide	0	0		2,167 (0.57)	
Comorbilidades:					
VIH/SIDA	2 (0.42)	0	2 (0.17)	160 (0.04)	<0.001
Abuso de Alcohol	0	0	0	4 (0.00)	
Anemia	7 (1.48)	27 (3.95)	34（2.94）	5,653 (1.48)	<0.001
Asma	8 (1.69)	14 (2.05)	22（1.90）	7,046 (1.84)	<0.001
Cáncer	2 (0.42)	6 (0.88)	8（0.69）	182 (0.05)	<0.001
Cerebro Vascular	2（0.42）	0	2（0.17）	117 (0.03)	<0.001
EPOC	9 (1.90)	17 (2.49)	26（2.25）	7,177 (1.88)	<0.001
Coagulopatía	4 (0.84)	13 (1.90)	17（1.47）	2,295 (0.60)	<0.001
Falla Cardiaca Cong	1（0.21）	1（0.15）	2（0.17）	99 (0.03)	
Demencia	0	0	0	3 (0.00)	
Depresión	0	0	0	3 (0.00)	
Diabetes Mellitus	13 (2.74)	17 (2.49)	30（2.59）	19,175 (5.02)	<0.001
Abuso de Drogas	0	1(0.15)	1（0.09）	346 (0.09)	
HTA	80 (16.88)	50 (7.31)	130 (11.23)	14,991 (3.92)	<0.001
Hipotiroidismo	34 (7.17)	39 (5.70)	73 (6.30)	24,685 (6.46)	<0.001
Enferm Hepática	0	3(0.44)	3（0.26）	853 (0.22)	<0.001
Compromiso fetal	0	0	0	0	<0.001
Embarazo Múltiple	30 (6.33)	21 (3.07)	51 (4.40)	6,834 (1.79)	<0.001
IAM	0	0	0	19 (0.00)	<0.001
Malformación Recién Nacido	59 (12.45)	43 (6.29)	102 (8.81)	66,354 (17.36)	<0.001
Obesidad	2（0.42）	7(1.02)	9(0.78)	7,250(1.90)	<0.001
Parálisis	0	0	0	57(0.01)	<0.001
Úlcera péptica	0	0	0	53(0.01)	<0.001
Enfermedad Vascular Periférica	0	0	0	31(0.01)	<0.001
Desorden Vascular Periférica	0	0	0	31(0.01)	<0.001
Enfermedad Renal	4(0.84)	0	4(0.35)	151(0.04)	<0.001

Enferm. Reumática	3(0.63)	6(0.88)	9(0.78)	780(0.20)	<0.001
Tabaquismo	32(6.75)	29(4.24)	61(5.27)	24,837(6.50)	<0.001
Venas Varicosas en MMII o pelvis	0	0	0	34(0.01)	
varicesmmiivh	0	0	0	3,234(0.85)	
Bajo peso y gestación acortada de recién nacido	0	0	0	5(0.00)	
Peso elevado y gestación prolongada	0	0	0	5(0.00)	
Pérdida de peso	0	0	0	0	
Enf. especificada con origen en el periodo perinatal (Solo sistema circulatorio)	0	0	0	17(0.00)	
Hipoxia intrauterina y asfixia intraparto	0	0	0	2(0.00)	

Tabla 2				
	Posparto (ECP)	**Posparto (EVP)**	**Posparto (ECVP)**	**Población general Posparto**
N participantes (%)	152 (32.07)	221 (32.31)	373 (32.21)	5,067（1.33）
Coste -€ (SD)	2627.36 (334.57)	2664.04 (348.95)	2649.09 (343.18)	2647.27（342.04）
Coste (total)	399358.3	588753.5	988111.7	
Edad –años (SD)	32.38 (6.06)	30.46 (6.77)	31.24（6.54）	32.13（5.80）
Estancia –días (SD)	5.03 (4.50)	4.13 (6.75)	4.49（5.95）	3.74（4.24）
N con IVC	0	1(0.45)	1（0.27）	27（0.53）
N con varices pélvicas	0	0	0	0
N con varices vulvares	0	0	0	2（0.04）
N con hemorroide	1（0.45）	0	1（0.27）	23（0.45）
Comorbilidades:				
VIH/SIDA	0	0	0	1（0.02）
Abuso de Alcohol	0	0	0	0
Anemia	0	14 (6.33)	14（3.75）	113(2.23)
Asma	4(2.63)	5 (2.26)	9（2.41）	82(1.62)
Cáncer	1(0.66)	2(0.9))	3（0.80）	14(0.28)
Cerebro Vascular	1(0.66)	0	1（0.27）	31(0.61)
EPOC	5(3.29)	6(2.71)	11（2.95）	90(1.78)
Coagulopatía	0	4(1.81)	4（1.07）	33(0.65)
Falla Cardiaca Cong	1(0.66)	1(0.45)	2（0.54）	21(0.41)
Demencia	0	0	0	0
Depresión	0	0	0	0
Diabetes Mellitus	0	0	0	33(0.65)

Abuso de Drogas	0	0	0	10(0.20)
HTA	23(15.13)	9(4.07)	32（8.58）	259(5.11)
Hipotiroidismo	7(4.61)	9(4.07)	16（4.29）	177(3.49)
Enferm Hepática	0	1(0.45)	1（0.27）	21(0.41)
Compromiso fetal	0	0	0	0
Embarazo Múltiple	0	0	0	0
IAM	0	0	0	1（0.02）
Malformación Recién Nacido	2(1.32)	2(0.9)	4（1.07）	46(0.91)
Obesidad	1(0.66)	1(0.45)	2（0.54）	62(1.22)
Parálisis	0	0	0	5(0.01)
Úlcera péptica	0	0	0	3(0.06)
Enfermedad Vascular Periférica	0	0	0	0
Desorden Vascular Periférica	0	0	0	0
Enfermedad Renal	1 (0.66)	0	1（0.27）	4 (0.08)
Enfermedad Reumática	1 (0.66)	1 (0.45)	2（0.54）	16 (0.32)
Tabaquismo	7(4.61)	9(4,07)	16（4.29）	202(3.99)
Venas Varicosas en MMII o pelvis	0	0	0	0
varicesmmiivh	0	1(0.45)	1（0.27）	27（0.53）
Bajo peso y gestación acortada de recién nacido	0	0	0	0
peso elevado y gestación prolongada	0	0	0	0
Pérdida de peso	0	0	0	0
Enfermedad especificada con origen en el periodo perinatal (solo sistema circulatorio)	0	0	0	0
Hipoxia intrauterina y asfixia intraparto	0	0	0	0

Tabla 3				
	Embarazo (ECP)	**Embarazo (EVP)**	**Embarazo (ECVP)**	**Embarazo Población general**
N participantes (%)	179	252	431	38,152
Coste -€ (SD)	2457.62 (315.80)	2329.80 (445.80)	2382.88（401.58）	2404.63 (421.33)
Coste (total)	439914.6	587108.5	1027023	
Edad –años (SD)	31.98 (6.16)	29.71 (6.93)	30.65（6.71）	31.01(6.28)
Estancia –días (SD)	3.93 (4.79)	3.44 (5.50)	3.65（5.22）	3.39(4.08)
N con IVC	0	0	0	86 (0.23)
N con varices pélvicas	0	0	0	0
N con varices vulvares	0	0	0	12 (0.03)
N con hemorroide	0	0	0	42 (0.11)
Comorbilidades:				
VIH/SIDA	1（0.56）	0	1（0.23）	19(0.05)
Abuso de Alcohol	0	0	0	1(0.00)
Anemia	1（0.56）	2(0.79)	3（0.70）	310(0.81)
Asma	1（0.56）	2(0.79)	3（0.70）	893(2.34)
Cáncer	0	2(0.79)	2（0.46）	37(0.10)
Cerebro Vascular	0	0	0	17(0.04)
EPOC	1(0.56)	3(1.19)	4（0.93）	925(2.42)
Coagulopatía	3（1.68）	5(1.98)	8（1.86）	207(0.54)
Falla Cardiaca Cong	0	0	0	16(0.04)
Demencia	0	0	0	1(0.00)
Depresión	0	0	0	1(0.00)
Diabetes Mellitus	7（3.91）	6（2.38）	13（3.02）	1,288(3.38)
Abuso de Drogas	0	0	0	52(0.14)
HTA	27(15.08)	21(8.33)	48（11.14）	2,129(5.58)
Hipotiroidismo	18(10.06)	12(4.76)	30（6.96）	1,923(5.04)
Enferm Hepática	0	1(0.40)	1（0.23）	104(0.27)
Compromiso fetal	0	0	0	0
Embarazo Múltiple	0	0	0	18(0.05)
IAM	0	0	0	4(0.01)
Malformación Recién Nacido	14(7.82)	8(3.17)	22（5.10）	2,070(5.43)
Obesidad	0	2(0.79)	2（0.46）	521(1.37)
Parálisis	0	0	0	10(0.03)
Úlcera péptica	0	0	0	8(0.02)
Enfermedad Vascular Periférica	0	0	0	5(0.01)
Desorden Vascular Periférica	0	0	0	0
Enfermedad Renal	1(0.56)	0	1（0.23）	27(0.07)
Enferm. Reumática	1(0.56)	3(1.19)	4（0.93）	101(0.26)

Tabaquismo	18(10.06)	8(3.17)	26（6.03）	2,346(6.15)
Venas Varicosas en MMII o pelvis	0	0	0	0
varicesmmiivh	0	0	0	86(0.23)
bajo peso y gestación acortada de recién nacido	0	0	0	2(0.01)
peso elevado y gestación prolongada	0	0	0	0
Pérdida de peso	0	0	0	0
Enfermedad especificada con origen en el periodo perinatal (solo sist circulatorio)	0	0	0	2(0.01)
Hipoxia intrauterina y asfixia intraparto	0	0	0	0

Tabla 4. tab dx1 if (ECPost ==1)&(CDM14==4),sort EMBARAZO			
Diagnóstico 1 ***Cie-9**** **(n (%)**	freq	percent	**Descripción**
644	54	30.17	Parto prematuro o amenaza de parto prematuro
642	22	12.29	Hipertensión que complica embarazo, parto y puerperio
646	22	12.29	Otras complicaciones del embarazo no clasificadas bajo otros conceptos
641	18	10.06	Hemorragia anteparto, desprendimiento de placenta y placenta previa
648	14	7.82	Otras enfermedades actuales de la madre clasificables bajo otros conceptos, pero que complican el embarazo, parto o puerperio
640	13	7.26	Hemorragia en fase temprana de embarazo
643	8	4.47	Vómitos excesivos en el embarazo
658	8	4.47	Otros problemas asociados con la cavidad y membranas amnióticas
654	6	3.35	Anomalías de los órganos y tejidos blandos de la pelvis
656	3	1.68	Otros problemas fetales y de placenta conocidos o sospechados que afectan al tratamiento de la madre
661	3	1.68	Anomalías de las contracciones uterinas

Tabla 5. tab dx1 if (EPPost ==1)&(CDM14==4),sort EMBARAZO			
Diagnóstico 1 ***Cie-9**** **(n (%))**	freq	percent	**Descripción**
644	102	40.48	Parto prematuro o amenaza de parto prematuro
646	47	18.65	Otras complicaciones del embarazo no clasificadas bajo otros conceptos
648	26	10.32	Otras enfermedades actuales de la madre clasificables bajo otros conceptos, pero que complican el embarazo, parto o puerperio
642	14	5.56	Hipertensión que complica embarazo, parto y puerperio
641	12	4.76	Hemorragia anteparto, desprendimiento de placenta y placenta previa
640	10	3.97	Hemorragia en fase temprana de embarazo
643	10	3.97	Vómitos excesivos en el embarazo
658	7	2.78	Otros problemas asociados con la cavidad y membranas amnióticas

661	7	2.78	Anomalías de las contracciones uterinas
649	4	1.59	Otras enfermedades o estados de la madre que complican el embarazo, parto y puerperio
652	4	1.59	Situación y presentación anómala del feto

Tabla 6. tab dx1 if (EPPost ==1)&(CDM14==5),sort POSTPARTO			
Diagnóstico 1 ***Cie-9**** **(n (%))**	freq	percent	**Descripción**
666	49	22.17	Hemorragia postparto
670	33	14.93	Infección puerperal grave
667	25	11.31	Placenta o membranas retenidas, sin hemorragia
648	22	9.95	Otras enfermedades actuales de la madre clasificables bajo otros conceptos, pero que complican el embarazo, parto o puerperio
646	18	8.14	Otras complicaciones del embarazo no clasificadas bajo otros conceptos
672	17	7.69	Fiebre de origen desconocido durante el puerperio
674	15	6.79	Otras complicaciones y complicaciones no especificadas del puerperio, no clasificadas bajo otros conceptos
675	10	4.52	Infecciones de la mama y del pezón asociadas con el parto
642	6	2.17	Hipertensión que complica embarazo, parto y puerperio
V24	6	2.17	Cuidados y examen postparto

Tabla 7. tab dx1 if (ECPost ==1)&(CDM14==5),sort POSTPARTO			
Diagnóstico 1 ***Cie-9**** **(n (%))**	freq	percent	**Descripción**
674	45	29.61	Otras complicaciones y complicaciones no especificadas del puerperio, no clasificadas bajo otros conceptos
670	24	15.79	Infección puerperal grave
666	22	14.47	Hemorragia postparto
642	12	7.89	Hipertensión que complica embarazo, parto y puerperio
672	11	7.24	Fiebre de origen desconocido durante el puerperio
675	10	6.58	Infecciones de la mama y del pezón asociadas con el parto

646	7	4.61	Otras complicaciones del embarazo no clasificadas bajo otros conceptos
648	7	4.61	Otras enfermedades actuales de la madre clasificables bajo otros conceptos, pero que complican el embarazo, parto o puerperio
667	4	2.63	Placenta o membranas retenidas, sin hemorragia
V24	3	1.97	Cuidados y examen postparto

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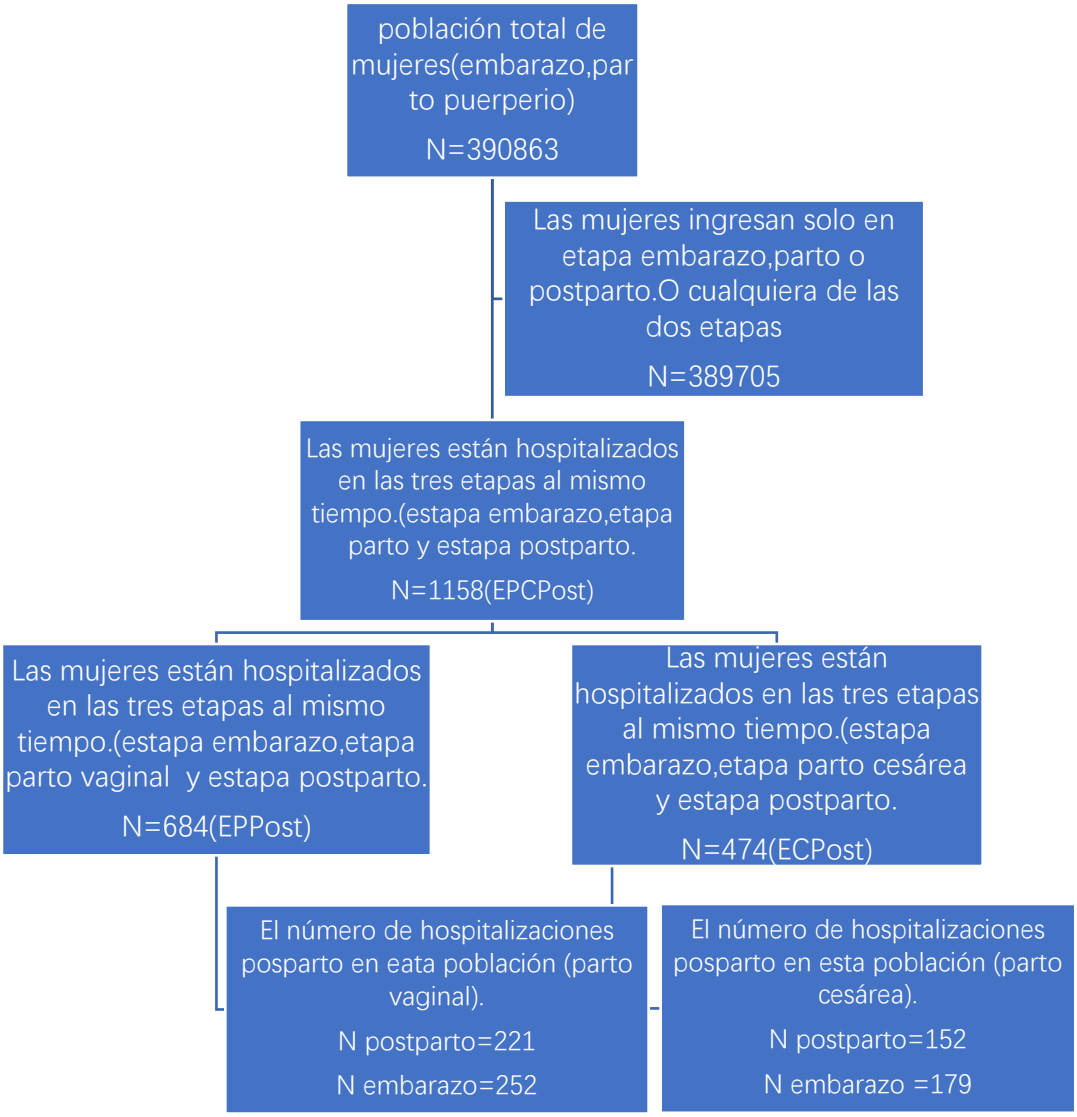
Grafico 1 Diagrama de flujo de la población del estudio



CEAP: clinical–etiology–anatomy–pathophysiology; CVD: Chronic venous disease.

TABLA 1. Last revision of CEAP classification on CVD.

Table 1. Last revision of CEAP classification on CVD.

Clinical (C) Classification		Etiologic (E) Classification		Anatomic (A) Classification		Pathophysiologic (P) Classification	
				As	Superficial		
				Old	New Description		
				1.	Tel Telangiectasia		
				1.	Ret Reticular veins		
				2.	GSVa Great saphenous vein above knee		
				3.	GSVb Great saphenous vein below knee		
				4.	SSV Small saphenous vein		
				AASV	Anterior accessory saphenous vein		
				5.	NSV Nonsaphenous vein		
				Ad	Deep		
				6.	IVC Inferior vena cava		
				7.	CIV Common iliac vein		
				8.	IIV Internal iliac vein		
		Ep	Primary	9.	EIV External iliac vein		
		Es	Secondary	10.	PELV Pelvic veins	Pr	Reflux
C0	No visible or palpable signs of venous disease	Esi	Secondary—intravenous	11.	CFV Common femoral vein	Po	Obstruction
C1	Telangiectasias or reticular veins	Ese	Secondary—extravenous	12.	DFV Deep femoral vein	Pr,o	Reflux and obstruction
C2	Varicose veins	Ec	Congenital	13.	FV Femoral vein	Pn	No pathophysiology identified
C2r	Recurrent varicose veins	En	No cause identified	14.	POPV Popliteal vein		
C3	Edema			15.	TIBV Crural (tibial) vein		
C4	Changes in skin and subcutaneous tissue secondary to CVD			15.	PRV Peroneal vein		
C4a	Pigmentation or eczema			15.	ATV Anterior tibial vein		
C4b	Lipodermatosclerosis or atrophie blanche			15.	PTV Posterior tibial vein		
C4c	Corona phlebectatica			16.	MUSV Muscular veins		
C5	Healed			16.	GAV Gastrocnemius vein		
C6	Active venous ulcer			16.	SOV Soleal vein		
C6r	Recurrent active venous ulcer			Ap	Perforator		
				17.	TPV Thigh perforator vein		
				18.	CPV Calf perforator vein		
				An			
				No venous anatomic location identified			

CEAP: clinical–etiology–anatomy–pathophysiology; CVD: Chronic venous disease.

Gráfico 2. Flowchart for the study population

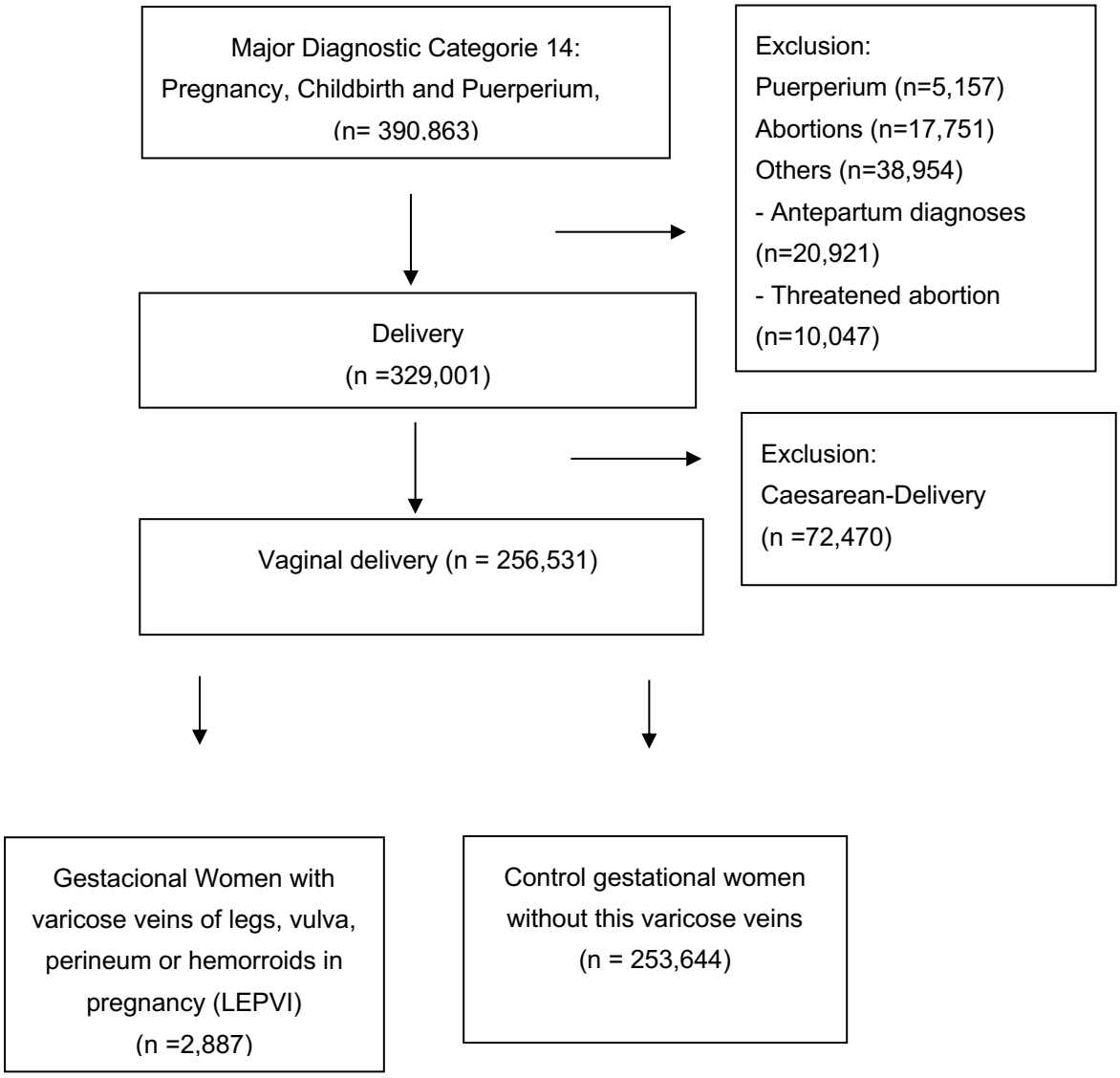


Tabla 1S: Códigos de la Clasificación Internacional de Enfermedades, Novena Revisión, Modificación Clínica (CIE 9 CM) utilizados para el diagnóstico

VARIABLE	**CODES IDC9-CM (2014 REV)**
AIDS/HIV	042x
Alcohol abuse	265.2, 291.1-291.3,291.5-291.9, 303.0, 303.9, 305.0, 357.5, 425.5, 535.3, 571.0-571.3, 980.x, V11.3
Anemia	280.0-280.9, 281.x
Asthma	493x
Cancer	200.x-202.x, 203.0, 196.x-199.x, 140.x-195.x,
Cerebrovascular disease	362.34, 430.x-438.x
Chronic pulmonary disease	416.8, 416.9, 490.x--493x, 494x-505.x, 506.4, 508.1, 508.8
Coagulopathy	286.x, 287.1, 287.3-287.5
Congestive heart failure	398.91, 402.01, 402.11, 402.91, 404.01, 404.03, 404.11, 404.91, 404.93, 425.4-425.9, 428.x
Dementia	290.x, 294.1, 331.2
Depression	296.2, 296.3, 296.5, 300.4, 309.0, 309.1, 301.12, 311
Diabetes	250.0- 250.9, 648.0, 648.8
Drug abuse	292.x, 304.x, 305.2-305.9, V65.42
Hypertension	401.x, 402.x-405.x 642.x
Hypothyroidism	240.9, 243.x, 244.x, 246.1, 246.8
Liver disease	070.22, 070.23, 070.32, 070.33, 070.44, 070.54, 070.6, 070.9, 456.0-456.2, 570.x, 571.x, 572.2-572.8, 573.3, 573.4, 573.8, 573.9, V42.7
Intrapartum fetal compromise	656.3, 656.7, 656.8
Multiple pregnancy	v27.2-v27.7
Myocardial infarction	410.x, 412.x
Newborn malformation (nm)	655x , 656x , 740x-759x
Obesity	278.0
Paralysis	334.1, 342.x, 343.x, 344.0-344.6, 344.9
Peptic ulcer disease excluding bleeding	531.x-534.x
Peripheral vascular disease	093.0, 437.3, 440.x, 441.x, 443.1-443.9, 557.1, 557.9, V43.4
Peripheral vascular disorders	093.0, 437.3, 440.x, 441.x, 443.1-443.9, 447.1, 557.1, 557.9, V43.4
Renal disease	403.01, 403.11, 403.91, 404.02, 404.03, 404.12, 404.13, 404.92, 404.93,582x, 583.0-583.7, 585.x, 586.x, 588.0, V42.0, V45.1, V56.x
Rheumatic disease	446.5, 710.0-710.4, 714.0-714.2, 714.8, 725.x
Smoke	989.84, 649.0, 305.1, V15.82
Varicose veins in the lower extremities or pelvis (LEPVI)	671.0, 671.1, 671.8