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Pharmacologie de la microcirculation : phénomène de Raynaud, troubles trophiques cutanés et hypertension artérielle pulmonaire

Charles Khouri

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Charles Khouri. Pharmacologie de la microcirculation : phénomène de Raynaud, troubles trophiques cutanés et hypertension artérielle pulmonaire. Biotechnologies. Université Grenoble Alpes, 2019. Français. NNT : 2019GREAS028 . tel-03092263

HAL Id: tel-03092263

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THÈSE

Pour obtenir le grade de

DOCTEUR DE LA COMMUNAUTE UNIVERSITE GRENOBLE ALPES

Spécialité : **Biotechnologie, instrumentation, signal et imagerie
pour la biologie, la médecine et l'environnement**

Arrêté ministériel : 25 mai 2016

Présentée par

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Thèse dirigée par le Pr **Jean-Luc Cracowski**,

et préparée au sein du **Laboratoire HP2 – Inserm U1042**
dans **l'École Doctorale Ingénierie pour la Santé, la Cognition et
l'Environnement**

Pharmacologie de la microcirculation : phénomène de Raynaud, troubles trophiques cutanés et hypertension artérielle pulmonaire

Thèse soutenue publiquement le **12 décembre 2019**,
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Résumé

La microcirculation désigne le sous-ensemble du système circulatoire où s'effectuent les échanges gazeux et liquidiens extracellulaires. Elle est composée des artérioles, des capillaires et des veinules. Plusieurs pathologies sont induites par une atteinte structurelle et/ou fonctionnelle primaire de cette microcirculation : le phénomène de Raynaud (PR), les troubles trophiques vasculaires et l'hypertension artérielle pulmonaire (HTAP). Les objectifs de ce travail sont d'étudier, de comprendre et d'identifier de nouvelles étiologies iatrogènes à ces pathologies microvasculaires, ainsi que d'évaluer et de comparer l'efficacité et la sécurité des traitements utilisés dans ces pathologies. Nous avons, à cette fin, réalisé plusieurs études à partir des bases de données de pharmacovigilances, de données d'essais cliniques et de la littérature.

Ce travail de thèse nous a permis d'explorer le rôle des médicaments dans ces pathologies microvasculaires, champs qui restait encore peu étudié dans la littérature. Ces travaux nous ont permis d'identifier de nombreuses classes pharmacologiques dont le rôle était encore non décrit dans ces pathologies. L'étude des mécanismes pharmacologiques à l'origine de ces effets indésirables permet également d'émettre de nouvelles hypothèses physiopathologiques à l'origine de ces maladies.

Les traitements utilisés dans ces différentes pathologies microcirculatoires sont à l'heure actuelle encore peu spécifiques et des travaux de recherche important doivent encore être réalisés afin de personnaliser la prise en charge des patients.

Mots clés : Phénomène de Raynaud, ulcères cutanés, hypertension artérielle pulmonaire, pharmacovigilance

Abstract

Pharmacology of microcirculation: Raynaud's phenomenon, trophic diseases and pulmonary arterial hypertension

Microcirculation refers to the subset of the circulatory system where extracellular gas and fluid exchanges take place. It is composed of arterioles, capillaries and venules. Several pathologies are induced by a primary structural and/or functional impairment of this microcirculation: Raynaud's phenomenon (RP), trophic vascular disorders and pulmonary arterial hypertension (PAH). The objectives of this work are to study, understand and identify new iatrogenic etiologies to these microvascular diseases, as well as to evaluate and compare the effectiveness and safety of treatments used in these diseases. We therefore conducted several studies using pharmacovigilance databases, clinical trial data and the literature.

This thesis work allowed us to explore the role of drugs in these microvascular pathologies, fields that were poorly studied in the literature yet. This work has allowed us to identify many pharmacological classes whose role was unknown in these diseases. The study of the pharmacological mechanisms underlying these adverse drug reactions also makes it possible to develop new pathophysiological hypotheses underlying these diseases.

The treatments used in these different microvascular diseases are currently not specific and important research work still needs to be carried out in order to personalize patient care.

Keywords: Raynaud's phenomenon, trophic vascular disorders, pulmonary arterial hypertension, drug safety

Remerciements

A M. le Professeur Jean-Louis Montastruc, merci d'avoir accepté de participer à ce jury. Votre présence est un immense honneur.

A M. les Professeurs Antoine Pariente et Michel Cucherat, merci beaucoup d'avoir accepté d'être les rapporteurs de ce travail. Vos travaux inspirent les miens chaque jour.

A Mme. le Professeur Sophie Blaise, merci Sophie d'avoir accepté de juger ce travail ainsi que pour tes nombreuses contributions tout au long de cette thèse.

A M. le Docteur Matthieu Roustit, quel plaisir de travailler avec toi. Merci pour tes innombrables remarques, relectures, idées qui ont largement participé à ces travaux.

A M. le Professeur Jean-Luc Cracowski, merci Jean-Luc pour la liberté et la confiance que tu as su me donner pendant ces 3 années. J'espère un jour arriver à encadrer mes étudiants avec autant de justesse.

Merci à tous les collègues et étudiants qui ont participé à ces travaux : Marion, Bruno, Sébastien, Zaza, Lucie, Dorothee et Camille.

Je remercie également tous ceux qui travaillent à mes côtés au CIC, au CRPV et à HP2.

A Pauline et Arthur, mes bonheurs quotidiens.

A ma famille, pour tout ce qu'on partage et partagera avec les nouveaux venus.

A mes amis pour tous les bons moments passés ensemble.

Et à tous ceux qui auront le courage de lire ce travail, qui je l'espère y trouveront un intérêt.

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ABREVIATIONS

GCs : guanylate cyclase soluble.

HTAP : hypertension artérielle pulmonaire

NO : monoxyde d'azote

PGI₂ : prostacycline

PR : phénomène de Raynaud

SDR : signaux de disproportionnalité

SSc : sclérodémie systémique

SSc-PR : phénomène de Raynaud associé à la sclérodémie

PARTIE 1. INTRODUCTION

1. Epidémiologie et physiopathologie des pathologies microvasculaires

La microcirculation désigne le sous-ensemble hétérogène du système circulatoire où s'effectuent les échanges gazeux et liquidiens extracellulaires. Elle est composée des artérioles, des capillaires et des veinules non visibles à l'œil nu, soit inférieurs à une taille de 150 µm. Ces petits vaisseaux, disséminés dans tous les tissus, forment un réseau qui se situe entre le système artériel et le système veineux. (1) Ce réseau microvasculaire joue un rôle primordial dans les échanges gazeux et nutritionnels, et dans la diapédèse leucocytaire. (2)

Une dysfonction microcirculatoire généralisée est retrouvée dans de nombreuses pathologies cardio-vasculaires, notamment dans le diabète et l'hypertension artérielle mais aussi dans les chocs septiques, elle est également retrouvée physiologiquement au cours du vieillissement. (2-4) Cependant, plusieurs pathologies sont induites par une atteinte structurelle et/ou fonctionnelle primaire de cette microcirculation : le phénomène de Raynaud, les troubles trophiques vasculaires et l'hypertension artérielle pulmonaire.

Le Phénomène de Raynaud

Le phénomène de Raynaud a été décrit pour la première fois en 1862 par Maurice Raynaud comme une asphyxie locale des extrémités. (5) Ce phénomène correspond en fait à vasoconstriction paroxystique, anormale, des extrémités, en réponse à un stress environnemental (généralement le froid, plus rarement l'humidité) ou émotionnel. (6) Il se manifeste typiquement par un changement de couleur des doigts, qui passent du blanc (phase de vasoconstriction excessive), au bleu (phase de cyanose tissulaire), puis au rouge (phase de reperfusion). Cette phase rouge est généralement douloureuse. Les trois phases ne sont pas systématiquement observées chez un même patient et ne sont pas indispensables au diagnostic. Chaque épisode peut durer de quelques minutes à plusieurs heures, la durée moyenne étant de l'ordre de 30 minutes. (7,8)

Le phénomène de Raynaud peut être idiopathique, également appelé primaire ; il est généralement bénin, c.à.d. n'entraînant pas de troubles trophiques, mais est à l'origine d'une gêne fonctionnelle parfois importante. Il touche 3 à 5 % de la population générale, avec une large prédominance féminine et des variations géographiques importantes. (9) Le phénomène de Raynaud peut également être secondaire à des pathologies auto-immunes comme des

connectivites (notamment la sclérodémie systémique (SSc) ou le lupus érythémateux disséminé), à une compression vasculaire, une artériopathie, une hyperviscosité sanguine ou à certains médicaments. (10) Beaucoup plus rare, le phénomène de Raynaud secondaire expose à des complications parfois graves, notamment chez les patients atteints de SSc, chez qui l'apparition d'ulcères digitaux est associée à une morbidité importante et représente la principale gêne fonctionnelle. (11)

La physiopathologie du phénomène de Raynaud est complexe et multifactorielle, mêlant une dysfonction de l'endothélium vasculaire, une altération des mécanismes de contrôles neuronaux du tonus vasculaire et de facteurs intravasculaires (Figure 1).

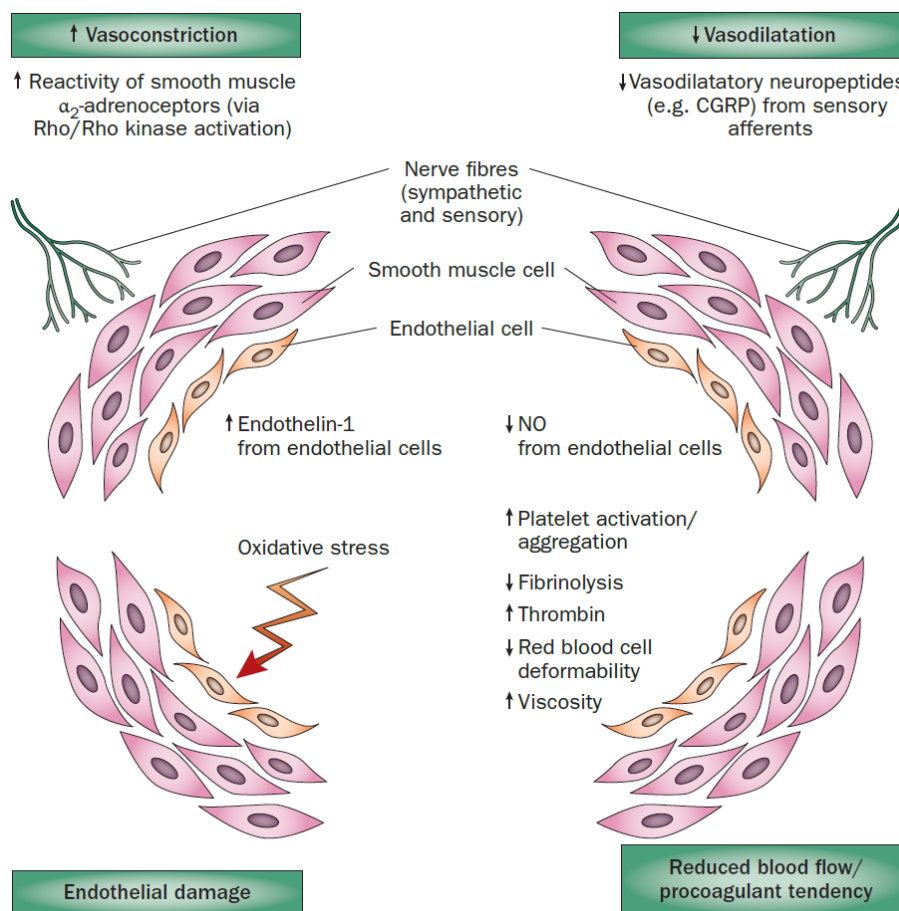


Figure 1. Représentation schématique des principaux éléments et mécanismes contribuant à la pathogenèse du phénomène de Raynaud. Extrait de (Herrick et al, Nat Rev Rheumatol. 2012) (10)

Les troubles trophiques cutanés

Sous le terme de troubles trophiques sont regroupées diverses atteintes engendrées par une insuffisance vasculaire artérielle ou veineuse, provoquant une diminution de la perfusion cutanée et à terme l'apparition d'ulcères. Les ulcères cutanés sont définis comme une lésion de la peau qui peut s'étendre jusqu'au tissu sous-cutané ou même jusqu'au niveau du muscle ou de l'os. En fonction de leur mécanisme physiopathologique les ulcères cutanés peuvent être classés en 3 groupes principaux : les ulcères de pression, les ulcères vasculaires (veineux ou artériels) et les ulcères neuropathiques ou diabétiques. (12) La prévalence des ulcères varie de façon importante en fonction de la population et du type d'ulcère. Les ulcères de pieds diabétiques, par exemple, affectent approximativement 25% de la population diabétique au cours de leur vie et représentaient 11% des ulcères en France en 2012. (13) La prévalence des ulcères de pression, quant à elle, est estimée entre 7.3 et 20% chez les personnes âgées. (14) Actuellement, 25 à 50% des ulcères cutanés ne sont pas cicatrisés après 6 mois de traitement optimal. Cette chronicisation affecte de façon importante la qualité de vie des patients et représente des coûts significatifs pour les systèmes de soins. (15)

L'hypertension artérielle pulmonaire

L'hypertension artérielle pulmonaire (HTAP) correspond au premier sous-groupe des hypertensions pulmonaires. Cette pathologie affecte la circulation artérielle, les artéριοles pré-capillaires et veineuse pulmonaire, ainsi que le ventricule droit. (16) L'HTAP est définie par une pression artérielle pulmonaire moyenne au repos supérieure à 20mmHg et une résistance vasculaire pulmonaire supérieure à 3 unités Wood. (17) Plusieurs étiologies peuvent l'engendrer, notamment des causes génétiques (mutation du gène BMPR2), des pathologies systémiques (connectivites, VIH, hypertension portale...), des causes iatrogènes (amphétamines, benfluorex, interférons...) ou idiopathiques. (17) L'HTAP est une pathologie rare avec une prévalence estimée dans la population générale entre 11 et 26 cas par millions d'habitants, avec une large prédominance féminine. Le pronostic de patients atteint d'HTAP s'est largement amélioré ces dernières années mais cela reste une pathologie sévère, la médiane de survie étant actuellement de 6 ans. (16)

La physiopathologie, illustrée Figure 2, est également très complexe et multifactorielle.

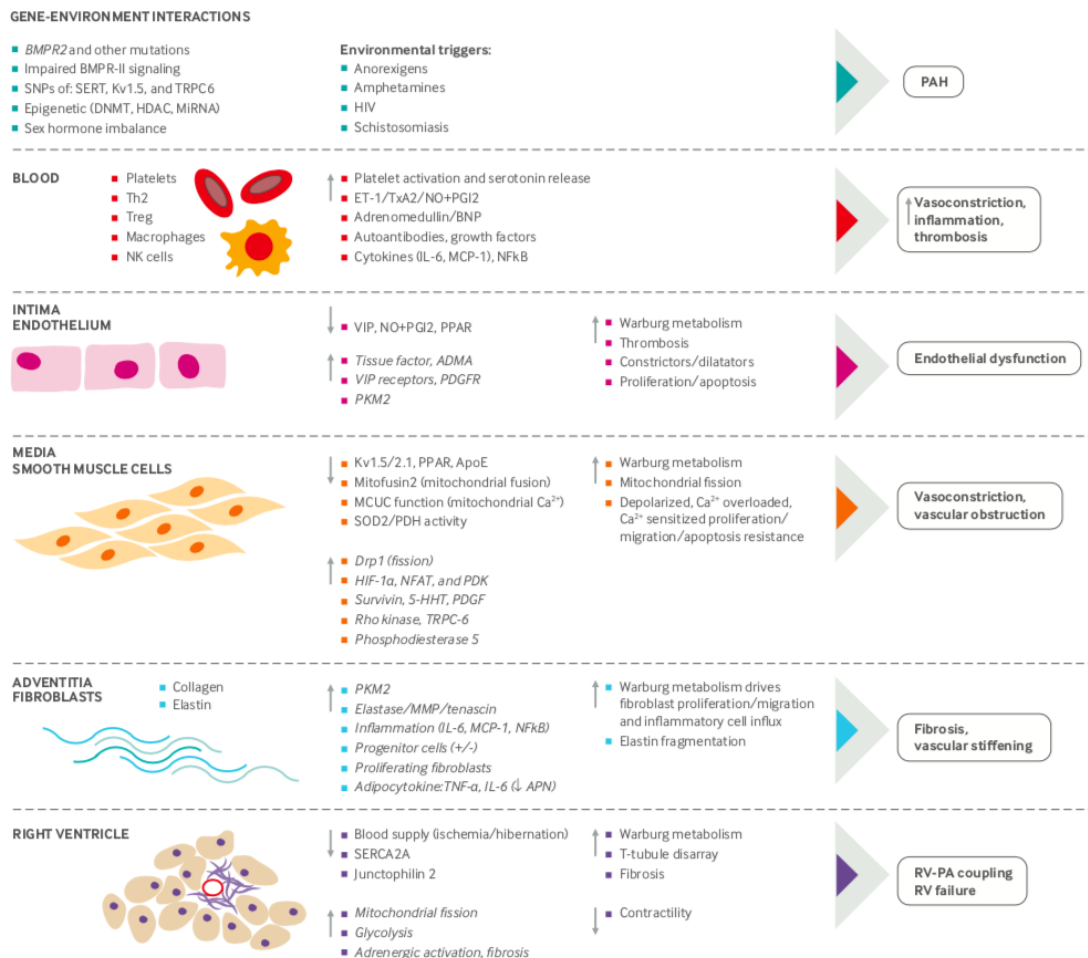


Figure 2. Mécanismes impliqués dans la pathogenèse de l'HTAP. Extrait de (Thenappan et al, BMJ.2018) (16)

La dysfonction endothéliale point commun entre ces 3 pathologies

Chacune de ces maladies possède une pathogenèse qui lui est propre (10,16,18–28). On retrouve cependant des similarités, avec une atteinte plus ou moins marquée des différents éléments suivants :

- une composante génétique notamment dans l'HTAP et le phénomène de Raynaud associé à la sclérodermie.
- une altération des éléments figurés du sang notamment plaquettaires et macrophagiques dans l'HTAP et le phénomène de Raynaud associé à la sclérodermie.
- Une dysfonction de l'endothélium vasculaire retrouvée de manière systématique dans toutes ces pathologies. Cette dysfonction endothéliale aboutit à une modification de la balance vasodilatation-vasoconstriction, à une augmentation de la perméabilité

vasculaire, à un phénotype pro-inflammatoire, pro-thrombotique et à une perte de communication avec les cellules musculaires lisses.

- Une réponse neurovasculaire anormale notamment dans le phénomène de Raynaud, voire une neuropathie dans les ulcères diabétiques.
- Un remodelage vasculaire notamment dans la sclérodermie systémique et l'HTAP.

Dans le tableau 1 sont résumés les principaux mécanismes physiopathologiques actuellement décrits dans le phénomène de Raynaud primaire, secondaire, dans l'HTAP et dans les troubles trophiques cutanés.

	PR primaire	SSc-PR	HTAP	Ulcère diabétique	Ulcère de pression	Ulcère vasculaire
Génétique		CMH 2, altération facteurs de transcriptions, dérégulation épigénétique	BMPR2, altération facteurs de transcriptions, dérégulation épigénétique			
Intra-vasculaire		↗ activation et agrégation plaquettaire, macrophages, LB, LT	↗ activation et agrégation plaquettaire et relargage de 5-HT, macrophages, LT, cellules NK	↗ activation et agrégation plaquettaire, PAI-1, Fibrinogène, facteur Von Willebrand		↗ macrophages
Endothelium vasculaire						
Balance vasoconstriction-vasodilatation	↘ NO ?	↘ NO (selon stade SSc). ↗ ET-1, ADMA, Ag2	↘ VIP, NO, PGI, canaux K (TASK1) ↗ ADMA, ET-1, Rho kinase, TRP-C, PDE-5, Ag2, PPAR	↘ NO, BH4 ↗ ET-1, ADMA	↘ NO, BH4, PGI2 ↗ AngII, ET-1, PDE-1, PGH2, TxA2, PGF2 α	↘ NO
Phénotype pro-inflammatoire		↗ cytokines (TGF-β, IL-13, IL-6, IFN1 γ), chimiokines (CCL2, CCL3, PF4)	↗ cytokines (IL-1α, IL-6, IL-8, IL-12), chimiokines (CCL2, MCP-1)	↗ CRP, cytokines (IL-6, IL-1β, TNFα), chimiokines	↗ cytokines (IL-6, TNF α, TGF-β), chimiokines	↗ cytokines (IL-1, TNF α, TGF-β1)
Facteurs de croissances		↗ PDGF, CTGF	↗ FGF2, VEGF, PDGF, EGF, NGF	↘ HIF/VEGF	↘ HIF/VEGF, PDGF	↗ PDGF, VEGF
Dysfonction de la barrière endothéliale		↗ VCAM-1, ICAM-1, E-selectin	↗ VCAM-1, ICAM-1, E-selectin	↗ VCAM-1, ICAM-1		↗ VCAM-1, E-selectin, L-selectin
Stress oxydatif		↗ ROS	↗ ROS	↗ ROS	↗ ROS	
Atteinte neuronale	↘ CGRP ↗ alpha-2C-adrenergic receptor (Rho kinase)	↘ CGRP ↗ alpha-2C-adrenergic receptor (Rho kinase)		↘ SP, NPY, CGRP ↗ CRF, α-MSH, NT		
Autre		↗ prolifération fibroblastique	↗ prolifération fibroblastique Dysfonction mitochondriale	↗ MMP-2, MMP-9, synthèse de collagène ↘ Prolifération fibroblastique	↗ MMP-2, synthèse de collagène ↘ Prolifération fibroblastique Dysfonction mitochondriale	↗ MMP, synthèse de collagène ↘ Prolifération fibroblastique

Tableau 1. Principaux mécanismes physiopathologiques actuellement décrits dans le PR primaire, secondaire, dans l'HTAP et dans les troubles trophiques cutanés. HTAP : hypertension artérielle pulmonaire ; PR : phénomène de Raynaud ; SSc-PR : phénomène de Raynaud associé à la sclérodermi

Des stratégies thérapeutiques communes

La pierre angulaire de la prise en charge pharmacologique des pathologies microvasculaires repose sur l'utilisation de traitements vasodilatateurs. En sus des antagonistes calciques, ces traitements vasodilatateurs agissent sur 3 grandes voies de signalisation : la voie du monoxyde d'azote (NO), la voie de la prostacycline et la voie de l'endothéline.

- **les antagonistes calciques** : Ils bloquent les canaux calciques de type L dans cellules musculaires lisses vasculaires et diminuent ainsi le flux calcique entrant. Ils possèdent donc une activité vasodilatatrice périphérique puissante. Ils sont indiqués en première intention dans le phénomène de Raynaud et dans l'HTAP chez les patients répondeurs aux tests de vasoréactivité. (29,30)

- **la voie du NO** : Le NO est produit par la NO-synthase endothéliale à partir d'un acide aminé, la L-arginine. Une fois libéré par les cellules endothéliales le NO pénètre dans la cellule musculaire lisse et active une enzyme cytoplasmique, la guanylate cyclase soluble (GCs). Cette enzyme est impliquée dans la production de guanosine monophosphate cyclique (GMPc) à partir de GMP, entraînant une relaxation des fibres musculaires lisses et ainsi une vasodilatation. Trois grandes classes pharmacologiques agissent sur cette voie.

- *les donneurs de NO* : peu utilisés par voie systémique en raison d'une importante tachyphylaxie (31), leur utilisation par voie locale, en hydrogel a été testée dans le phénomène de Raynaud (32). De plus la découverte récente d'une voie entero-salivaire de production de NO à partir de nitrates alimentaires ouvre la voie à l'étude de l'impact d'une supplémentation nutritionnelle chez les patients atteints d'une dysfonction vasculaire (33).

- *les inhibiteurs de la phosphodiesterase-5* : L'inhibition de la phosphodiesterase-5 bloque la dégradation de GMPc dans les cellules musculaires lisses et conduit ainsi au maintien de la relaxation vasculaire induite par la voie du NO. Ils sont actuellement recommandés en première ligne dans le phénomène de Raynaud, au même titre que les antagonistes calciques, et dans l'HTAP. (29,30)

- *les activateurs/stimulateurs de la GCs* : La GCs est une enzyme hème-dépendante qui catalyse la production de GMPc sous l'effet du NO. Les stimulateurs de la GCs potentialisent donc l'effet du NO mais peuvent également activer cette enzyme de façon NO-indépendante.

Le riociguat, chef de file de cette classe pharmacologique récente, est recommandé en première ligne dans l'HTAP et en cours de d'évaluation dans la sclérodémie et dans les ulcères digitaux (NCT02915835).

- **la voie de la prostacycline** : La prostacycline et ses dérivés sont de puissants vasodilatateurs qui agissent en se liant aux récepteurs IP et augmentent la production d'adénosine monophosphate cyclique (AMPC) dans la cellule musculaire lisse vasculaire.

- *les analogues de la prostacycline* : l'iloprost est un analogue stable de la prostacycline. Il est utilisé en première intention dans les troubles trophique des patients atteints de sclérodémie. (29) L'epoprostenol et le treprostinil sont en plus utilisés et recommandés dans les HTAP sévères. (30)

- *les agonistes des récepteurs IP de structure chimique non prostanoides*: plus récemment ont été développés des analogues du récepteur à la prostacycline IP, le selexipag. Ce dernier a été testé récemment dans le phénomène de Raynaud, sans démontrer son efficacité, mais il est indiqué dans l'HTAP. (29,34)

- **la voie de l'endothéline** : l'endothéline-1 est vasoconstricteur endogène puissant dont l'implication dans la physiopathologie de la SSc et de l'HTAP est clairement établi. L'endothéline-1 exerce son effet vasoconstricteur en activant deux sous-types de récepteurs (ET_A et ET_B) présents au niveau des cellules musculaires lisses.

- *les antagonistes des récepteurs à l'endothéline* : le bosentan a démontré une activité dans la prévention de la survenue des ulcères digitaux des patients atteint de sclérodémie mais n'a pas d'action curative. Le bosentan, l'ambrisentan, et le macitentan sont indiqués dans l'HTAP, les deux premiers étant commercialisés en France. (29,35)

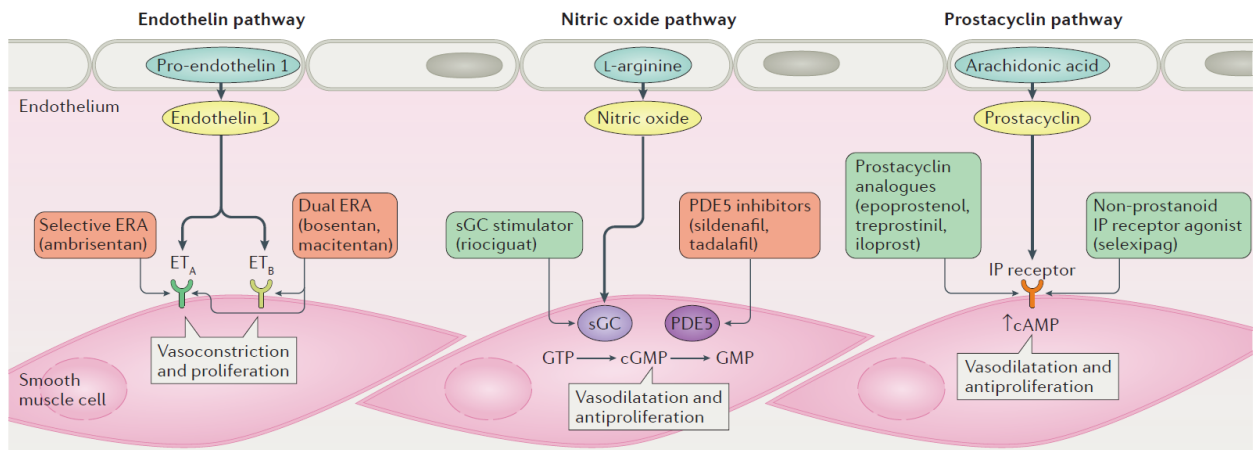


Figure 3. Cibles moléculaires des différentes classes thérapeutiques approuvées dans l'HTAP. Extrait de (Lau et al, Nat Rev Cardiol. 2017) (36)

L'un des objectifs principaux de ce travail de thèse est de d'identifier puis d'évaluer de nouvelles étiologies médicamenteuses impliquées dans l'apparition ou l'aggravation de ces pathologies microvasculaires.

2. Méthodes en Pharmacovigilance

Au-delà de la démonstration d'efficacité, la définition du profil d'effets indésirables est un enjeu majeur du développement d'un médicament et de son évaluation après commercialisation. Avant sa première introduction chez l'homme ce profil peut être prédit à partir de la structure de la molécule, de son appartenance à une classe thérapeutique connue et à partir des données de toxicologie animales. Les essais cliniques apportent ensuite une grande quantité d'informations permettant d'appréhender les effets indésirables fréquents, dose dépendants (effets indésirables de type A) mais sont souvent incapables d'identifier des effets indésirables inattendus et plus rares (type B) ou retardés (type C). Après commercialisation des informations provenant d'une multitude grandissante de sources (bases de pharmacovigilance, bases de données de santé, réseaux sociaux...) sont utilisées pour approfondir les connaissances sur profil de sécurité d'un médicament (apprécier l'incidence des effets indésirables connus en population réelle et en identifier de nouveaux).

Nous pouvons définir deux étapes distinctes de l'identification de nouveaux effets indésirables médicamenteux :

- la détection, qui consiste à identifier de potentiel nouveaux effets indésirables qu'on appellera signaux de pharmacovigilance.
- l'évaluation qui consiste à confirmer ou infirmer ces signaux, à quantifier le risque et son impact dans la population.

A cette fin de nombreuses méthodes ont été développées, elles sont adaptées au type de données disponibles et à l'objectif de détection ou d'évaluation de l'effet indésirable d'intérêt.

Littérature/Essais cliniques

Les essais cliniques sont avant tout conçus pour évaluer l'efficacité d'un nouveau médicament par rapport à un traitement de référence ou un placebo; la détection et l'évaluation du profil d'effets indésirables, même si elle est obligatoire, reste un objectif secondaire. De plus, ils n'incluent au mieux que quelques milliers de patients sélectionnés, suivis et surveillés de manière optimale (posologie, dosage, observance) afin de maximiser l'efficacité du nouveau traitement. Les populations particulières (enfants, femmes enceintes, personnes âgées ou polyopathologiques) sont généralement exclues de sorte que l'échantillon de patients inclus dans un essai clinique est rarement représentatif de la population utilisatrice du médicament en vie réelle. (37,38) Même les plus grands essais cliniques ne permettent pas de garantir, avec une puissance suffisante, la détection d'un effet indésirable dont la probabilité de survenue est faible (Tableau 2). (39) Dernier aspect, la durée des essais cliniques, souvent courte, est inadéquate pour évaluer la sécurité d'un médicament utilisé à long terme. (4)

Tableau 2. Probabilité d'observer au moins 1 cas d'un effet indésirable en fonction de la taille de l'échantillon et la probabilité de survenue de l'évènement. Extrait de (Bouvenot et al. Lavoisier 2006) (39)

Nombre de sujets inclus	Probabilité de survenue de l'évènement						
	1/100	2/1 000	1/1 000	2/10 000	1/10 000	2/100 000	1/100 000
100	63 %	39%	9,5%	5%	1%	0,5%	0%
500	99 %	63%	39%	9,5%	5%	1%	0,5%
1 000	1	99%	63%	39%	9,5%	5%	1%
5 000	1	1	99%	63%	39%	9,5%	5%
10 000	1	1	1	99%	63%	39%	9,5%
50 000	1	1	1	1	99%	63%	39%
100 000	1	1	1	1	1	99%	63%

Des méthodes méta-analytiques sont couramment utilisées pour combiner plusieurs essais cliniques et en augmenter la puissance statistique. (41,42) L'utilisation de méta-analyses a notamment un intérêt pour détecter des effets indésirables trop rares pour être mis en évidence dans les études originelles, ou pour lesquels la fréquence n'augmente que légèrement par rapport à la fréquence basale et dont les étiologies sont multiples (cancer, évènements cardiovasculaires...). (43–45) Elles sont donc principalement utilisées confirmer un signal de pharmacovigilance et en quantifier le sur-risque. Cependant leur utilité est actuellement débattue compte tenu du grand nombre de biais pouvant affecter ce type de méta-analyse en raison de la faible qualité méthodologique et du manque de transparence des études publiées. (46,47) Voici par exemple dans cette figure (Figure 4) représenté le nombre d'effets indésirables de deux essais cliniques rapportés dans des sources publiques (publications, résumés de conférences, bases d'enregistrement d'essais cliniques...) et non publiques (données individuelles des essais, rapport d'essai clinique...) pour lesquels suffisamment de données sont présentes pour pouvoir réaliser une méta-analyse. (48) Il apparait clairement que le nombre d'effets indésirables rapportés dans les sources publiquement accessibles est nettement inférieur au nombre réel d'effets indésirables survenus dans un essai clinique.

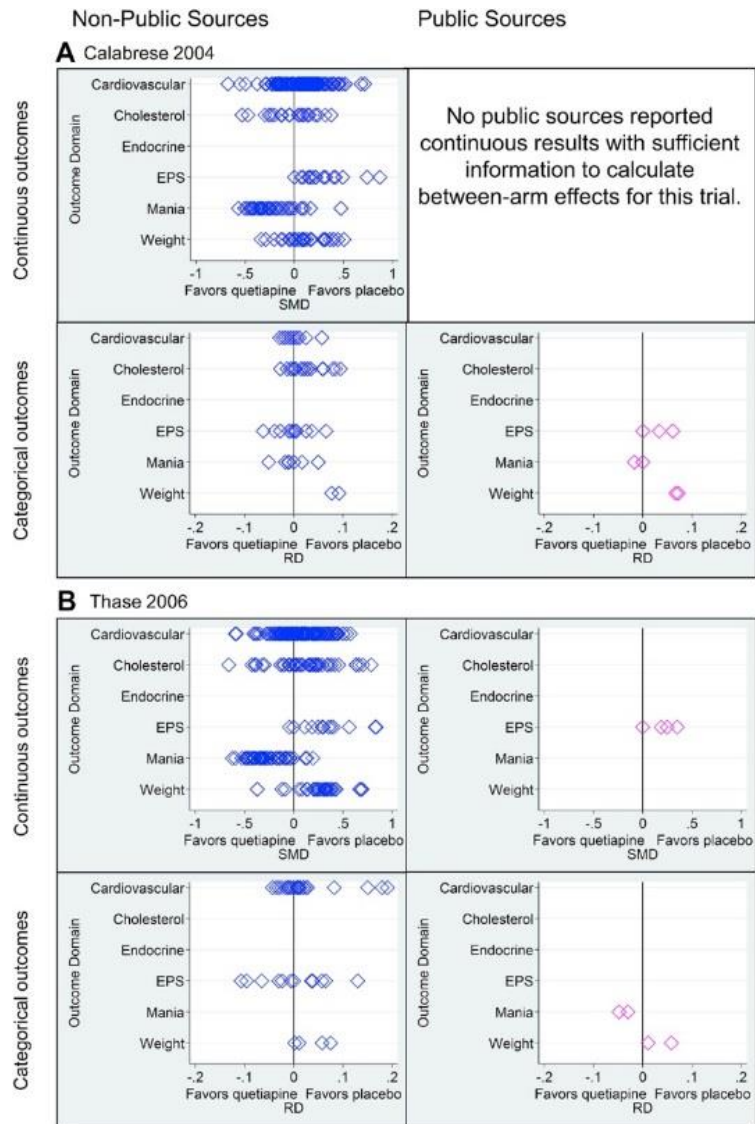


Figure 4. Comparaison du nombre d'effets indésirables méta-analysables (classés par type) entre les sources publiques (publications, résumés de conférences, bases d'enregistrement d'essais cliniques...) et non publiques (données individuelles des essais, rapport d'essai clinique...) pour deux essais cliniques. Extrait de (Mayo-Wilson et al., J Clin Epidemiol. 2019) (48)

Des recommandation du Council for International Organizations of Medical Sciences (CIOMS) et de la FDA sur la conduite de méta-analyses d'effets indésirables à partir d'essais cliniques ont récemment été publiées. (34,37) Voici une synthèse des différents biais pouvant affecter la conduite de méta-analyses d'effets indésirables et des recommandations actuelles afin de prendre en compte ces biais. (45,49–52)

Tableau 4. Synthèse des différents biais pouvant affecter la conduite de méta-analyses d'effets indésirables (EI) et des recommandations actuelles afin de prendre en compte ces biais.

Biais	Recommandations
Essais cliniques	
Design <ul style="list-style-type: none"> - durée des essais non adéquate pour certains EI - population sélectionnée - sortie prématurée de l'essai, censure « informative » et déplétion des susceptibles - effets indésirables entraînant un biais dans l'aveugle - définition des sorties d'essais variable en fonction des essais - schémas de doses flexibles parfois utilisées 	<ul style="list-style-type: none"> - définir une période à risque d'EI - examiner des durées de suivi, temps d'exposition, raison d'exclusions entre les groupes (peut constituer une raison de non-inclusion dans la méta-analyse si non équilibré), à partir des données individuelles idéalement.
Effets indésirables <ul style="list-style-type: none"> - pas de définition a priori des EI, ni de comité d'adjudication - modification de la façon de rapporter un EI au cours de l'essai - données sur les facteurs de risque d'EI non disponibles - report sélectif des EI et exprimés en nombre de patients ou en nombre d'évènements - reports uniquement des EI suspects ou de tous les EI (distinction difficile à faire dans les publications) - données de sécurité en per protocole - pas d'informations sur la fréquence, gravité et temps de survenue - des EI peuvent être présents dans plusieurs catégories (EI graves+ EI de type particulier) 	<ul style="list-style-type: none"> - multiplier les sources de données (registres d'essais cliniques/ rapport d'essai sponsors/protocoles) pour identifier/sélectionner les EI d'intérêt - contacter le sponsor pour s'assurer que l'évènement n'a pas eu lieu s'il n'est pas rapporté dans un essai - utilisation des données individuelles pour harmoniser l'adjudication des EI par un comité indépendant - utilisation d'EI « dur » pour minimiser les biais de mesure ; exclure les EI peu spécifiques et difficile à vérifier objectivement - préférer l'utilisation de tous les EI car non biaisé par l'interprétation de l'investigateur
Meta-analyses	
Sélection des essais <ul style="list-style-type: none"> - biais de publication - biais de sélection - pas d'harmonisation de la terminologie des EI - double comptage des volontaires - problème de l'inclusion de l'essai qui a généré le signal de sécurité 	<ul style="list-style-type: none"> - utiliser des méthodes de recherches bibliographiques spécifiques aux EI (voir (52)) - sélection des études sur la qualité et critères d'inclusions en aveugle des résultats. Critères d'inclusion à définir a priori en prenant en compte la qualité méthodologique de l'étude, la qualité de la

		« vérification » des EI, l'exposition, la durée de suivi, la population, le comparateur, l'équilibre entre les groupes (durée de suivi et d'exposition) et la disponibilité des données individuelles - analyses de sensibilité en excluant l'essai à l'origine du signal.
Pertinence des essais inclus	- différence de design, protocoles, indication, critères d'inclusion/exclusions, dose et durée de traitement, et de définition/vérification des EI	- privilégier la qualité et l'homogénéité des essais inclus sur la quantité
Analyses statistiques	- difficultés de calcul engendrées par les 0 et par les faibles taux d'évènements - si exclusion des études avec 0 événements surestimation à tort de la fréquence de l'EI -multiplicité des tests statistiques -probable hétérogénéité importante	- pré-spécification du plan d'analyse et de ses hypothèses - utilisation de méthodes adaptées aux évènements rares et aux 0 (méthodes bayésiennes ou méthode de Mantel-Haenszel par exemple) plutôt que d'introduire une correction de continuité - utilisation d'effet aléatoire recommandé - choix du modèle en fonction du type d'EI (patients vs évènements) - évaluer la dose-réponse - vérification de la réplicabilité des résultats prospectivement dans un essai - analyses de sensibilité pour évaluer la robustesse des résultats (variation de la définition de l'évènement, de la fenêtre temporelle, population et méthodes d'analyse)

A partir de ces constats, la FDA définit 3 niveaux de preuves associées à ces méta-analyses d'effets indésirables, que l'on peut résumer ainsi :

- les plus fiables sont les méta-analyses dont le protocole est publié avant l'inclusion des études, et dont le design des études incluses prend en compte ces objectifs de « safety ». Les données individuelles sont disponibles avec des critères de jugements et des périodes d'exposition bien définies et adéquates.

- le niveau suivant regroupe les méta-analyses dont le protocole a été pré-spécifié utilisant des données d'essais cliniques designés pour d'autres objectifs mais dont l'exposition et les critères de jugements sont fiables et adaptés à la question. Le protocole et l'inclusion des études sont réalisées en aveugle des résultats.

- le reste des méta-analyses est considéré de faible niveau de preuve.

La très grande majorité des méta-analyses d'effets indésirables publiées ne suivent pas ces recommandations et sont clairement identifiées comme problématiques dans une démarche de prise de décision par la FDA. Elles peuvent néanmoins servir de première étape à la planification d'une méta-analyse de meilleure qualité, à partir des données individuelles par exemple, ou à compléter l'étude d'un effet indésirable qui est généralement réalisé à partir de multiples sources (bases de pharmacovigilances, études sur bases de données médicales...).

Au-delà de l'évaluation de signaux par des méta-analyses, des méthodes de détection de signaux ont été développées en utilisant les données de la littérature. (53,54) Elles sont utilisées seules ou en combinaison avec des méthodes de disproportionnalité sur base de données de notification spontanée pour en augmenter les performances. Le challenge principal de ce type d'analyse est d'identifier le nombre de cas rapportés de manière fiable avec un médicament d'intérêt. Pour ce faire certains auteurs ont développé des algorithmes de machine learning afin de trier les références de la littérature ; la complexité de ces analyses limitent encore l'utilisation large de ce type d'approches (53).

Bases de pharmacovigilance

Les bases de pharmacovigilances ont été créées dans l'objectif de générer des signaux de disproportionnalité (SDR) à partir de la notification spontanée d'effets indésirables. Trois bases internationales sont actuellement largement utilisées par les autorités de santé et pour la recherche, la base de la Food and Drug Administration (FAERS), la base de l'Agence

Européenne du Médicament (EudraVigilance), et la base de l’OMS (Vigibase[®]) gérée par l’Uppsala Monitoring Center. Différentes méthodes ont été développées afin de quantifier ces SDR et de prioriser leur évaluation. Plusieurs mesures de disproportionnalité sont utilisées en pharmacovigilance mais il n’existe pas de gold standard en termes de performance, d’efficacité et de fiabilité. (55–58) Quatre types de méthodes de disproportionnalité se distinguent :

- les méthodes fréquentistes ou classiques

Elles mesurent l’association entre un médicament et un effet indésirable basée sur une augmentation relative de la proportion de cas rapportés de l’effet indésirable d’intérêt par rapport aux autres médicaments et aux autres effets indésirables. Ces méthodes sont basées sur le même principe de calcul en utilisant un tableau de contingence 2x2 (Figure 5). (59,60) Le Reporting Odds Ratio (ROR) et le Proportional Reporting Ratio (PRR) sont deux mesures classiques de disproportionnalité largement utilisées, le PRR est notamment utilisé en routine par EudraVigilance Data Analysis System (EVDAS) pour la détection de SDR. (61–63) Bien que ces différentes approches présentent des propriétés différentes, elles donnent des résultats similaires lorsque le nombre de cas observés est supérieur ou égal à 3. (56,58)

	Adverse event of interest	All other adverse event of interest	Total
Drug of interest	a	b	a+b
All other drug of interest	c	d	c+d
Total	a+c	b+d	a+b+c+d

$$\text{Reporting Odds Ratio (ROR)} = \frac{a / c}{b / d} = \frac{a d}{b c}$$

$$95\% \text{ confidence interval (CI)} = e^{\ln(\text{ROR}) \pm 1.96 \sqrt{\frac{1}{a} + \frac{1}{b} + \frac{1}{c} + \frac{1}{d}}}$$

$$\text{Proportional Reporting Ratio (PRR)} = \frac{a / a+b}{b / c+d}$$

Figure 5. Formules de calculs des Proportional Reporting Ratio et Reporting Odds Ratio à partir d’un tableau de contingence. Extrait de (Hasegawa et al. PLOS ONE. 2017) (64)

- *les méthodes bayésiennes*

Le Multi-Item Gamma Poisson Shrinker (GPS) utilisé par la FDA (65) et le Bayesian Confidence Propagation Neural network (BCPN) (66) utilisé par l’OMS sont deux exemples d’analyses bayésiennes. Ces mesures permettent d’estimer la probabilité (probabilité à posteriori) qu’un effet indésirable survienne avec l’utilisation d’un médicament (55). Elles sont notamment intéressantes lorsque le nombre de cas rapportés est faible.

- *les méthodes multivariées*

Afin de prendre en compte les co-prescriptions et interaction médicamenteuses dans l’estimation des SDR, l’utilisation de régressions logistiques a été proposée en 2008 sur la base FAERS. (67) Puis des méthodes multivariées à partir d’estimateurs bayésiens ont été plus récemment développés comme le regression-adjusted GPS (mixe entre une régression logistique et le GPS) ou l’algorithme Monte Carlo expectation-maximization (modification du GPS) afin de prendre en compte les co-prescriptions. (68,69)

- *les méthodes d’intelligence artificielle*

Récemment plusieurs méthodes de machine learning ont été testées pour détecter des SDR dans les bases de pharmacovigilances : les règles d’association, les forêts aléatoires et la régression logistique de Monte Carlo. Ces méthodes sont dérivées des études de « genome-wide association » visant à déterminer une association entre un phénotype et un variant génétique à partir de très larges données.

Une étude récente a comparé la valeur pronostique positive et négative de toutes ces méthodes à partir de la base FAERS et d’un jeu de données comprenant des paires effets indésirables - médicaments vrais positifs et vrais négatifs (Figure 6). (70)

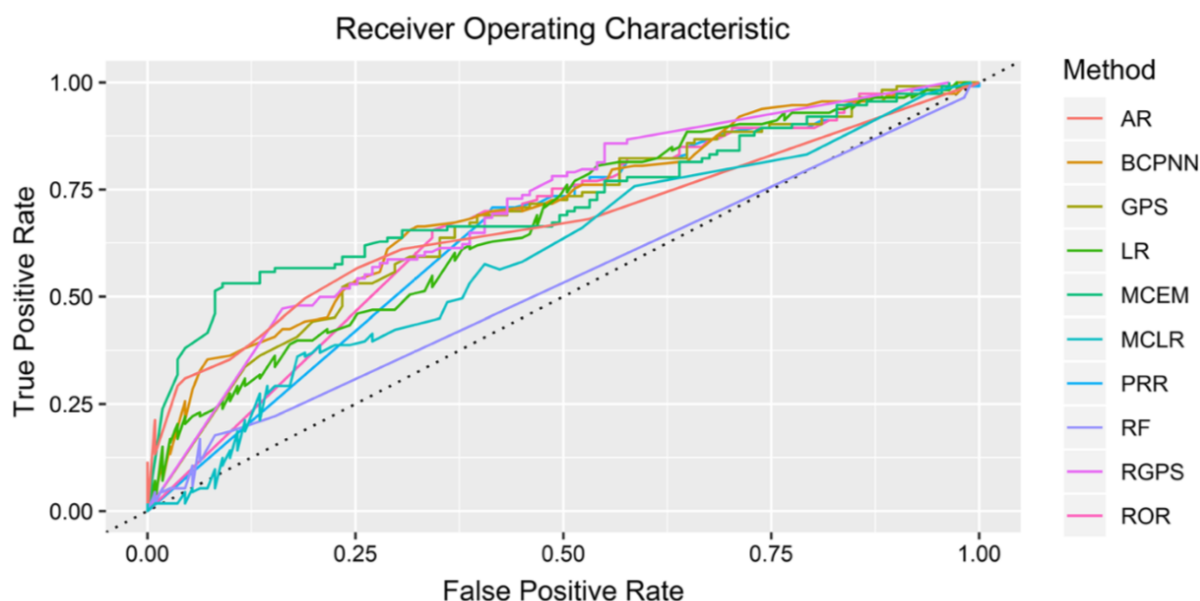


Figure 6. Courbes ROC des différentes méthodes de détection de signaux. Extrait de (Pham et al., *Drug Saf.* 2019). (70)

AR: association rules; *BCPNN*: Bayesian confidence propagation neural network; *GPS*: gamma Poisson shrinkage; *LR*: logistic regression; *MCEM*: Monte Carlo expectation maximization; *MCLR*: Monte Carlo logic regression; *PRR*: proportional reporting ratio; *RF*: random forests; *RGPS*: regression-adjusted GPS; *ROR*: reporting odds ratio.

Au total, les résultats sont relativement comparables, mais suggèrent une supériorité des méthodes bayésiennes dans la détection des SDR par rapport aux méthodes fréquentistes et de machine learning.

L'une des principales problématiques de ces analyses est la prise en compte des biais inhérents à la notification spontanée des effets indésirables. La notification d'effets indésirables aux systèmes de pharmacovigilance est en effet conditionnée par de nombreux facteurs comme la nature de l'effet indésirable, sa gravité, sa nouveauté ainsi que la nouveauté du médicament en cause, sa médiatisation, son temps de survenue après l'instauration du traitement mais également par le type de déclarant (patient/ professionnel de santé). (71–73) Ainsi, seule une faible proportion des EI présentés par les patients est notifiée, de plus, la qualité et l'exhaustivité des informations sont très hétérogènes selon le rapporteur. Les bases de notification spontanée sont donc soumises à une sous-notification et à une notification sélective importante. (59,74–77) De nombreuses méthodes ont été développées

afin d'identifier et de prendre en compte cas biais. (73,78) Cependant, leur impact sur les performances des analyses de disproportionnalité en fonction du type de médicament-effet indésirable étudié, est encore mal défini. En dépit de ces limites, la notification spontanée a réussi à démontrer sa capacité à identifier de nouveaux EI, particulièrement s'ils sont rares, mais permet difficilement l'identification EI retardés de type C. (59)

Au-delà de la détection des signaux de disproportionnalité les bases de pharmacovigilances ont été utilisées pour d'autres objectifs. La détection d'interaction médicamenteuse est une des applications classiques de ces bases de données. Les méthodes appliquées pour la détection de ces interactions font appel à des méthodes variées, fréquentistes (modèle additif ou multiplicatif des PRR-ROR) (79,80), des comparaison de temps d'apparition des EI (81) ou des méthodes plus complexe de machine learning. (82) L'étude de tendance de notification après une alerte dans les médias ou le retrait du marché de certains substance est également possible. (83,84) Un des développements récents est la corrélation de SDR à des données de pharmacodynamie afin de générer des hypothèses mécanistiques. (85–89) Ces méthodes nécessitent néanmoins un travail méthodologique afin d'évaluer la pertinence de l'approximation d'un risque d'effet indésirable par une métrique de disproportionnalité. Enfin, de nombreux travaux ont été réalisés afin d'identifier des syndromes et des effets de classes et non pas des EI isolés dans les bases de pharmacovigilances. (90–93) Les méthodes utilisées font appel aux méthodes de clustering, analyse en classe latente, analyses en réseaux... Ces méthodes intéressantes sont encore peu utilisées en pharmacovigilance et peuvent permettre, en association notamment à des méthodes intelligence artificielle, de faire du repositionnement de drogues ou de prédire le profil d'effets indésirables d'un nouveau médicament à partir de ses caractéristiques chimiques ou pharmacologiques.

Bases de données de santé

Les bases de données en santé ont classiquement été utilisées en pharmacovigilance pour confirmer/évaluer des signaux à l'aide de méthodes basées sur les designs de l'épidémiologie : cas témoins, cohortes, études transversales... (91,94)

L'un des enjeux actuels est le développement de méthodes permettant la détection de signaux en routine sur ces bases de données. (95) De nombreux travaux ont récemment été publiés notamment dans le cadre de projets comme Observational Medical Outcomes Partnership (OMOP), (96) Innovative Medicines Initiative's (IMI) PROTECT, (97) Exploring and

Understanding Adverse Drug Reactions (EU-ADR), (98) ou Asian Pharmacoepidemiology Network (AsPEN). (99) Les méthodes utilisées sont basées sur plusieurs schémas différents : des analyses de disproportionnalité, des designs dérivés des méthodes classiques de pharmaco-épidémiologie (designs auto contrôlés ou cohortes), des analyses en symétrie de séquence, des méthodes bayésiennes, des arbres de décision ou sur des méthodes de machine learning et d'intelligence artificielle. (95,100) Très peu d'études ont comparé les performances de ces approches pour la détection de signaux ; cela dit les méthodes paraissant les plus intéressantes actuellement en termes de faisabilité et de performances sont les analyses en symétrie de séquence et les designs autocontrôlés. (95,100) Le développement de ces méthodes pourra à terme compléter efficacement la détection de signaux sur les bases de pharmacovigilance avec l'avantage de pouvoir calculer l'incidence de ces EI, et pourrait être plus efficace notamment pour la détection d'EI retardés de type C.

Autres types de données

La diversité des données utilisées en pharmacovigilance est de plus en plus importante à mesure que les méthodes et les puissances de calcul augmentent. L'utilisation des forums, média sociaux, données chimiques et biologiques, données de la littérature à l'aide d'algorithmes d'intelligence artificielle est maintenant possible. (101–108) Cette multiplication des sources de données permet d'étudier différents types d'EI, de corroborer des signaux entre les sources et d'en augmenter les performances.

3. Objectifs de la thèse

Les objectifs de ce travail sont 1-d'étudier, de comprendre et d'identifier de nouvelles étiologies iatrogènes à ces pathologies microvasculaires : phénomène de Raynaud, troubles trophiques et HTAP et 2-d'évaluer et de comparer l'efficacité et la sécurité des traitements utilisés dans ces pathologies.

L'organisation des différentes études de cette thèse est représenté Figure 7. Ce travail sera divisé en quatre parties. Dans la première partie de ce travail nous nous intéresseront aux travaux portant sur le phénomène de Raynaud ; puis dans une seconde partie sur l'HTAP et ensuite sur les troubles trophiques ; enfin nous aborderons les travaux méthodologiques et perspectives de ce travail de thèse.

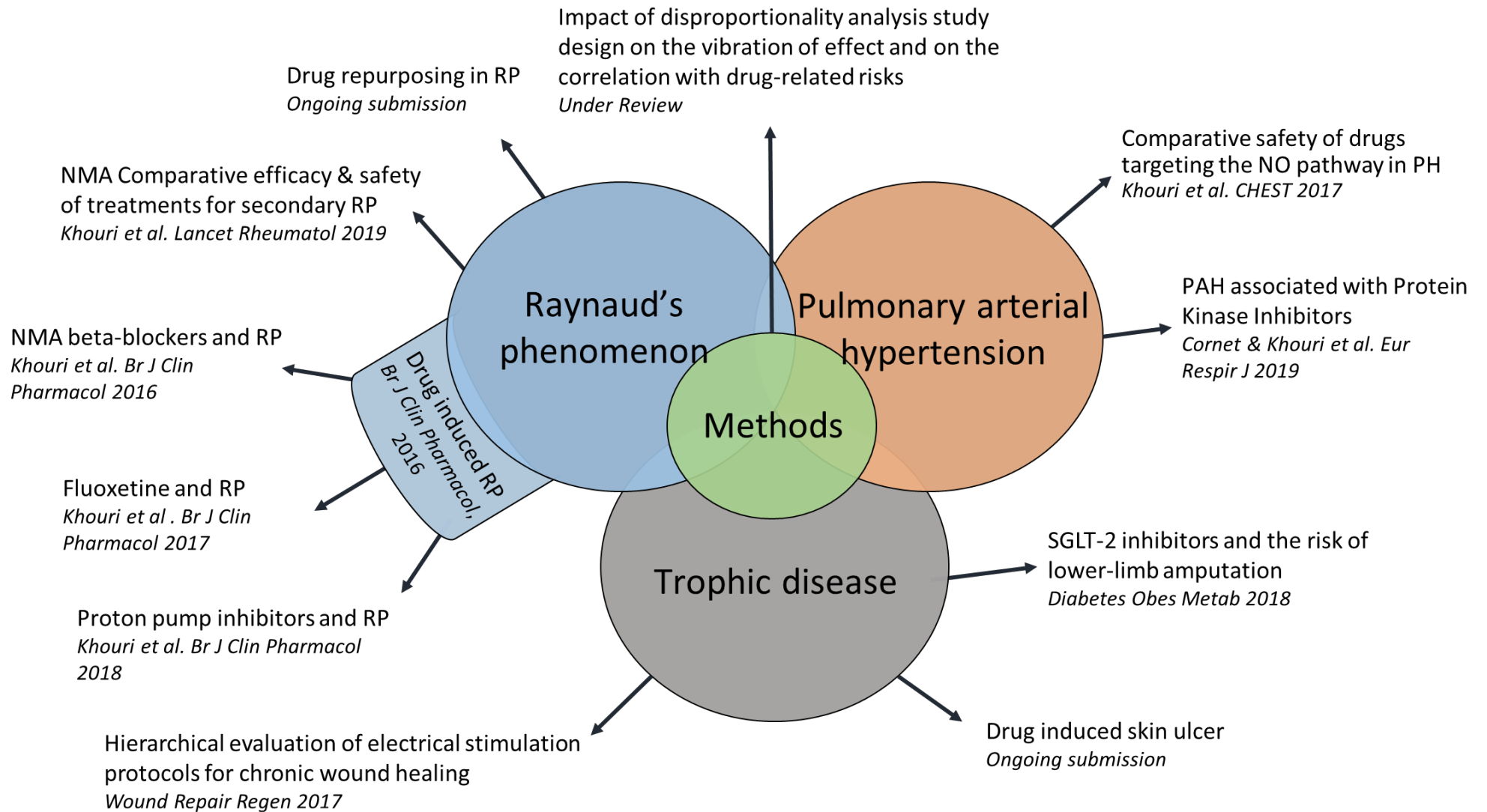


Figure 7. Organisation des études dans la thèse. NMA: network meta-analysis; NO: nitric oxide; PAH: pulmonary arterial hypertension; PH: pulmonary hypertension; RP: Raynaud's phenomenon.

PARTIE 2. LE PHÉNOMÈNE DE RAYNAUD

1. Drug-induced Raynaud's phenomenon: beyond β -adrenoceptor blockers: Drug-induced Raynaud's phenomenon.

Quelques mois après la mise sur le marché des bêta-bloquants l'apparition de phénomènes de Raynaud ont été observés chez certains patients (109). Depuis, d'autres classes médicamenteuses ont été identifiées comme pouvant provoquer ou aggraver un phénomène de Raynaud préexistant ; notamment les chimiothérapies alkylantes, la clonidine ou les dérivés de l'ergot. (6,9,10) Cependant de nombreux cas ont été rapportés dans la littérature avec d'autres médicaments et aucune revue systématique n'avait été réalisée sur le sujet. L'objectif de ce travail était donc de faire un état des lieux des étiologies iatrogènes induisant ou aggravant un phénomène de Raynaud. Ce travail a été réalisé à partir d'une revue systématique des données de la littérature. Nous avons, de plus, tenté de synthétiser ces données via une approche mécanistique et d'apprécier le niveau de preuve associé à chacune des classes médicamenteuses identifiées.

SYSTEMATIC REVIEW

Drug-induced Raynaud's phenomenon: beyond β -adrenoceptor blockers

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Received 21 January 2016; **revised** 12 February 2016; **accepted** 14 February 2016

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Keywords β -adrenoceptor blockers, peripheral vasoconstriction, Raynaud's phenomenon

AIM

Drug-induced Raynaud's phenomenon (RP) has long been associated with the use of different drugs, including cancer chemotherapy or β -adrenoceptor blockers. However, sources report extremely variable prevalence and the level of evidence for each class is heterogeneous. Moreover, new signals are emerging from case reports and small series. Our objective was therefore to review available evidence about this adverse drug effect and to propose a mechanistic approach of drug-induced RP.

METHODS

A systematic review of English and French language articles was performed through Medline (1946–2015) and Embase (1974–2015). Further relevant papers were identified from the reference lists of retrieved articles.

RESULTS

We identified 12 classes of drugs responsible for RP, with a variety of underlying mechanisms such as increased sympathetic activation, endothelial dysfunction, neurotoxicity or decreased red blood cell deformability. Cisplatin and bleomycin were associated with the highest risk, followed by β -adrenoceptor blockers. Recent data suggest a possible involvement of tyrosine kinase inhibitors (TKI), through an unknown mechanism.

CONCLUSION

Drug-induced RP is a probably underestimated adverse drug event, with limited available evidence regarding its prevalence. Although rare, serious complications like critical digital ischaemia have been reported. When these treatments are started in patients with a history of RP, careful monitoring must be made and, if possible, alternative therapies that do not alter peripheral blood flow should be considered.

Introduction

Raynaud's phenomenon (RP) is characterized by transient ischaemia of the extremities in response to environmental stress or emotions [1]. It typically manifests as changes to the fingers, with pallor (vasospasm and decreased blood

flow), cyanosis (deoxygenation of the static venous blood) and rubor (reperfusion), often accompanied by pain. RP can be primary (i.e. idiopathic) or secondary to an underlying cause. In both cases, abnormalities of the cutaneous microcirculation are primarily involved in the pathophysiology of RP [2].

The prevalence of RP in the general population varies between 0.5 and 19%, with major geographic variability [3–6]. While primary RP is the most frequent form (80–90%) [7], RP may also be secondary to various auto-immune diseases (such as systemic sclerosis (SSc), systemic lupus erythematosus, vasculitis, etc.), or other systemic diseases [1]. Several drugs with peripheral vascular effects leading to decreased microvascular perfusion may induce or aggravate RP. Drug-induced RP probably goes unrecognized because of the limited knowledge of this side effect.

Literature reviews and textbooks usually have comprehensively reviewed drugs that have long been known to be responsible for RP [8]. However, new signals are emerging from numerous case reports. Yet, to our knowledge, no systematic review has been performed and little is known about the prevalence and the level of evidence of drug-induced RP. Our objective in the present work was therefore to summarize available evidence and to propose a mechanistic approach of drug-induced RP.

Methods

The MEDLINE database was searched for English or French language articles published between January 1946 and May

2015 using the following search terms: 'Raynaud disease/chemically induced' [MESH] and 'raynaud' AND 'clonidine', 'betablocker', 'ergot alkaloid', 'dopaminergic agonist', 'selective serotonin re-uptake inhibitors', 'sympathomimetic drugs', 'chemotherapy', 'tyrosine kinase inhibitors', 'interferon' and 'ciclosporin'. Further relevant papers were identified from the reference lists of retrieved articles. We used the Oxford Centre for Evidence-based Medicine - Levels of Evidence to graduate the strength of the link between RP and drug classes [9]. Among 253 records screened, 131 full texts were assessed for eligibility and included in the review (Figure 1).

Results

Drugs enhancing vasoconstriction

β-adrenoceptor blockers. β-adrenoceptor blockers have long been known as causing drug-induced RP, but data about its prevalence are scarce. Analysis of the Framingham Heart study data identified β-adrenoceptor blocker use as the most common cause of secondary RP (34.2% of secondary RP). A meta-analysis published in 2012 that included 13 studies (1012 patients) found a prevalence of 14.7% in patients receiving β-adrenoceptor blockers [4]. However, the studies

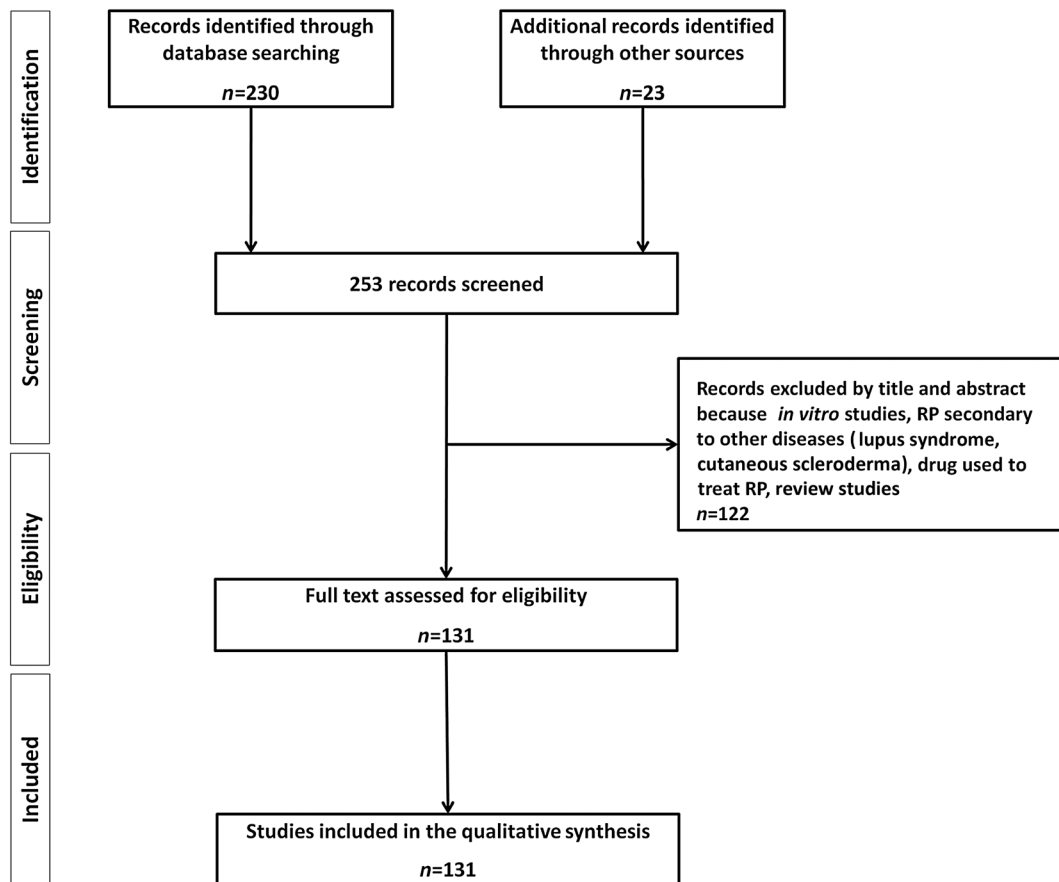


Figure 1

Flow diagram of studies included in the review

were old (1971 to 1984) and of varying quality. A network meta-analysis of prospective randomized controlled trials revealed a prevalence of peripheral vasoconstriction among patients treated with β -adrenoceptor blockers of 7% (1966/28072), whereas 4.6% (555/12060) and 1.7% (305/17492) of patients treated with placebo or active control experienced this adverse effect, respectively ($P < 0.001$) (Khouri *et al.*, submitted).

The pathophysiology of this side effect remains unclear. Studies exploring the effect of β -adrenoceptor blockers on patients with primary RP failed to show any worsening of their symptoms [10–13]. There is no evident explanation for this discrepancy, but the studies have small sample size.

The influence of the ancillary properties of β -adrenoceptor blockers (e.g. intrinsic sympathomimetic activity, β_1 -selectivity, vasodilator activity) should theoretically influence their propensity to induce peripheral vasoconstriction, although studies report conflicting results [13–16]. The recent network meta-analysis conducted by our group suggests that β -adrenoceptor blockers are a heterogeneous class. High affinity for β_1 -adrenoceptors does not protect from RP while ancillary properties such as intrinsic sympathomimetic activity and vasodilator properties seem to be protective (Khouri *et al.*, submitted to British Journal of Clinical Pharmacology).

Clonidine. RP induced by clonidine is a well-known adverse reaction, described since many years although its frequency is not known [17]. In patients with RP, cold-amplified α_{2c} -adrenoceptors mediated vasoconstriction is increased [18, 19]. It has been identified that skin vasoconstriction in response to local cooling is mediated by the translocation of α_{2c} -adrenoceptors to the vascular smooth muscle cells surface, through a pathway involving RhoA–Rho kinase [20]. In cold situations, clonidine direct α_{2c} -vascular agonism may become pre-eminent on the usually desired central reduction of the adrenergic tone.

Ergot alkaloids. Ergotamine and its derivatives are used to treat migraine disorders and cluster headache [21]. They display affinity for a wide variety of receptors including those for 5-HT (serotonin), dopamine and norepinephrine [22]. They are partial agonists of various serotonergic receptors and the usual response of blood vessels to 5-HT is contraction [23]. More precisely, they exert a central vasoconstrictor effect through serotonergic 5-HT_{1B/1D} receptors, which are mostly in the cranial vessels and at therapeutic dose exert only a weak constricting effect on peripheral blood vessels [24]. However 5-HT₂ agonism seems to be the main effector of their peripheral serotonergic vasoconstrictor effect. Moreover, they are α_1 -, α_2 -adrenergic and dopaminergic D₂-receptor agonists. Numerous case reports illustrating this effect are found in the literature [25, 26]. However, the accountability of ergot alkaloids in RP is difficult to assess because of a significantly higher prevalence of RP in the migraine population [27, 28]. Furthermore, the peripheral vasoconstriction caused by ergot alkaloids is sometimes interpreted as RP. ‘Ergotism’ is rarely observed (estimated incidence is 0.1%), but the prolonged vasoconstriction can lead to gangrene.

In contrast, other drugs targeting serotonin receptors such as triptans, selective agonists of 5-HT_{1B/1D}, do not induce vasoconstriction of extremities and RP.

Dopaminergic agonists. RP cases have been reported following the use of bromocriptine, another ergot alkaloid [29–32]. One report describes severe RP with vascular morphological injury (presence of megacapillary on nailfold capillaroscopy) attributed to 6 years of treatment with bromocriptine [31]. Bromocriptine is mainly a dopaminergic agonist. At low doses it has vasodilative properties resulting from D₁-receptor activation and leading to the well-identified orthostatic hypotensive state. At high doses it exhibits α_1 -adrenoceptor properties [33] and peripheral release of catecholamines both resulting in vasoconstriction. Moreover, direct activation of α_2 -adrenoceptors by bromocriptine has been described and could explain increased sensitivity to cold [34], like clonidine. Microvascular injury with long term use of bromocriptine has also been suspected. [31] Nevertheless, a large case–control study (542 cases and 2155 controls) did not support the association between dopamine agonists and an increased risk of ischaemic events requiring hospitalization [35]. Unfortunately this study did not provide detailed information on RP.

Surprisingly, two cases of erythromelgia have been described with bromocriptine, in association with calcium channel blockers [36, 37]

Selective serotonin re-uptake inhibitors (SSRIs). Contradictory effects of SSRIs on peripheral vasoreactivity have been reported. On the one hand, SSRIs have been proposed as a treatment for RP, following the observation of the relief of patients with erythromelgia or RP with fluoxetine and sertraline [38], or paroxetine and escitalopram [39]. Indeed, fluoxetine blocks the uptake of serotonin by platelets and decreases the amount of serotonin that is released during platelet activation/aggregation, which may explain the favourable outcome in patients with primary or secondary RP participating in an open randomized clinical trial [40]. On the other hand, other authors have described a deleterious association between RP and the SSRIs, fluoxetine [41, 42], fluvoxamine [43], citalopram [44] and milnacipran [45], together with the relief of erythromelgia symptoms [46]. A case of emerging RP 2 days after beginning tergaserod treatment, a partial 5-HT₄ serotonin receptor agonist, has also been described [47].

Currently this discrepancy between vasoconstriction and vasodilatation remains unexplained. Some authors suggested that endothelial damage is necessary for the development of a vasoconstrictive effect during SSRI treatment [48]. In a healthy vascular bed it has been proposed that blocking serotonin re-uptake could increase free plasma serotonin concentrations and produce, in stasis conditions, a local accumulation of serotonin, exacerbating vasoconstriction through 5-HT₂ receptors that may worsen RP [44]. In contrast, SSRIs decrease the amount of serotonin that is released during platelet activation/aggregation. For example fluoxetine is known to deplete platelet serotonin by 95% [49]. Individual variability in metabolism or in signalling serotonin pathways could explain this variability in response to SSRIs [40].

Stimulants. Central stimulation of the dopaminergic and noradrenergic system is responsible for the peripheral release of catecholamines leading to vasoconstriction. Cases of RP induced by central nervous system stimulants have been reported [50]. A retrospective case-control study investigated whether medications used for the treatment of attention deficit hyperactivity disorder (ADHD) were associated with the development of RP. Sixty-four children were enrolled in the study (32 cases with RP and 32 age and gender matched control patients) and a significant association between the presence of RP and past or current use of ADHD stimulants (methylphenidate and dextroamphetamine) was found [51]. Atomoxetine, a selective norepinephrine re-uptake inhibitor, was excluded from this study because it was not considered as a central nervous system stimulant. However the case of dose dependent RP following the use of atomoxetine on a girl has recently been described [52]. Two cases of RP induced by reboxetin, an inhibitor of norepinephrine re-uptake, have been described [47]. Amphetamine-like drugs have also been associated with the emergence of RP and vasculopathy, as has phentermine, a weak sympathomimetic agent, used most commonly as an appetite suppressant in the treatment of obesity [53].

Ciclosporin. A study assessing the prevalence of RP in 100 renal transplant patients treated with ciclosporin monotherapy who were then transferred to prednisolone and azathioprine observed the development of *de novo* symptoms in 39% of patients on the introduction of ciclosporin. After withdrawal of ciclosporin, symptoms improved in 89% [54]. Moreover four case studies of RP induced by ciclosporin use have been described. Three of them appeared a few days after ciclosporin introduction and totally disappeared after cessation [55, 56]. The second case was dose related but persistent RP symptoms were observed after cessation of ciclosporin [57]. The mechanism for ciclosporin induced RP remains unclear. A vasospastic effect of ciclosporin on both the macro and microcirculation has been shown [58] leading to systematic monitoring of hypertension or acute renal failure in the early treatment phase. Furthermore, changes in the viscosity of the blood, a decrease in the deformability of red blood cells and an increase in the aggregation of platelets can also be induced by ciclosporin use and contribute to RP [55].

It is worth noting that drugs increasing blood viscosity such as erythropoietins or intravenous immunoglobulins are not a known cause of RP. Much about the physiopathology of drug-induced RP remains to be learnt.

Sympathomimetics. Digital necrosis was described following the localized use of lidocaine/epinephrine in a patient with primary RP [59]. Data concerning sympathomimetic nasal decongestants (pseudoephedrine, phenylephrine) are scarce. Thus, pharmacologic properties of these drugs and their poor clinical benefit suggest that they should be contraindicated in patients with scleroderma-related RP [60].

Toxic substances. Among recreational drugs, RP with ischaemic finger necrosis was attributed to cocaine abuse in the case of in a 37-year-old man [61]. Cocaine has a potent

vasoconstrictor effect through its α_2 -adrenoceptor activity. In animal studies it has also been shown to alter prostaglandin production with disproportionate increases in thromboxane in rabbit endothelium resulting in vasospasm, platelet activation and thrombus formation [62, 63], although in some cases cocaine vasculopathy is more likely to be related to a Buerger-like syndrome [64–66] as described with cannabis use or arsenic exposure [67–69].

Endothelium damage and/or neurotoxicity

Cancer chemotherapies. The link between RP and chemotherapies has long been clearly identified. First descriptions of chemotherapy-induced RP were related to treatments for testicular cancer [70–72]. A study in 1995 that included 90 patients treated with cisplatin-based chemotherapy for more than 1 year after testicular cancer found that 37% of them had developed RP after four cycles of chemotherapy combining cisplatin, bleomycin and vinblastine [73]. RP typically appeared 3 to 6 months after the start of chemotherapy and often persisted for several years [74]. The risk factors identified for the development of RP were high cumulative doses of bleomycin and a combination of bleomycin with vinblastine rather than etoposide.

Furthermore, a trend towards the increased prevalence of RP was observed in patients who received bleomycin as a bolus compared with continuous infusion. No significant correlation was seen with the cumulative or single doses of cisplatin, etoposide or vinblastine, serum magnesium concentrations during or after chemotherapy or a history of smoking [73].

These results were confirmed by the follow-up of a cohort study that included 739 patients treated for testicular cancer between 1982 and 1992. Patients were divided between chemotherapy ($n = 384$) and non-chemotherapy ($n = 355$) groups. The prevalence of RP was significantly higher among patients who received chemotherapy (20.7% vs. 1.7%, $P < 0.001$) [75]. Once again, a significant relationship between the cumulative dose of bleomycin and the prevalence of RP was found (OR 2.98, 95% CI, 2.286, 3.388, $P < 0.001$); $P < 0.001$). Thirteen percent of patients still suffered from RP 10 years after having received a cumulative bleomycin dose of $< 180\,000$ IU (corresponding approximately to three cycles of cisplatin-etoposide-bleomycin), 24.6% after a cumulative dose of 180 000 IU to 360 000 IU, and 29% after a cumulative dose $> 360\,000$ IU. A large observational study [76] including 1409 testicular cancer survivors found a prevalence of RP among the chemotherapy group of 39%. The cancer chemotherapy associated Vinca alkaloids, cisplatin and bleomycin. The odds ratios for Raynaud-like phenomena in those who received one to four cycles of chemotherapy compared with those who received no chemotherapy were 2.9 [95% CI, 2.2, 3.9] and 8.0 [95% CI, 4.4, 14.7] if they received more than five cycles of chemotherapy. When these drugs have been used to treat Kaposi's sarcoma RP has also been described [77–81]. Nevertheless, emergence of severe RP with digital necrosis after a single cycle of doxorubicin, bleomycin, vincristine and dacarbazine chemotherapy, with a cumulative dose of only 40 000 IU of bleomycin has also been described [82]. Cases describing the occurrence of RP

after the local injection of bleomycin to treat warts have also been reported [83–87].

While RP has been associated with cisplatin-based chemotherapies the immutability of cisplatin itself remains unclear [73, 88]. A recent meta-analysis of cisplatin-based chemotherapies included 24 studies ($n = 2479$ patients) and found a prevalence of RP of 24% (95% CI, 17.5, 31.3) [72]. However, cisplatin was almost always associated with bleomycin and Vinca alkaloids making imputability difficult. Agents targeting the VEGF-VEGFR axis are associated with hypertension, thromboembolic events and induce microvascular rarefaction [89], but their use is not associated with RP and peripheral vasoconstriction.

Among other cancer chemotherapies that could be responsible for RP, there is limited evidence for gemcitabine [90–92], vincristine [93], 5-fluorouracil [94], oxaliplatin [95], tegafur and uracil [96] and cyclophosphamide-methotrexate-5-fluorouracil adjuvant therapy [97].

The pathophysiology of RP induced by cancer chemotherapies is not well understood and is probably multifactorial. Some studies showed an exaggerated response to cold not only in patients with RP but also in patients without finger symptoms before testicular chemotherapy [74, 98]. An increased central sympathetic vasoconstrictor reflex and an impaired non-neurogenic vasomuscular, auto-regulation was highlighted in patients suffering from RP syndrome after chemotherapy when compared with the control group (patients without RP after chemotherapy) [99]. Currently, one of the main mechanisms proposed is through the vascular damage induced by chemotherapy, i.e. endothelial dysfunction that persists after chemotherapy [75]. Indeed, some authors [100] showed that microalbuminuria, considered to be a sign of endothelial damage, was significantly higher in patients who received testicular cancer chemotherapy [83]. Another possible mechanism is the neurotoxicity of chemotherapies toward arteriolar tone regulation, particularly through hypomagnesemia related to cisplatin administration leading to dysregulation of vascular smooth muscle tone [101]. RP would appear at the same time as the tubular damage. It is interesting to note that bleomycin is used to induce a sclerodermic phenotype in animals [102], scleroderma being the main aetiology of secondary RP.

Occupational and/or environmental exposure. For some time vinyl chloride exposure has been linked to RP [103]. The vascular endothelial toxicity of vinyl chloride has been shown by angiographic studies of arteries in the hand and by capillaroscopy [104, 105]. The prevalence of RP in vinyl chloride workers ranges from 6 to 33% [106]. In 1980 a prospective exposed/non-exposed cohort study showed a strong association between vinyl chloride exposure and RP ($P < 0.006$) [107].

Drugs increasing blood viscosity and enhancing vasoconstriction

Interferons (IFN). RP is a known side effect of treatment with interferon supported by numerous cases reports [41, 108–116]. On direct questioning of patients taking IFN [117], symptoms of RP were reported by more than half. Analysis of 24 case reports of RP associated with interferon [118] highlighted that

IFN α is the most common substance implicated ($n = 14$), followed by IFN γ ($n = 5$) and IFN β ($n = 3$). The treatment period was variable and lasted from 2 weeks to 49 months (mean 15.5 months). Clinical findings varied from mild and transient vasospasm (1 h after injection) to digital necrosis in 14 cases. Outcomes were known for 15 patients. Spontaneous recovery occurred for 50% of them after withdrawal of the drug. The remaining patients needed specific medication and six amputations were necessary, underlining the severity of this adverse reaction. A recent meta-analysis [119] with six eligible studies and 183 patients estimated the prevalence of RP in patients taking interferon to be 13.6% (95% CI 0.026, 0.313).

Currently, the pathophysiology of this reaction is not fully understood. However numerous hypotheses have been proposed: a direct vasospastic effect [113, 120], increasing levels of intracellular fibroblast growth factor in endothelial cells leading to proliferation of these cells and increasing angiogenesis [121] and induction or exacerbation of a dormant collagen disease [109]. Although some case reports of RP induced by interferons are described without any immune deficiency [122] it is known that interferon therapy can be related to an autoimmune disease [123, 124]. Increasing blood viscosity by induction of serum cryoprecipitation [125], deposition of immune complexes [126] and arterial occlusion by thrombi due to the procoagulant activity of interferon [112, 113] have been proposed. A study of 108 patients with SSc found a higher level of IFN- γ in patients with associated RP and suggested a pathogenic role of INF- γ in SSc patients with RP, but this role still remains unclear [127].

Unknown mechanisms

Tyrosine kinase inhibitors. The relationship between tyrosine kinase inhibitors (TKI) and RP is complex. Experimental studies have shown that receptors with a tyrosine kinase activity may play a role in the exaggerated vasoconstriction in response to cold [128]. On one hand, a pilot study [129] that included three SSc patients treated with 100 mg day⁻¹ of imatinib for 6 months showed improvement of their RP [86]. Indeed in each patient, RP was attenuated at around 3 months and had completely disappeared at 6 months. On the other hand, exactly the opposite reaction has been described with other TKIs. Emergence of RP during the first week of treatment with nilotinib has been described in two patients [130]. One of them experienced improvement after the treatment was switched to imatinib, with recurrence of RP on the reintroduction of nilotinib. Another patient experienced recurrent RP with nilotinib [131]. Erlotinib had also been implicated in the case of a 72-year-old patient suffering from scleroderma and secondary RP who experienced digital necrosis 20 days after starting daily oral treatment of 150 mg [132]. Erlotinib was promptly discontinued and treatment with calcium channel blockers, nitrates and anti-platelet drugs was initiated. After 3 weeks of therapy, the digital lesion was completely healed. Erlotinib was scored as producing a probable adverse drug reaction (7/10 on the Naranjo scale).

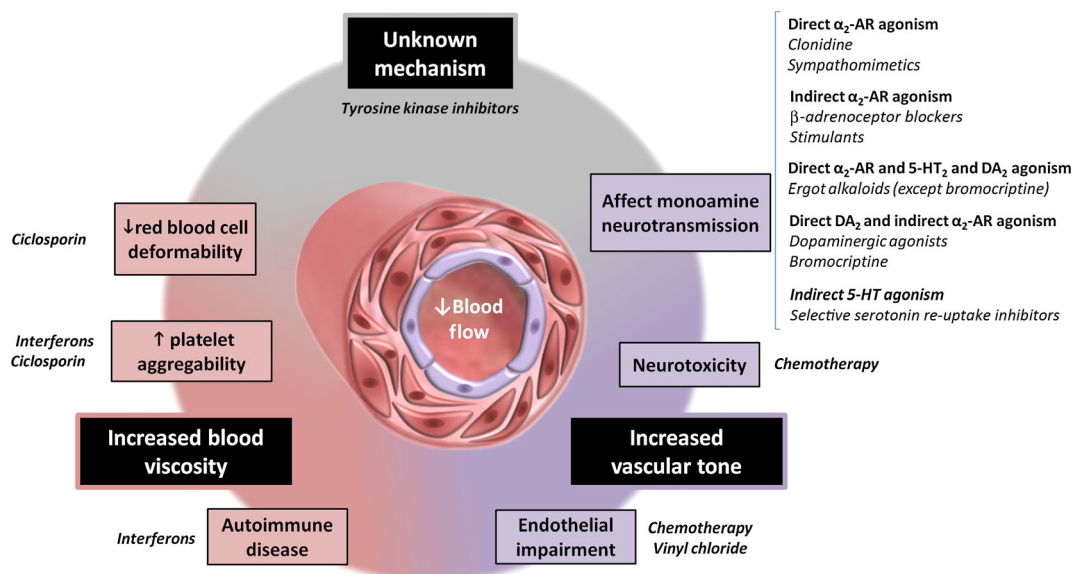
Other. In the literature sporadic case reports of RP potentially induced by drugs can be found, such as the two

Table 1

Most relevant prevalence and level of evidence (defined by the Oxford Centre for Evidence-based Medicine - Levels of Evidence) of the association between each drug and RP

Mechanism	Drug	Prevalence	Level of evidence
Enhancing vasoconstriction	Clonidine	Unknown	C
	β -adrenoceptor blockers	7%	A
	Ergot alkaloids	0.1%	C
	Dopaminergic agonists	Unknown	D
	SSRIs	Unknown	D
	Sympathomimetic drugs	Unknown	B
	Ciclosporin	Unknown	B
Endothelial damage	Chemotherapy	20.7–37%	A
	Vinyl chloride	6–33%	A
Drugs increasing blood viscosity and enhancing vasoconstriction	Interferons	13.6%	B
Unknown mechanism	Tyrosine kinase inhibitors	Unknown	D

A: systematic review (with homogeneity) of RCTs or individual RCT (with narrow confidence interval); B: cohort or case control studies; C: Case series; D: Expert opinion or troublingly inconsistent or inconclusive studies of any level; RCT: randomized controlled trial.

**Figure 2**

Schematic representation of some of the key mechanisms contributing to the pathogenesis of iatrogenic Raynaud's phenomenon

cases of fluorescein induced RP [133, 134], sulfasalazine [135, 136], propofol [137] and amphotericin B [138] without being able to determine the pathophysiological mechanism. Some paradoxical reactions following the repeated administration of iloprost [139] or yohimbine [140], a selective α_2 adrenergic antagonist, have even been described.

To our knowledge, no pathophysiological mechanism has been identified yet.

Conclusion

RP is complex, multifactorial and not fully understood yet. This present review summarises the prevalence and level of evidence of the association between drugs and RP (Table 1). Microvascular impairment is a key feature of its pathophysiology. Only symptomatic treatment with vasodilators such as calcium channel blockers or phosphodiesterase-5

inhibitors has been proposed as a treatment for RP. Logically, vasoconstrictors have long been known to induce or aggravate RP. Increased vascular tone may be related to increased sympathetic activation, but also to endothelial dysfunction or neurotoxicity. Other mechanisms include decreased red blood cell deformability and increased platelet aggregation, both leading to increased blood viscosity (Figure 2). The need for future high quality research including prospective and vascular physiology studies to clarify these mechanisms is obvious. Indeed, this review highlights the lack of available evidence regarding the prevalence of drug-induced RP, as well as the heterogeneity of its clinical presentation. This probably contributes to the underestimation of drug-induced RP, as well as the fact that RP is a usually benign condition. However, such an adverse event may rarely lead to serious complications like critical digital ischaemia. Therefore, when these treatments are started in patients with a history of RP, careful monitoring must be made and, if possible, alternative therapies that do not alter peripheral blood flow should be considered.

Competing Interests

They authors do not have any conflict of interest that might bias the present work.

Acknowledgement

We thank Dr Alison Foote (Clinical Research Centre, Grenoble University Hospital) for critically reading and correcting the manuscript.

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2. Peripheral vasoconstriction induced by β -adrenoceptor blockers: a systematic review and a network meta-analysis

Lors de la revue de la littérature présentée précédemment, nous avons remarqué que plusieurs questions restaient en suspens à propos d'une des classes médicamenteuses la plus connue pour provoquer/aggraver des phénomènes de Raynaud, les bêta-bloquants :

- s'agit-il d'un effet de classe ou spécifique à certaines caractéristiques pharmacodynamiques ?
- quel est le mécanisme d'action exact des phénomènes de Raynaud induits par les bêta-bloquants ?
- quel est l'amplitude du sur-risque de phénomène de Raynaud en fonction du type de bêta-bloquants ?

Afin de tenter de répondre à ces questions nous avons réalisé une méta-analyse en réseau à partir d'une revue systématique de tous les essais cliniques qui ont été publiés avec des bêta-bloquants.

META-ANALYSIS

Peripheral vasoconstriction induced by β -adrenoceptor blockers: a systematic review and a network meta-analysis

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Received 22 January 2016; **revised** 1 April 2016; **accepted** 14 April 2016

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Keywords β -adrenoceptor blockers, peripheral vasoconstriction, Raynaud's phenomenon

AIM

Peripheral vasoconstriction has long been described as a vascular adverse effect of β -adrenoceptor blockers. Whether β -adrenoceptor blockers should be avoided in patients with peripheral vascular disease depends on pharmacological properties (e.g. preferential binding to β_1 -adrenoceptors or intrinsic sympathomimetic activity). However, this has not been confirmed in experimental studies. We performed a network meta-analysis in order to assess the comparative risk of peripheral vasoconstriction of different β -adrenoceptor blockers.

METHOD

We searched for randomized controlled trials (RCTs) including β -adrenoceptor blockers that were published in core clinical journals in the Pubmed database. All RCTs reporting peripheral vasoconstriction as an adverse effect of β -adrenoceptor blockers and controls were included. Sensitivity analyses were conducted including possibly confounding covariates (latitude, properties of the β -adrenoceptor blockers, e.g. intrinsic sympathomimetic activity, vasodilation, drug indication, drug doses). The protocol and the detailed search strategy are available online (PROSPERO registry CRD42014014374).

RESULTS

Among 2238 records screened, 38 studies including 57 026 patients were selected. Overall, peripheral vasoconstriction was reported in 7% of patients with β -adrenoceptor blockers and 4.6% in the control groups ($P < 0.001$), with heterogeneity among drugs. Atenolol and propranolol had a significantly higher risk than placebo, whereas pindolol, acebutolol and oxprenolol had not.

CONCLUSION

Our results suggest that β -adrenoceptor blockers have variable propensity to enhance peripheral vasoconstriction and that it is not related to preferential binding to β_1 -adrenoceptors. These findings challenge FDA and European recommendations regarding precautions and contra-indications of use of β -adrenoceptor blockers and suggest that β -adrenoceptor blockers with intrinsic sympathomimetic activity could be safely used in patients with peripheral vascular disease.

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- β -adrenoceptor blockers are known to induce peripheral vasoconstriction, probably according to their pharmacological properties (e.g. preferential binding to β_1 -adrenoceptors, intrinsic sympathomimetic activity or vasodilator effect). However, this has never been confirmed in experimental studies.

WHAT THIS STUDY ADDS

- Our results suggest that β -adrenoceptor blockers have variable propensity to enhance peripheral vasoconstriction. Moreover, ancillary properties of β -adrenoceptor blockers widely influence this peripheral vasoconstriction: ISA and vasodilator effect are protective, whereas preferential binding to β_1 -adrenoceptors does not protect from peripheral vasoconstriction.
- These findings challenge FDA and French recommendations regarding precautions and contra-indications of use of β -adrenoceptor blockers, and suggest that β -adrenoceptor blockers with intrinsic sympathomimetic activity could be safely used in patients with Raynaud's phenomenon.

Introduction

β -adrenoceptor blockers have long been known to cause drug-induced peripheral vasoconstriction, especially Raynaud's phenomenon (RP), which was described as an adverse effect of β -adrenoceptor blockers 40 years ago [1]. Among the aetiologies of the syndrome, β -adrenoceptor blockers have usually appeared as the primary cause of drug-induced RP in recent state-of-the-art reviews and textbooks [2–6]. However, little is known about the exact prevalence of β -adrenoceptor blocker induced peripheral vasoconstriction. Analysis of the Framingham heart study identified β -adrenoceptor blocker use as the most common cause of secondary RP (34.2% of secondary RPs) [7]. More recently, a meta-analysis including 13 studies found a prevalence of RP of 14.7% in patients receiving β -adrenoceptor blockers [8]. However, the number of included studies was low and this simple meta-analysis did not permit to hierarchizing the vasoconstrictor effect of the different β -adrenoceptor blockers. The exact mechanism leading to peripheral vasoconstriction induced by β -adrenoceptor blockers remains incompletely understood. Antagonism of β_2 -adrenoceptors, which are responsible for peripheral arteriolar vasodilatation, has long been thought to be the main mechanism. This led to the contra-indication of non-selective β -adrenoceptor blockers in patients with RP. However, this hypothesis is challenged by clinical observations of RP occurring in patients taking β -adrenoceptor blockers with higher affinity for β_1 -adrenoceptors [1, 9]. In addition, in patients with primary RP, no differences in skin or muscular blood flow could be detected between propranolol, a non-selective β_2 -adrenoceptor blocker and metoprolol, a β_1 -adrenoceptor blocker [10]. Moreover, the involvement of β_2 -adrenoceptors in the pathogenesis of RP is not currently upheld [11].

Another hypothesis to explain peripheral vasoconstriction due to β -adrenoceptor blockers would involve the vasoconstrictor sympathetic reflex mediated by baroreceptors in response to the decrease in cardiac output following β -adrenoceptor blocker intake [12]. In accordance with this hypothesis, β -adrenoceptor blockers with intrinsic sympathomimetic activity (ISA) have a less pronounced effect on cardiac output, and may even decrease peripheral resistance during chronic treatment, therefore inducing less peripheral vasoconstriction [12]. However, limited evidence supports this hypothesis in patients with Raynaud's phenomenon, and available data are conflicting [13–15].

The paradox is that a considerable number of large, randomized, controlled trials have been conducted in the past decades that should provide sufficient evidence to clarify the implication of β -adrenoceptor blockers in induced peripheral vasoconstriction, such as RP. In the past few years, the development of sophisticated methods such as the combination of direct and indirect comparisons in network meta-analyses has been successfully applied to identify class adverse drug events [16].

Our objective in the present work was therefore to perform a systematic review and a network meta-analysis of randomized clinical trials to assess the effect of β -adrenoceptor blockers on peripheral vascular disease. We aimed at comparing the risk of peripheral vasoconstriction induced by the different β -adrenoceptor blockers according to their pharmacological properties (ISA, β_1 -selectivity, vasodilators and non-selective).

Methods

This systematic review complies with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analysis) statement guideline [17]. The protocol and systematic search strategy of the review has been documented online before starting the study (PROSPERO registry, CRD42014014374).

Objectives and outcomes

The primary objective of our study was to assess and compare the effect of β -adrenoceptor blockers on peripheral vascular disease.

Secondary objectives were to compare the risk of peripheral vasoconstriction induced by the different β -adrenoceptor blockers according to their pharmacological properties (ISA, β_1 -selectivity, vasodilators and non-selective), assess the influence of the year of study publication, the latitude, the way of reporting RP, the dosage and indication for β -adrenoceptor blockers on the risk of peripheral vasoconstriction.

Study identification, selection and data extraction

We searched for randomized controlled trials (RCTs) including β -adrenoceptor blockers that were published in core clinical journals in the Pubmed database. The following terms were sought: acebutolol, atenolol, betaxolol, bisoprolol, carvedilol, celiprolol, labetalol, metoprolol, nadolol, nebivolol, oxprenolol, pindolol, propranolol, sotalol and β -adrenoceptor blockers.

Applied filters were (Comparative Study [ptyp] OR Clinical Trial[ptyp]) AND jsubsetaim[text]. We also searched Google Scholar, the reference lists of relevant Cochrane reviews [18–20] and the reference list of the Trial Result-centre (<http://www.trialresultscenter.org>). There was no restriction on language or publication date. One reviewer (CK) screened titles and abstracts for inclusion. Then two authors (MR and CK) independently reviewed the full text of potentially relevant articles to check inclusion criteria using a standardized form. Eligibility criteria included parallel or crossover RCTs comparing the previously listed β -adrenoceptor blockers to control groups (placebo or any active comparator), for at least 4 weeks and reporting RP or any relevant symptom related to peripheral vasoconstriction. Despite the high prevalence of RP, standardized diagnostic criteria have not been used in these trials. Therefore, we used the term ‘peripheral vasoconstriction’ rather than ‘Raynaud’s phenomenon’.

Independent assessment of risk of bias was made by the same reviewers according to the Cochrane Handbook for Systemic Reviews of Interventions [21]. The risk of bias was rated as low, unclear or high for the following items: randomization, blinding, incomplete outcome data, selective reporting. The overall risk of bias for each trial was defined as high risk if more than three high risk criteria were met, moderate risk if two to three high risk criteria were met and low risk if one or less high risk criterion was met.

Then, the same two reviewers independently extracted data and appraised the quality and content of included studies using the Grading of Recommendation Assessment, Development and Evaluation (GRADE) recommendations for network meta-analysis [22]. These recommendations permit to appraise the quality of each direct and indirect pairwise comparisons of the network meta-analysis considering the average risk of bias [23], inconsistency [24], indirectness [25], imprecision [26] and publication bias [27]. Finally we rated their quality as very low, low, moderate or high. Special attention was paid to the way used to record the side effects (spontaneous reporting, medical visit or questionnaire).

The following data were extracted: year, country(ies) and latitude where the study was conducted, sample size, methodology, Raynaud’ phenomenon as a non-inclusion criteria in the trial, indication of the β -adrenoceptor blocker, follow-up period, β -adrenoceptor blocker dosage and treatment duration, nature of the peripheral vascular effect reported and frequency of outcomes (prevalence and/or withdrawals).

Statistical analysis

The primary objective was to compare the number of events in the different treatment arms with a frequentist approach. We used an arcsine transformation as it enables one to include empty cells in the analysis (i.e. taking into account study arms without any event), without continuity corrections [28]. We also provided odds ratios (OR) for easier interpretation, with a + 1 continuity correction for empty cells. Meta-regressions were performed to take into account covariates of interest, i.e. the year of study publication, the latitude, the way of reporting RP, as well as the dosage and indication for β -adrenoceptor blockers. A Bayesian approach was used to compute the rankograms as well as indirect effects (using the node-splitting algorithm). The rankograms

represent the probability of each β -adrenoceptor blocker to be the greatest inducer of peripheral vasoconstriction.

Statistical analysis was performed with R statistical software (version 3.2.0). The metafor package (v1.9–4, www.metafor-project.org) was used for frequentist analyses and the gemtc package (with the rjags Gibbs sampler) [29] for the Bayesian approach. We used a Mantel–Haenszel method with a random effect model to provide pooled OR of the risk of peripheral vasoconstriction according to the pharmacological properties of β -adrenoceptor blockers vs. placebo, using RevMan (Version 5.1, The Cochrane Collaboration, 2011). Confidence or credibility intervals are given for all measures and represented in forest plots. We used *t*-test to compare frequencies between groups when necessary. All tests and confidence or credibility intervals were two-sided. *P* values <0.05 were considered as significant.

Results

Characteristics of studies and patients

The literature search yielded a total of 2238 references. The main reasons for excluding records were that studies were *in vitro* studies, or were not randomized clinical trials, or were RCTs that did not report the incidence of peripheral vasoconstriction. Thirty-eight studies finally fulfilled the eligibility criteria [30–67]. (Figure 1).

All studies were RCTs with study duration ranging from 4 to 468 weeks and included a total of 57 026 patients. Most of the trials were multicentre and parallel, conducted in Europe or North America, examined a β -adrenoceptor blocker as an antihypertensive treatment and included an active comparator (27/38). For more than half of them, the presence of RP was a non-inclusion criterion (20/38). The characteristics of included studies are presented in Table 1.

The risk of bias is reported in supplementary on-line Figure S1. Eight studies were considered as having a high risk of bias.

Overall prevalence of peripheral vasoconstriction

The prevalence of peripheral vasoconstriction was highly dependent on the way in which adverse events were reported: 13.47% with a questionnaire (systematic approach) and 6.02% for spontaneous reports. In the placebo group, the prevalence was 8.1% with a questionnaire and 4.84% with spontaneous reporting.

Network and methodological quality of available comparisons

Thirty-four direct comparisons between β -adrenoceptor blockers and controls were available. Controls mostly included placebo, angiotensin-converting enzyme blockers/angiotensin receptor blocker, α -adrenoceptor blockers and thiazide diuretics. The network of available comparisons is represented in Figure 2.

The quality of evidence according to GRADE recommendations are presented in Supplementary Table S1. Discrepancies between the mean qualities of evidence for each β -adrenoceptor blocker were obvious and are presented in Table S2. When combining β -adrenoceptor blockers

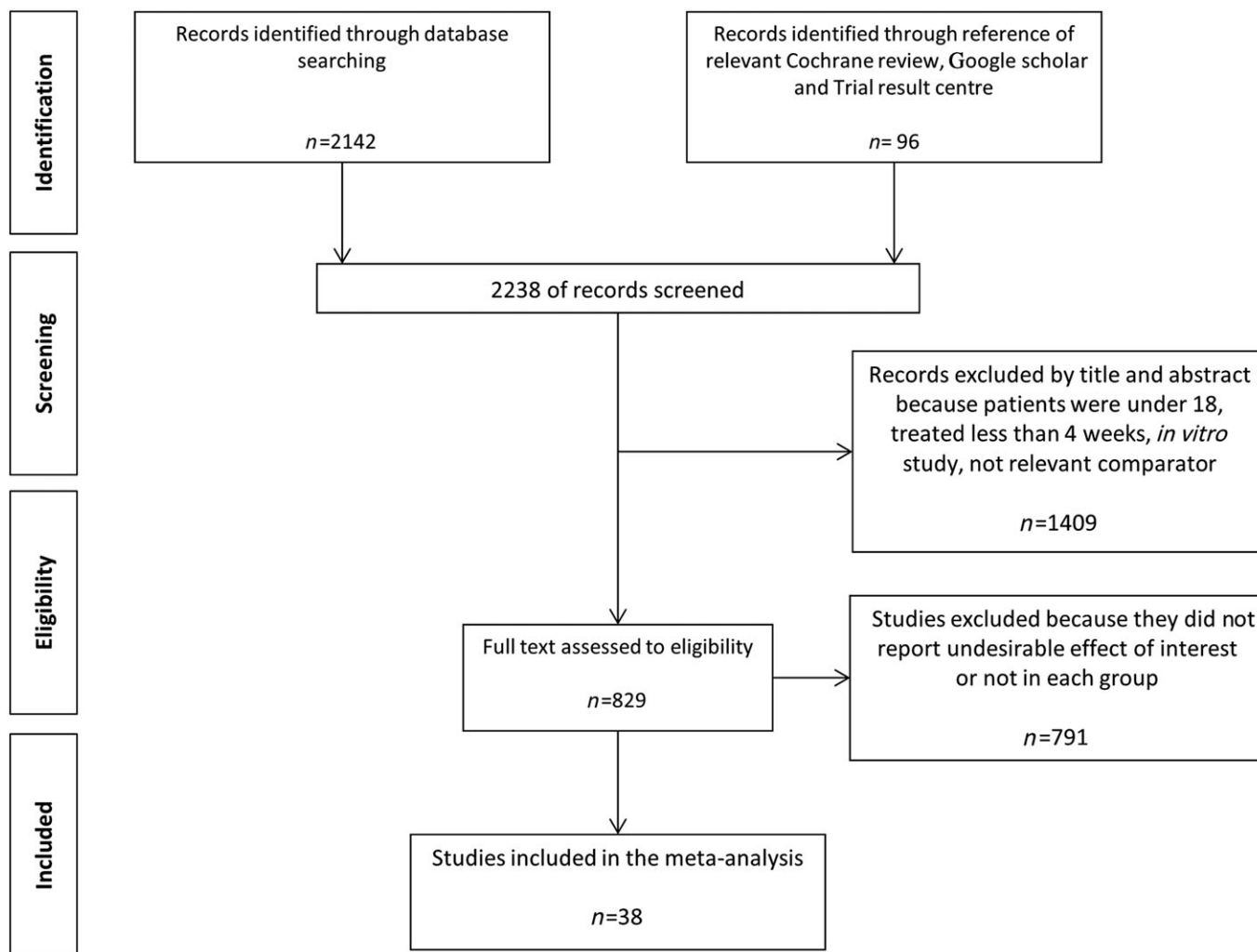


Figure 1

PRISMA flow diagram

depending of their pharmacologic properties overall mean qualities of each group were moderate for β -adrenoceptor blockers owning ISA and β_1 -selectivity, just below the moderate threshold for non-selective β -adrenoceptor blockers and low for vasodilator β -adrenoceptor blockers. Moreover, the percentages of high qualities studies included in each group were comparable, except for the vasodilator group (Table S3).

Peripheral vasoconstriction induced by β -adrenoceptor blockers

The prevalence of peripheral vasoconstriction among patients treated with β -adrenoceptor blockers was 7% (1966/28 072), whereas 4.6% (555/12 060) and 1.7% (305/17 492) of patients treated with placebo or active control experienced peripheral vasoconstriction, respectively ($P < 0.001$).

The network meta-analysis of direct and indirect comparisons between the different β -adrenoceptor blockers revealed differences between drugs (Figure 3, supplementary Figure S2). Propranolol (moderate quality evidence) and atenolol (moderate quality evidence) significantly increased the risk of peripheral vasoconstriction. Continuity correction

for empty cells allowed calculating ORs of 3.0 (1.4–6.6) and 2.0 (0.9–4.7) for propranolol and atenolol, respectively.

Influence of pharmacologic properties of β -adrenoceptor blockers on peripheral vasoconstriction

We categorized β -adrenoceptor blockers into four non-exclusive groups (non-selective, β_1 -selective, ISA and vasodilators), depending on their secondary properties (presented in Table 2). The OR of peripheral vasoconstriction in each group was 2.53 (1.39–4.61), 1.67 (1.29–2.17), 1.24 (0.7–2.19), respectively. Only β_1 -selective and non-selective β -adrenoceptor blockers were associated with an increased risk of peripheral vasoconstriction when compared with placebo (Figure 4).

Sensitivity analyses

Univariate meta-regressions did not show any significant effect of study latitude ($P = 0.18$), drug indication [hypertension ($P = 0.24$), ischaemia ($P = 0.27$), other ($P = 0.71$)], drug doses [low ($P = 0.67$), normal ($P = 0.86$), high ($P = 0.82$)],

Table 1

Study characteristics

Study	Country	Indication	Treatment	n	Methodology	Exclude RP	Exposure (weeks)	Double-blind	Peripheral vasoconstriction symptom
DiBianco <i>et al.</i> [30]	USA	Angina	Acebutolol, propranolol, placebo	46	Crossover	No	9	Yes	Cold extremities
Dahlöf <i>et al.</i> [31]	UK-Scandinavian	HT	Atenolol, CCB	19 257	Parallel	Yes	287	No	Peripheral coldness
Dahlöf <i>et al.</i> [32]	USA-UK-Scandinavian	HT	Atenolol, ACE/ARB	9193	Parallel	Yes	209	Yes	Cold extremities
Talseth <i>et al.</i> [33]	Norway	HT	Atenolol, α -adrenoceptor blocker	164	Parallel	No	157	Yes	Peripheral ischaemia
NASR Committee [34]	UK	HT	Atenolol, CCB	410	Parallel	Yes	12	Yes	Peripheral ischaemia or pain
Ott <i>et al.</i> [35]	Denmark	HT	Atenolol, α -adrenoceptor blocker	126	Parallel	No	20	Yes	Cold extremities
Fairhurst [36]	International	HT	Atenolol, bevantolol, placebo	229	Parallel	No	12	Yes	Peripheral vascular side effect
Helgeland <i>et al.</i> [37]	Norway	HT	Atenolol, ACE/ARB, thiazide	400	Parallel	Yes	14	Yes	Cold extremities
Rubin <i>et al.</i> [38]	UK	HT	Atenolol, placebo	85	Parallel	Yes	36	Yes	Raynaud's phenomenon
Julian <i>et al.</i> [39]	UK	MI	Sotalol, placebo	1456	Parallel	No	26	Yes	Cold extremities
Hansteen <i>et al.</i> [40]	Norway	MI	Propranolol, placebo	560	Parallel	Yes	52	Yes	Cold hands and feet
Persson <i>et al.</i> [41]	Sweden	MI	Metoprolol, xamoterol	121	Parallel	No	52	Yes	Cold extremities
Greenberg <i>et al.</i> [42]	UK	HT	Propranolol, thiazide, placebo	7241	Parallel	No	256	No	Raynaud's phenomenon
BHATR Group [43]	USA	MI	Propranolol, placebo	3837	Parallel	Yes	109	Yes	Cold hands, feet
Silberstein <i>et al.</i> [44]	Norway	Migraine	Propranolol, placebo	191	Parallel	Yes	261	Yes	Peripheral coldness
Leren <i>et al.</i> [45]	France	Angina	Propranolol, α -adrenoceptor blocker	23	Crossover	No	8	Yes	Cold hands and feet
Pascal & Raies [46]	USA	VB	Propranolol, placebo	230	Parallel	Yes	62	No	Raynaud's phenomenon
Moltzer <i>et al.</i> [47]	Italy	HT	Metoprolol, ACE/ARB	16	Crossover	Yes	16	No	Cold extremities
Metra <i>et al.</i> [48]	Scotland	HF	Metoprolol, carvedilol	150	Parallel	Yes	100	Yes	Raynaud's phenomenon
Herrick <i>et al.</i> [49]	UK	HT	Atenolol, ACE/ARB	162	Parallel	Yes	12	Yes	Cold extremities
Taylor <i>et al.</i> [50]	UK	HF	Oxprenolol, placebo	1103	Parallel	No	209	Yes	Cold extremities
UKPDS [51]	Germany	DT2	Atenolol, ACE/ARB	758	Parallel	No	438	Yes	Cold hands and feet
The DTS Group [52]	Europe	MI	Atenolol, placebo	1473	Parallel	Yes	157	Yes	Cold extremities
The IPPSSH Group [53]	Sweden	HT	Oxprenolol, placebo	6357	Parallel	No	209	Yes	Cold extremities
Ekborn <i>et al.</i> [54]	Sweden	HT	Metoprolol, atenolol, pindolol, placebo	1021	Parallel	No	52	Yes	Cold hands and feet
Garden <i>et al.</i> [55]	UK	VB	Propranolol, placebo	81	Parallel	No	104	Yes	Cold extremities
Nielsen <i>et al.</i> [56]	Denmark	HT	Atenolol, ACE/ARB	36	Parallel	No	183	No	Cold extremities
Beevers <i>et al.</i> [57]	UK	HT	Atenolol, ACE/ARB, placebo	288	Parallel	Yes	8	Yes	Cold extremities
Khattar <i>et al.</i> [58]	UK	HF	Carvedilol, ACE/ARB	57	Parallel	No	12	Yes	Cold peripheries
Mc Neil <i>et al.</i> [59]	Australia	HT	Metoprolol, pindolol, atenolol, labetalol	29	Crossover	No	10	Yes	Cold extremities
Pasotti <i>et al.</i> [60]	Italy	HT	Pindolol, metoprolol	16	Crossover	Yes	12	Yes	Cold extremities
Iliuta <i>et al.</i> [61]	Romania	CABPG	Betaxolol, metoprolol	1352	Parallel	No	4	Yes	Cold extremities
Vandenburch <i>et al.</i> [62]	UK	HT	Propranolol, α -adrenoceptor blocker	60	Parallel	Yes	10	No	Cold extremities
Dety <i>et al.</i> [63]	International	Angina	Propranolol, trimetazidine	149	Parallel	Yes	12	Yes	Cold extremities
Salonen <i>et al.</i> [64]	Finland	HT	Betaxolol, placebo	60	Crossover	No	4	Yes	Cold hands and feet

(continues)

Table 1
(Continued)

Study	Country	Indication	Treatment	n	Methodology	Exclude RP	Exposure (weeks)	Double-blind	Peripheral vasoconstriction symptom
Bühler et al. [65]	Europe	HT	Bisoprolol, atenolol	94	Crossover	Yes	8	Yes	Cold extremities
De Muinck et al. [66]	Europe Nord	Angina	Bisoprolol, atenolol	175	Parallel	No	12	Yes	Cold extremities
Pedersen et al. [67]	Denmark	HT	Metoprolol, thiazide	20	Crossover	No	8	Yes	Cold extremities

HT: hypertension; MI myocardial infarction; HF heart failure; VB: variceal bleeding; T2DM: type two diabetes mellitus; CAPPG: coronary artery bypass grafting. CCB: calcium channel blockers; ACE/ARB: angiotensin converting enzyme inhibitors /angiotensin II receptor blockers.

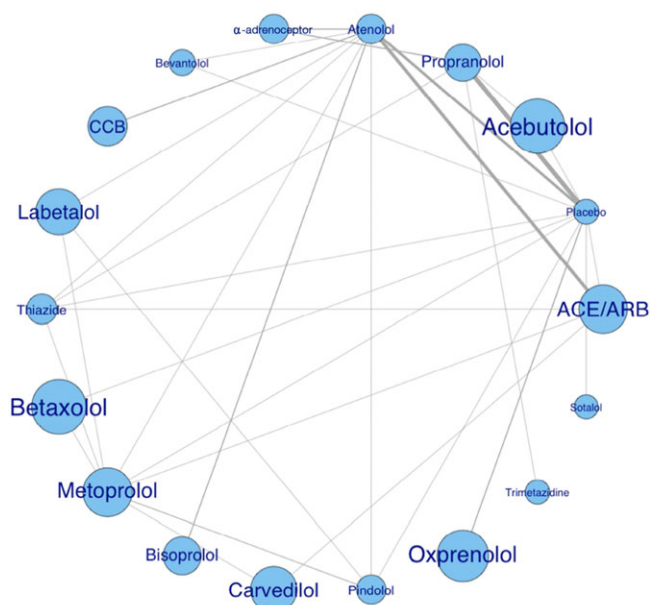


Figure 2

Network of available comparisons between the different β -adrenoceptor blockers and controls. Size of node is proportional to number of trials participants and thickness of the lines is proportional to number of trials that included the direct comparisons. CCB calcium channel blockers; ACE/ARB angiotensin converting enzyme inhibitors/angiotensin II receptor blockers

duration ($P = 0.06$), year of publication ($P = 0.19$), way of reporting adverse effect ($P = 0.39$) and RP as a non-inclusion criterion for the trial ($P = 0.21$).

Discussion

In our study 7% of the 28 072 patients taking β -adrenoceptor blockers suffered from RP or cold extremities, whereas only 4.6% did so when on placebo. We showed that β -adrenoceptor blockers represent a highly heterogeneous family regarding their propensity to induce RP, and some ancillary properties such as a vasodilator effect or ISA are somewhat protective, while β_1 -selectivity is not.

The present work brings additional information to what was known about the prevalence of peripheral vasoconstriction induced by β -adrenoceptor blockers. The prevalence of 7% found in our study is lower than in the studies assessing it in the general population. A general practice based study in the UK found that 14.5% of patients responding to a postal survey and 19% of patients attending surgeries have RP-related symptoms. [68]. A community based study from the US reported RP in 11% of women and 8% of men [69]. In a recent meta-analysis, the prevalence of RP in patients receiving β -adrenoceptor blockers was 14.7% [8]. Included studies were clinical cohort, or case-control studies and for most of them RP symptoms were also reported using a questionnaire. This is close to what we found in studies reporting adverse effects with a questionnaire (prevalence of 13.5%) [38, 39, 53, 54, 56, 59, 65]. In this meta-analysis the influence of the way to report symptoms on the prevalence of

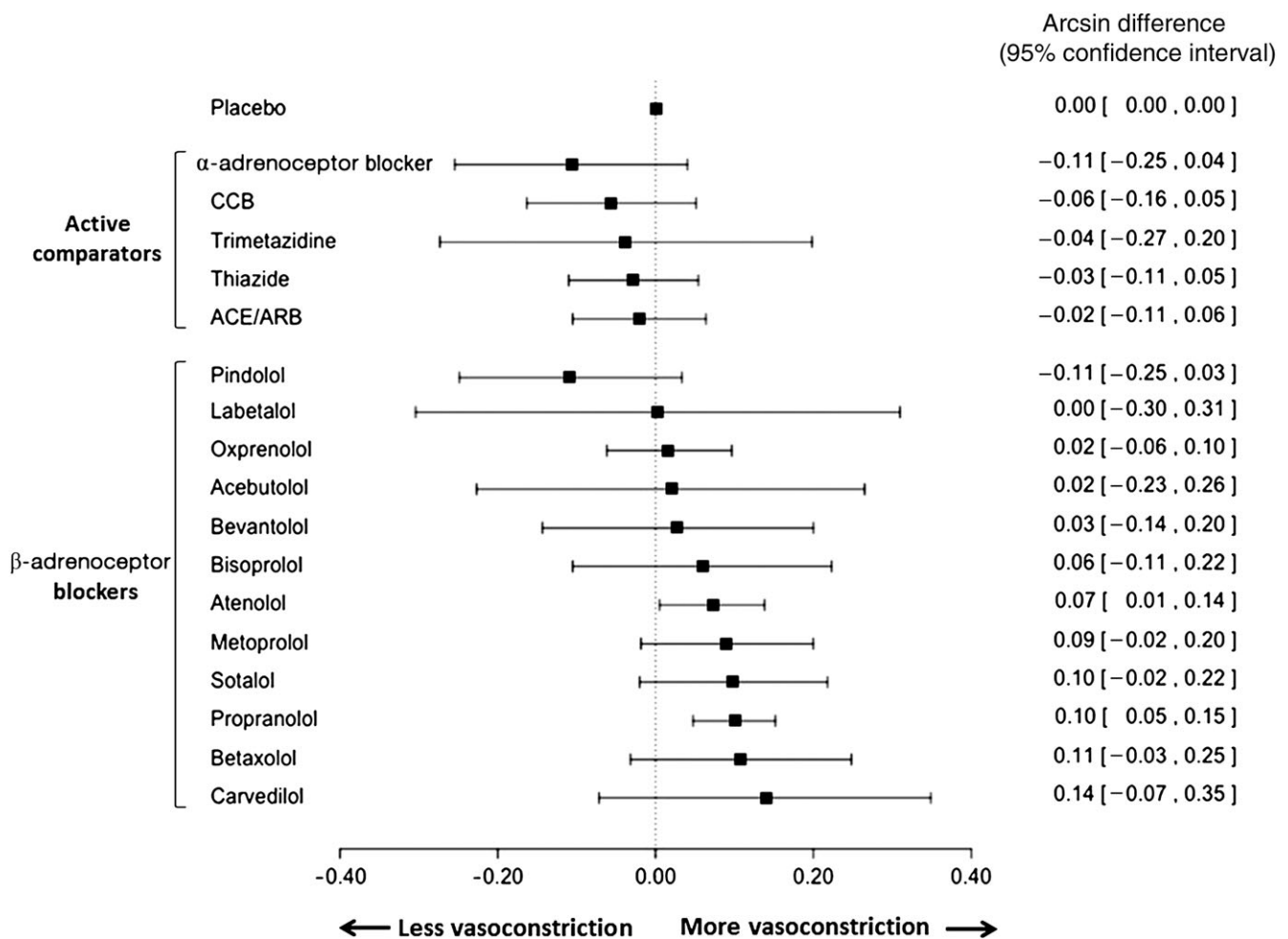


Figure 3

Forest plot, effect size estimated through the arcsin difference. CCB calcium channel blockers; ACE/ARB angiotensin converting enzyme inhibitors /angiotensin II receptor blockers

peripheral vasoconstriction was obvious (13.47% with a questionnaire vs. 6.02% for spontaneous reports), although non-significant, and should certainly be assessed in every meta-analysis focusing on side effects. Another explanation of the low prevalence observed in the present work was that RP was a non-inclusion criterion in 20 out of the 38 studies included. Although including or not patients with RP in trials obviously changes prevalence, it does not affect the general conclusion of the network meta-analysis. Finally, one should admit that there was considerable heterogeneity between studies [49, 56]. This variability probably reflects differences in the definition of RP or cold extremities and, in most cases, the lack of objective criteria to assess peripheral vasoconstriction.

Cold hands and RP were rapidly linked to the use of the first β -adrenoceptor blocker, propranolol [70]. Propranolol is a non-selective β_1 - and β_2 -adrenoceptor antagonist devoid of ISA and vasodilator activity. Activity on β_2 -adrenoceptors was first incriminated in the pathophysiology of peripheral vasoconstriction related to β -adrenoceptor blocker intake. Indeed, β_2 -adrenoceptors are involved in the vasodilator tone of blood vessels in skeletal muscle. However, studies did not show any difference in the frequency of the feeling of cold

hands according to β_1 -selectivity of β -adrenoceptor blockers [71–73]. Based on a large basis of available evidence, our study further shows that drugs with higher affinity for β_1 - than for β_2 -adrenoceptors, such as atenolol, also induce significantly more peripheral vasoconstriction than placebo.

There is also a rationale for a link between ISA and the reduction of peripheral vasoconstriction. Indeed, β -adrenoceptor blockers with ISA induce smaller falls in cardiac output and do not lead to the same baroreceptor-dependent reflex vasoconstriction as that observed with β -adrenoceptor blockers devoid of ISA [12, 74]. Pindolol is the β -adrenoceptor blocker with the highest ISA, followed by acebutolol, celiprolol and oxprenolol. Yet, in our study these β -adrenoceptor blockers are among those inducing the least peripheral vasoconstriction-related symptoms. This is consistent with experimental data showing that brachial artery infusion of pindolol leads to a dose-dependent increase of forearm blood flow, that may be reduced by concomitant infusion of propranolol [75]. The ISA of pindolol is so large that stimulation of β_2 -adrenoceptors is produced, leading to vasodilatation and the relaxation produced by pindolol or celiprolol can partly be antagonized by pretreatment with propranolol or

Table 2

β -adrenoceptor blockers characteristics. We excluded β -adrenoceptor blockers used only for ophthalmic use in France: alprenolol, carteolol, levobunolol, metipranolol, metipranolol, penbutolol and timolol

β -adrenoceptor blocker	β_1 -selectivity	ISA*	MSA**	Vasodilator activity	Half-life (h)	Mean usual hypertension dose (mg day ⁻¹)	Clinical application	Contra-indication with RP in USA	Contra-indication with RP in France	Contra-indication with RP in UK
•Propranolol	0	0	++	0	3.5–6	160	Hypertension, angina pectoris, migraine, hyperthyroidism, arrhythmias	No	Yes	Severe form
•Nadolol	0	0	0	0	14–24	160		No	Yes	No
•Sotalol	0	0	0	0	12	160–320		No	Yes	Yes
•Metoprolol	+	0	0	0	3–4	100–200	Hypertension, angina pectoris, arrhythmias	Severe form	Severe form	Severe form
•Atenolol	+	0	0	0	6–9	50–100		Precaution	Severe form	Severe form
•Betaxolol	+	0	0	Ca ⁺⁺ entry blockade	14–22	20		No	Severe form	Precaution
•Nebivolol	+	0	0	Nitric oxide release	11–30	5–10		Precaution	Severe form	Severe form
•Bisoprolol	+	0	0	0	9–12	5–10		Precaution	Severe form	Severe form
•Bevantolol	+	0	+	α_1 -adrenoceptor blockade, Ca ⁺⁺ entry blockade	2	150–300		Not available	Not available	Not available
•Xamoterol	+	+	0	0				Not available	Not available	Not available
•Pindolol	0	++	0	0	3–4	15	Hypertension, migraine, arrhythmias	No	Yes	Yes
•Acebutolol	+	+	+	0	3–4	400		Precaution	Severe form	Severe form
•Oxprenolol	0	+	+	0	1–2	320		Not available	Yes	Severe form
•Celiprolol	+	+	–	β_2 -adrenoceptor agonist, nitric oxide release	4–5	200–400		Not available	No	Severe form
•Carvedilol	0	0	0	α_1 -adrenoceptor blockade, Ca ⁺⁺ entry, blockade, antioxidant activity	7–10	25–50	Heart failure	Precaution	Yes	Precaution
•Labetalol	0	0	0	α_1 -adrenoceptor blockade	5	400	HTA	No	No	Severe form

0 = absent or low, + = moderate; ++ = high; – = no information; *ISA = intrinsic sympathomimetic activity; **MSA = membrane-stabilizing activity.

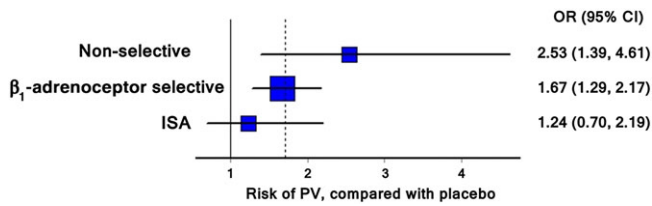


Figure 4

Comparison of the risk of peripheral vasoconstriction according to the pharmacological properties of β -adrenoceptor blockers. Only direct comparisons vs. placebo were included and a random effect model was used. ISA intrinsic sympathomimetic activity

sotalol [76–78]. Several clinical studies have previously reached similar conclusions. Direct comparison between pindolol and propranolol showed a decreased risk of peripheral vasoconstriction with pindolol [79]. A UK study including 7659 patients with hypertension in general practice found that peripheral vasoconstriction-related symptoms were more pronounced in patients taking β -adrenoceptor blockers than other hypertensive treatment (4.1% vs. 0.2%), but that patients taking β -adrenoceptor blockers with ISA complained less frequently than those on other β -adrenoceptor blockers (3.1% vs. 5.2%) [72].

Interestingly, in our study bevantolol and labetalol, two β -adrenoceptor blockers with vasodilator activity through α_2 -adrenoceptor antagonism, are among drugs inducing the least peripheral vasoconstriction. In line with our results, α_2 adrenoceptor-induced vasoconstriction is increased in patients with Raynaud's phenomenon and selective inhibition of α_2 -adrenoceptors reduces digital artery vasospastic attacks [2, 11]. Furthermore, we did not find any study implicating nebivolol and celiprolol, two β -adrenoceptor blockers with vasodilator activity through nitric oxide release, suggesting that patients taking these β -adrenoceptor blockers did not complain of peripheral vasoconstriction symptoms although large randomized controlled trials including thousands of patients and assessing the efficacy of nebivolol such as SENIORS study exist [80].

Overall, the results of this work challenge the relevance of the contraindication of β -adrenoceptor blockers in patients with peripheral vascular disease (Table 2). In the USA, propranolol, nadolol, sotalol, betaxolol, pindolol and labetalol are not contraindicated. Metoprolol is contraindicated in severe forms of peripheral circulatory disorder and precaution is recommended for atenolol, nebivolol, bisoprolol, acebutolol and carvedilol in patients with peripheral vascular disease. In France, carvedilol, nadolol, oxprenolol, pindolol, propranolol and sotalol are contraindicated in patients with RP. Acebutolol, betaxolol, bisoprolol, metoprolol and nebivolol are contraindicated only in severe forms, whereas celiprolol and labetalol are not contraindicated. It appears that contraindications vary between countries and that they do not seem to be based on available evidence.

Network meta-analysis is a relevant approach in pharmacovigilance, especially to test the homogeneity of a class adverse effect. Although this methodological approach is becoming more accessible thanks to the availability of dedicated statistics packages, its use remains limited in safety studies. The development of approaches and recommendations to appraise the quality of a treatment effect estimated

from a network meta-analysis participates toward standardizing practices. To our knowledge, this is the first network meta-analysis with a safety purpose that uses the GRADE recommendation to assess the quality of direct and indirect comparisons. This approach includes assessment of five items for each pairwise comparison: risk of bias [23], inconsistency [24], indirectness [25] and imprecision [26] and publication bias [27]. The risk of bias for each pairwise comparison was assessed in the light of the weight of each study involved, as advised in GRADE recommendations. In general, the risk of bias was relatively low in the studies that we included and overall the quality of direct comparisons was reasonable. Heterogeneity was >40% in only 2/34 pairwise comparisons reflecting consistency of our results. However, many pairwise comparisons based on indirect comparisons have a low level of evidence. The exchangeability property of the included studies in this network meta-analysis was respected because no interaction between the effect estimate and the factors known to modify the risk of peripheral vasoconstriction (e.g. duration of treatment, drug dose, drug indication, year of publication, way of reporting adverse effect and RP as a non-inclusion criterion for the trial) was highlighted in the sensitivity analysis.

Another limitation is that we reduced our literature searches in the PubMed database to 'core clinical journals' only, possibly leading to a publication bias. However this study did not aim to assess an efficacy criterion of β -adrenoceptor blockers for which exhaustivity would have been mandatory. Indeed, we supposed that no clinical trial was unpublished or stopped because of RP or cold extremities. This restriction was imposed by the impressive amount of available data when considering β -adrenoceptor blockers. We were unable to consider all β -adrenoceptor blockers in our analysis, as well designed RCTs were lacking for some drugs.

Finally, the number of studies that reported peripheral vasoconstriction-related symptoms in the publication was low. Indeed, as it is often considered as well-known and benign, peripheral vasoconstriction-related symptoms may be omitted in study reports and thus only <5% of eligible studies were included in our analysis. This stresses the need for making data from clinical trials widely available for further analyses with safety purposes.

Conclusion

While peripheral vasoconstriction-related symptoms induced by β -adrenoceptor blockers have long been known to be side effects, this network meta-analysis provides evidence that this should not be considered as a homogeneous class effect. Ancillary properties such ISA and vasodilator effects are protective. On the other hand, a higher affinity for β_1 -adrenoceptors does not protect from RP, which challenges current recommendations and contraindications.

Competing Interests

All authors have completed the Unified Competing Interest form and declare no support from any organization for the submitted work, no financial relationships with any organizations that might have an interest in the submitted work

in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work.

We thank Dr Alison Foote (Grenoble Clinical Research Center) for editing the manuscript.

Contributors

CK, TJ, SB, PC, JLC and MR wrote manuscript, MR designed research, CK and MR performed the research and TJ analyzed the data.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

<http://onlinelibrary.wiley.com/doi/10.1111/bcp.12980/supinfo>.

Table S1 Quality ratings following GRADE recommendations for comparison of peripheral vasoconstriction induced by β -adrenoceptor blockers. CCB calcium channel blockers; ACE/ARB angiotensin converting enzyme inhibitors /angiotensin II receptor blockers

Table S2 Number of direct and indirect comparisons included in the network meta-analysis, average GRADE quality rating summary and percentage of high quality studies for each β -adrenoceptor blocker

Table S3 Number of direct and indirect comparisons included in the network meta-analysis, mean GRADE quality rating summary and percentage of high quality studies for each pharmacologic group of β -adrenoceptor blocker. ISA intrinsic sympathomimetic activity. VD vasodilator activity

Figure S1 The risk of bias summary

Figure S2 Rankograms represent for each treatment on the horizontal axis the 18 possible ranks (from left to right the risk of peripheral vasoconstriction decreases) and on the vertical axis the probability to achieve each rank. CCB calcium channel blockers; ACE/ARB angiotensin converting enzyme inhibitors/angiotensin II receptor blockers

3. Fluoxetine and Raynaud's phenomenon: friend or foe?

En réalisant la revue systématique sur les étiologies iatrogènes du phénomène de Raynaud nous avons également remarqué que les inhibiteurs sélectifs de la recapture de la sérotonine étaient à la fois recommandés dans la prise en charge du phénomène de Raynaud alors qu'ils étaient décrits chez certains patients comme pouvant être à l'origine de l'apparition ou de l'aggravation de leur phénomène de Raynaud. (29,110) Nous avons tenté dans ce court article d'émettre des hypothèses afin d'expliquer ce phénomène paradoxal.

LETTER TO THE EDITOR

Fluoxetine and Raynaud's phenomenon:
friend or foe?

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Received 11 January 2017; **Revised** 7 April 2017; **Accepted** 16 April 2017

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Keywords fluoxetine, Raynaud's phenomenon, systemic sclerosis

Tables of Links

TARGETS	
G protein-coupled receptors [2]	Enzymes [4]
5-HT _{2A} receptor	Adenylate cyclase
5-HT _{2B} receptor	eNOS
5-HT _{1B} receptor	Nitric oxide (NO)-sensitive (soluble) guanylyl cyclase
5-HT ₇ receptor	
CGRP receptor	
Voltage-gated ion channels [3]	
Calcium-activated potassium channels	
Voltage-gated calcium channels	

LIGANDS
Fluoxetine
Nifedipine

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY [1], and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 [2–4].

Whether fluoxetine, a selective serotonin reuptake inhibitor, is an effective treatment for Raynaud's phenomenon (RP) has been debated for about 20 years. Based on one positive efficacy trial [5] and some preliminary observations [6], fluoxetine is recommended in RP secondary to systemic sclerosis (SSc), after failure of calcium channel blockers [7]. However, when one looks closely at the available

evidence, the lack of a homogeneous effect of fluoxetine in RP patients is obvious.

The crossover study comparing the efficacy of nifedipine and fluoxetine in 56 patients with primary or secondary RP showed a significant improvement in the Raynaud's condition score (RCS) [4.35 (0.39) vs. 2.3 (0.35); $P = 0.0002$] and daily frequency of attacks [2.98 (0.31) vs. 1.7 (0.25);

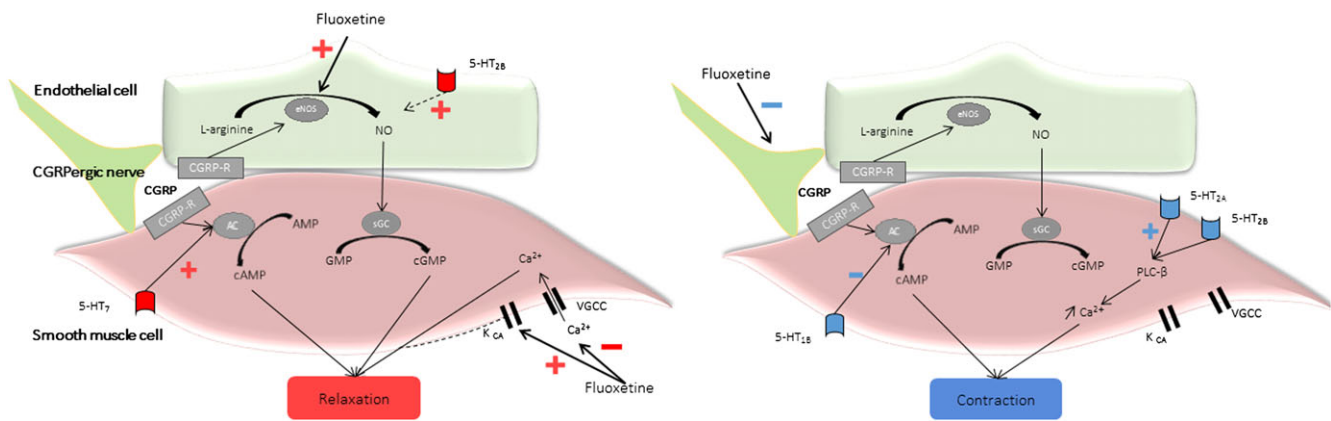


Figure 1

Co-existing vasodilator-vasoconstrictor pharmacodynamic effects of fluoxetine. AC, adenylyl cyclase; AMP, adenosine monophosphate; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; CGRP, calcitonin gene-related peptide; eNOS, endothelial NO synthase; GMP, guanosine monophosphate; K_{Ca}, calcium-sensitive potassium channel; NO, nitric oxide; PLC-β, phospholipase C beta; R-CGRP, calcitonin gene-related peptide receptor; sGC, soluble guanylate cyclase; VGCC, voltage-gated calcium channels

$P = 0.003$] [4]. However, when looked at more carefully, subgroup analysis showed a significant benefit for RCS and the frequency of attacks in primary RP, while only RCS was significantly improved in patients with secondary RP. Likewise, the secondary criterion of percentage of rewarming after cold challenge was positive in primary RP [33.4% ($\pm 7.5\%$) vs. 58.8% ($\pm 8.7\%$); $P = 0.03$] but negative in secondary RP [31.6% ($\pm 6.4\%$) vs. 31.2% ($\pm 8.2\%$); $P = 0.97$]. Furthermore, we could hypothesize that the antidepressant activity of fluoxetine may have a significant impact on a subjective measurement of self-reported outcomes such as RCS. Owing to the discovery of thrombocyte dysfunction correlated with an increase in intraplatelet serotonin in RP, the antiaggregant effect of fluoxetine was hypothesized to be the main mechanism [8]. However, later studies using antithrombotic drugs were disappointing and evidence of their benefit in RP is now limited [9].

The involvement of the serotonergic pathway in vascular tone is complex; serotonin causes direct vasoconstriction through 5HT_{2A}, 5HT_{2B} and 5HT_{1B} receptors [10]. Experimental data also suggest that serotonin released from adrenergic nerves inhibits calcitonin gene-related peptide-containing nerve-dependent vasodilation [11]. By contrast, vasodilation is mediated through 5HT₇ and 5HT_{2B} receptors, located on smooth muscle cells and on the endothelium, respectively [10]. Endothelium-dependent vasodilation would be secondary to increased nitric oxide (NO) bioavailability, through enhanced endothelial NO synthase activity [11, 12]. Mechanisms underlying direct activity on smooth muscle cells may involve activation of calcium-sensitive potassium channels [13] and inhibition of voltage-gated calcium channels [12] (Figure 1).

Whether fluoxetine increases, through the reduction in serotonin reuptake into platelets, or decreases, through the sequestration of serotonin at the intestinal level, the plasma serotonin concentration is still controversial [14]. However, this probably has a limited impact, considering that vasomodulation mediated by fluoxetine is not dependent on plasma serotonin concentration [15].

In light of the clinical discrepancies described above, we raise the hypothesis that in SSC, endothelial dysfunction could explain the reduced vasodilator effect of fluoxetine, and could even switch the balance between vasoconstriction and vasodilation.

We therefore believe that there is insufficient scientific evidence to recommend fluoxetine as a treatment in SSC-related RP. A well-designed, double-blinded clinical trial that properly stratifies patients according to RP aetiology would address this question.

Competing Interests

All authors have completed the Unified Competing Interest form and declare no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

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4. Proton pump inhibitors and Raynaud's phenomenon: is there a link ?

A la suite de la découverte récente d'une action potentialisatrice des inhibiteurs de la pompe à proton sur une enzyme, l'asymmetric dimethylarginine (ADMA), que l'on pense être impliquée dans la physiopathologie du phénomène de Raynaud, nous avons réalisé une étude de disproportionnalité sur la base de pharmacovigilance de l'OMS afin d'identifier de potentiels signaux de disproportionnalités de phénomène de Raynaud avec cette classe médicamenteuse. (10,111)

LETTER TO THE EDITOR

Proton pump inhibitors and Raynaud's phenomenon: is there a link?

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Received 9 March 2018; **Revised** 15 June 2018; **Accepted** 18 June 2018

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Keywords drug safety, proton pump inhibitors, Raynaud's phenomenon

Proton pump inhibitors (PPI) are a worldwide used drug class for the treatment of gastric reflux. Recent epidemiological studies have raised concern about the increased cardiovascular risk of long-term PPI use [1, 2]. One of the hypothesized pathophysiological mechanism is that PPI inhibit the degradation of plasma **asymmetric dimethylarginine (ADMA)** through inhibition of the **dimethylarginine dimethylaminohydrolases**, which largely metabolize ADMA [3]. ADMA is an endothelial nitric oxide synthase (NOS) competitive inhibitor, which is a prognostic biomarker of major cardiovascular events [4, 5]. Moreover, recent data show that treatment with esomeprazole increased superoxide anions production, decreased endothelial and inducible NOS expression and accelerates human endothelial senescence by reducing telomere length [5]. Such mechanisms are also involved in the pathophysiology of Raynaud's phenomenon (RP) [6]. However, whether the use of PPI is associated with an increased risk of developing or aggravating RP has never been explored.

To further address this issue, we performed a disproportionality analysis in the WHO pharmacovigilance database VigiBase®. Our objective was to compare the Proportional Risk Ratio (PRR) of RP associated with PPI and with histamine H2 antagonists, used as control. PRR is the ratio between the rate of reporting of one effect among all reports for a given drug and the rate of reporting of the same effect among all reports for all drugs in the database [7]. We extracted all individual cases safety reports (ICSRs) of RP associated with PPI (ATC A02BC) and H2 antagonists (ATC A02BA), considered as either suspect or concomitant medication. When a report was associated with both PPIs and H2 antagonists, it was counted in each drug class. The cut-off for signal detection was defined as a lower boundary of the PRR 95% confidence interval greater or equal to 1, and number of

reports greater or equal to 3, according to the European Medicines Agency [8]. Frequencies of reports between drugs were compared using the χ^2 test, and a P value < 0.05 was considered significant.

Among the 16 403 009 ICSRs reported in VigiBase® on 2018.02.01, 753 854 were related to PPI use and 269 663 to H2 antagonists. We identified 253 reports of RP associated with PPI and 48 with H2 antagonists. The PRR was above signal detection for the PPI drug class and for each PPI, whereas the cut-off for signal detection was not reached for H2 antagonists (Figure 1). The difference in PRR between the two groups was statistically significantly. Characteristics of RP reports are presented in Supplementary Table 1. We observed no difference in potential effect modifiers (age, sex, concomitant medications or pathology known to induce RP) between groups.

In conclusion, our results reveal a positive disproportionality signal of RP for PPIs, with a significantly higher reporting rate than for H2 antagonists. However, our analysis relies on a limited number of reports and is exposed to biases inherent to pharmacovigilance studies, such as selective reporting, media bias and limited ability to control for potential confounders. Further large and well-controlled epidemiological studies are therefore needed to better characterize the risk of RP with PPI.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY [9], and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 [10].

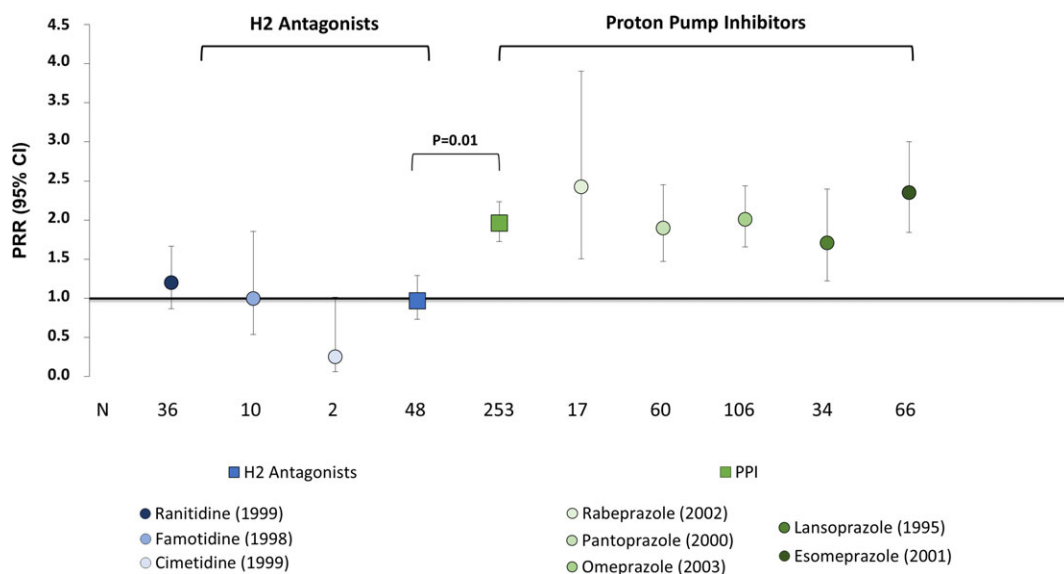


Figure 1

Proportional Reporting Ratio (PRR) of Raynaud's phenomenon associated with proton pump inhibitors and H2 antagonists drug classes and main belonging drugs (with 95% confidence intervals). For each drug, the FDA approval date is provided. P value of the χ^2 test comparing frequencies of reports between drug classes. N, number of reports

Competing Interests

There are no competing interests to declare.

The authors would like to thank the Uppsala Monitoring Centre that provided and gave permission to use the data analysed in the present study. Results and conclusions are those of the authors and not necessarily those of the National Centers, UMC or WHO.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

<http://onlinelibrary.wiley.com/doi/10.1111/bcp.13697/supinfo>

Table S1 Pooled data retrieved from Vigibase® reports of Proton Pump Inhibitors associated with Raynaud's phenomenon compared to H2 antagonists

5. Comparative efficacy and safety of treatments for secondary Raynaud's phenomenon: systematic review and network meta-analysis of randomized trials.

A la suite de la publication récente des dernières recommandations de la société européenne de rhumatologie dans la prise en charge du phénomène de Raynaud secondaire à la sclérodémie systémique, l'absence de données comparatives sur l'efficacité et la sécurité des différentes classes pharmacologiques utilisées dans le traitement du phénomène de Raynaud nous a paru évidente. (29) Nous avons donc réalisé une méta-analyse en réseau afin de comparer l'efficacité et la sécurité de toutes les classes médicamenteuses qui ont été testées dans le phénomène de Raynaud associé à la sclérodémie.

Comparative efficacy and safety of treatments for secondary Raynaud's phenomenon: a systematic review and network meta-analysis of randomised trials



Charles Khouri, Marion Lepelley, Sebastien Bailly, Sophie Blaise, Ariane L Herrick, Marco Matucci-Cerinic, Yannick Allanore, Ludovic Trinquart, Jean-Luc Cracowski, Matthieu Roustit

Summary

Background Several pharmacological treatments are available for secondary Raynaud's phenomenon, but there is uncertainty regarding the best options. We aimed to assess and compare the benefits and harms of treatments available for secondary Raynaud's phenomenon.

Method We did a systematic review and network meta-analysis of randomised controlled trials (RCTs) of pharmacological treatments. We searched for systematic reviews published in MEDLINE and the Cochrane Database of Systematic Reviews up to Jan 31, 2017, and for RCTs published from inception to Sept 24, 2019 in MEDLINE, Embase, and ClinicalTrials.gov. We included double-blind RCTs (parallel or crossover) that compared two or more pharmacological treatments or placebo in patients with secondary Raynaud's phenomenon. Individual patient data were obtained for one unpublished RCT. Three researchers independently screened the texts and extracted the data. Efficacy outcomes included severity (on a ten-point scale), daily frequency, and mean duration of Raynaud's phenomenon attacks. We also examined tolerability and acceptability. Pairwise meta-analyses and Bayesian random-effects network meta-analyses were used to synthesise data. This study is registered with PROSPERO (CRD42017057518).

Findings We included 58 RCTs in the analysis, comprising 3867 patients (3540 [91.5%] with secondary Raynaud's phenomenon) and 15 classes of drugs. Phosphodiesterase 5 (PDE5) inhibitors were more effective than placebo for frequency (mean difference -0.36 [95% credibility interval -0.69 to -0.04]), severity (-0.34 [-0.66 to -0.03]), and duration (-3.42 [-6.62 to -0.29]) of attacks (low to moderate level of evidence). Calcium channel blockers (CCBs) were superior to placebo for frequency (-0.35 [-0.67 to -0.02]) and severity (-0.84 [-1.25 to -0.45]) of attacks (low level of evidence). For severity of attacks, selective serotonin-reuptake inhibitors (-1.54 [-2.68 to -0.41]; very low level of evidence) and oral prostacyclin receptor agonists (-0.48 [-0.80 to -0.16]; low level of evidence) were superior to placebo. No other drug classes were significantly superior to placebo with regard to efficacy outcomes. Compared with placebo, tolerability was lower for PDE5 inhibitors (incidence rate ratio for serious adverse events or early study exit due to adverse events 3.30 [95% CrI 1.49 to 7.55]) and CCBs (3.13 [1.33 to 7.04]). For all outcomes, global heterogeneity and between-study variance ranged from low ($I^2=0\%$ and $\tau^2=0.0$ for attack severity and duration) to moderate ($I^2=41\%$ and $\tau^2=0.2$ for tolerability). The overall risk of bias was judged to be low in 22 (38%), high in ten (17%), and unclear in 26 (45%) RCTs.

Interpretation PDE5 inhibitors and CCBs are the most effective pharmacological options, albeit with moderate efficacy and a low level of evidence. Current evidence does not support the use of any other drug in secondary Raynaud's phenomenon.

Funding None.

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Introduction

Raynaud's phenomenon is the occurrence of paroxysmal episodes of a localised deficiency in cutaneous perfusion, most often in response to cold or emotional stress.¹ It affects 3–5% of the general population, with substantial geographical variations.² Raynaud's phenomenon can be primary (idiopathic) or secondary to a connective tissue disease, especially systemic sclerosis. Raynaud's phenomenon is present in up to 95% of patients with systemic sclerosis,² and is the earliest sign of vasculopathy in such patients. Systemic sclerosis-related microvascular

impairment is associated with significant morbidity (eg, ulcers and gangrene) and functional disability.³

The 2016 European League Against Rheumatism (EULAR) recommendations advise oral calcium-channel blockers (CCBs) as first-line therapy for systemic sclerosis-related Raynaud's phenomenon, and phosphodiesterase 5 (PDE5) inhibitors for patients with systemic sclerosis with severe Raynaud's phenomenon or those who do not respond to CCBs.⁴ CCBs and PDE5 inhibitors have been shown to have moderate efficacy in reducing the frequency and the severity of Raynaud's phenomenon in

Lancet Rheumatol 2019;
1: e237–46

See [Comment](#) page e200

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Research in context**Evidence before this study**

Raynaud's phenomenon can be secondary to connective tissue diseases, especially systemic sclerosis, where it represents the most frequent and earliest sign of vasculopathy. The updated European League Against Rheumatism guidelines recommend oral calcium-channel blockers (CCBs) or phosphodiesterase 5 (PDE5) inhibitors as first-line therapy for systemic sclerosis-related Raynaud's phenomenon. However, whether one treatment is superior over the other is unknown because of the lack of direct or indirect comparisons. Other drugs (such as endothelin receptor antagonists, prostacyclin analogues, or non-prostanoid agonists of the prostacyclin receptor) have been proposed, but the place of these options within the treatment strategy remains unclear. We searched PubMed for meta-analyses on the treatment of secondary Raynaud's phenomenon published between database inception and Aug 8, 2019. Using the search terms "Raynaud's phenomenon" AND ("meta-analysis" or "network meta-analysis"), we found several meta-analyses that have assessed the efficacy of the different treatments used in systemic sclerosis-related Raynaud's phenomenon, especially CCBs and PDE5 inhibitors. However, to our knowledge, none has combined direct and indirect comparisons through a network meta-analysis approach to assess and compare the efficacy and tolerability of all available treatments for secondary Raynaud's phenomenon.

Added value of this study

We did a network meta-analysis combining direct and indirect comparisons to assess and compare the efficacy and tolerability of all available treatments for secondary Raynaud's phenomenon. We included 58 RCTs (3867 patients) and 15 classes of drugs. We also had access to the full dataset of the largest, unpublished trial on a PDE5 inhibitors in Raynaud's phenomenon (NCT01090492), which we reanalysed and included in our meta-analysis. Our results show that CCBs and PDE5 inhibitors are superior to placebo for most efficacy outcomes, with similar but moderate treatment effects, and with a low to moderate level of evidence. For all other oral treatments, the efficacy is not consistent across outcomes, or the level of evidence is low to very low.

Implications of all the available evidence

The two treatments recommended as first-line therapy (CCBs and PDE5 inhibitors) are both superior to placebo but the treatment effect is below the minimal clinically important difference. For all other drugs, our results highlight that the available evidence is too weak to support any recommendation. Our results thus challenge the clinical relevance of these treatments, emphasising the pressing need for the development of new therapeutic strategies, including non-pharmacological interventions.

patients with systemic sclerosis.^{5,6} Intravenous iloprost also reduces the frequency and severity of attacks, and is recommended for severe Raynaud's phenomenon secondary to systemic sclerosis when oral therapies (including CCBs and PDE5 inhibitors) have failed.^{4,7} Other treatments such as fluoxetine have been tested in patients with systemic sclerosis-related Raynaud's phenomenon. However, whether any treatment is superior to another remains unknown as few randomised controlled trials (RCTs) have directly compared these different treatments. In addition, the place of newer agents, such as endothelin receptor antagonists and oral prostacyclin receptor agonists (including prostacyclin analogues and non-prostanoid agonists), among treatment options remains unclear.^{8,9}

To compare the efficacy and safety of all pharmacological treatments that have been tested in systemic sclerosis-related Raynaud's phenomenon, we did a systematic review of RCTs with network meta-analyses.

Methods**Overview**

We did a systematic review and network meta-analysis in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analysis statement guidelines.^{10,11} The study is registered with PROSPERO (CRD42017057518) and the protocol and systematic search strategy are available online.

Literature searches

We searched for narrative or expert reviews, systematic reviews, and meta-analyses indexed in MEDLINE or the Cochrane Database of Systematic Reviews on treatments for secondary Raynaud's phenomenon or for systemic sclerosis-related digital ulcers, published from inception to Jan 31, 2017. We screened all trials included in the reviews we found. In addition, we searched for RCTs published from database inception to Sept 24, 2019 in MEDLINE, Embase, the ClinicalTrials.gov registry, and the Addis Insight database. Finally, we asked key opinion leaders in the field. No language restriction was applied. Details of the search strategy are available in the appendix (pp 3–4).

Study selection and data extraction

Double-blind RCTs were eligible if they met the following criteria: had a parallel or crossover design; included patients with secondary Raynaud's phenomenon; compared two or more pharmacological treatments or a treatment versus placebo; and reported at least one outcome of interest. A crossover trial was eligible only if there was a washout period of 1 week or more. Trials that included patients with primary or secondary Raynaud's phenomenon were eligible if outcome data were reported separately for those with secondary Raynaud's phenomenon, or if more than 50% of patients had secondary Raynaud's phenomenon.

See Online for appendix

For the study protocol and search strategy see http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42017057518

All reviews were screened independently by two investigators for relevance and all RCTs identified. Titles, abstracts, and subsequently the full texts of all RCTs (identified from reviews or our de novo search) were evaluated independently for eligibility by three investigators. Disagreements were resolved by consensus.

The following data were extracted for each selected RCT: study characteristics (year of publication, country or countries, latitude [for single-country studies only], funding source, follow-up duration, study design, and primary outcome); participant characteristics (age, sex ratio, aetiology of Raynaud's phenomenon, proportion of smokers, disease duration, baseline frequency, severity and mean duration of Raynaud's phenomenon attacks); and details of the intervention (add-on therapy, dosage, treatment duration, and therapeutic class).

We found unpublished results for two RCTs in ClinicalTrials.gov. For one RCT (NCT01090492) of a PDE5 inhibitor, for which posted results pertained to the per-protocol population, we obtained access to the individual patient data through the sponsor and reanalysed the intention-to-treat population (appendix p 5).

Outcomes

We prespecified three efficacy outcomes: mean daily frequency of Raynaud's phenomenon attacks; mean severity of Raynaud's phenomenon attacks measured using the Raynaud's Condition Score (RCS), a visual analogue scale, or any other severity score; and mean duration of each attack. For mean severity of attacks, when several measures were used, we prioritised RCS because it is a more comprehensive measure of Raynaud's phenomenon severity, taking into account disability and impact on quality of life.^{12,13} If RCS was not reported, other severity scores were extracted and converted into the ten-point scale used for the RCS. Tolerability was defined by the proportion of patients who reported a serious adverse event or dropped out of the study early because of adverse events. Finally, we assessed acceptability, defined as all-cause discontinuation, which encompasses both efficacy and tolerability.

Data synthesis

All pharmacological treatments were grouped into 15 therapeutic classes: α -adrenoceptor antagonists, antioxidants, anti-interleukin 6, angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers, botulinum toxin type A, CCBs, endothelin receptor antagonists, PDE5 inhibitors, phosphodiesterase 3 inhibitors, intravenous prostacyclin analogues, oral prostacyclin receptor agonists (prostacyclin analogues or non-prostanoid agonists), selective serotonin-reuptake inhibitors (SSRIs), soluble guanylate-cyclase stimulators, topical nitric oxide donors, and thromboxane synthase inhibitors. When a study reported several groups with different doses of the same drug, these groups were merged.

We measured treatment effect using the mean difference for efficacy outcomes and incidence rate ratios (IRR)

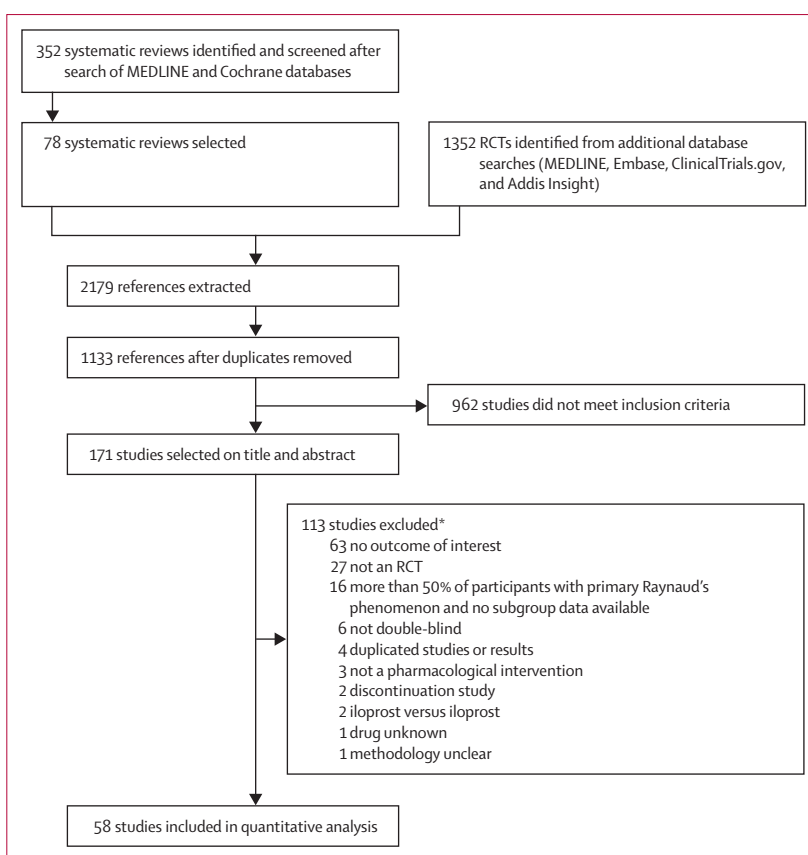


Figure 1: Study selection

RCT=randomised controlled trial. *Some studies had more than one reason for exclusion.

for safety outcomes. We first did pairwise meta-analyses of placebo-controlled RCTs to estimate the effect of each drug class versus placebo. We then did network meta-analyses to summarise direct and indirect evidence. We used a Bayesian approach using Markov Chain Monte Carlo simulation with non-informative prior distributions. A normal likelihood was used for continuous outcomes and the Poisson distribution with a logarithm link function for safety outcomes. Given the heterogeneity in RCTs and in-patient characteristics included in the meta-analyses, we used random-effects models to draw results (appendix p 6).

Publication bias was investigated by constructing funnel plots in a pairwise meta-analysis when more than ten studies were included in the meta-analysis, and by using Egger's regression test to assess for funnel plot asymmetry. In addition, the influence of a small-study effect was investigated using a network meta-regression model according to sample size.

Treatment classes were ranked according to the lower boundary of the mean rank 95% credibility interval (CrI), as previously described.¹⁴

We did a trial sequential network meta-analysis to assess if the amount of information to date was sufficient to support the conclusions.¹⁵

	Trials, n	Patients, n	Mean age, years (SD)	Women, n (%)	Mean disease duration, years (SD)	Secondary Raynaud's phenomenon, n (%)	Mean severity of attacks (SD)*	Mean daily frequency of attacks (SD)	Mean duration of attacks, min (SD)
α-adrenoceptor antagonists	4	280	47.8 (19.7)	203 (72%)	10.2 (9.5)	169 (60%)	1.79 (0.46)	1.96 (1.88)	15.60 (10.81)
Antioxidants	4	195	49.6 (10.0)	130 (66%)	9.4 (6.0)	195 (100%)	6.11 (2.88)	1.76 (1.97)	NR
Anti-interleukin 6	1	87	49.5 (12.3)	67 (77%)	NR	87 (100%)	NR	NR	NR
Angiotensin-converting enzyme inhibitors or angiotensin receptor blockers	1	210	54.5 (12.0)	178 (85%)	4.5 (5.5)	210 (100%)	NR	NR	NR
Botulinum toxin type A	1	40	51.9 (12.3)	31 (78%)	15.6 (9.3)	40 (100%)	NR	NR	NR
Calcium-channel blockers	14	342	45.2 (11.5)	283 (83%)	10.5 (8.5)	249 (73%)	4.58 (2.19)	3.05 (6.26)	13.46 (8.57)
Endothelin receptor antagonists	6	901	50.5 (12.7)	742 (82%)	12.3 (9.8)	901 (100%)	3.84 (2.74)	NR	NR
Phosphodiesterase 3 inhibitors	1	21	42.0 (13.0)	16 (75%)	12.0 (8.0)	21 (100%)	NR	NR	NR
Phosphodiesterase 5 inhibitors	12	556	47.8 (11.7)	479 (86%)	9.6 (7.1)	536 (96%)	3.92 (2.50)	3.01 (2.16)	15.94 (17.42)
Intravenous prostacyclin analogues	7	295	48.5 (13.1)	232 (79%)	11.4 (9.6)	263 (89%)	4.69 (2.15)	3.62 (2.39)	NR
Oral prostacyclin analogues or non-prostanoid prostacyclin receptor agonists	7	693	49.4 (11.0)	640 (84%)	13.6 (10.3)	764 (100%)	3.39 (1.73)	3.53 (2.09)	21.16 (21.70)
Selective serotonin-reuptake inhibitors	1	53	NR	42 (79%)	NR	27 (51%)	4.35 (2.84)	2.98 (2.26)	NR
Soluble guanylate-cyclase stimulators	1	17	51.0 (18.0)	13 (76%)	11.0 (7.9)	17 (100%)	5.40 (1.60)	4.30 (1.70)	NR
Topical nitric oxide donors	1	219	45.9 (11.4)	203 (93%)	NR	150 (68%)	3.34 (2.07)	2.80 (1.81)	NR
Thromboxane synthase inhibitors	2	35	37.9 (11.7)	27 (77%)	9.9 (11.7)	23 (66%)	NR	NR	NR

NR=not reported. *Rated from 0 to 10.

Table: Baseline characteristics of study participants by drug class

All statistical analyses were done with R version 3.3.4 (with packages *gemtc*, *sequentialnma*, *meta*, *netmeta*, and *rjags*) and JAGS version 3.4.0.^{15–20} More details on statistical analysis are available on supplementary material (appendix p 6).

Quality of evidence assessment

Quality assessment was done by two reviewers independently. Discrepancies were discussed to reach a consensus. We judged the quality of each study using the Cochrane Collaboration's tool for assessing risk of bias in RCTs (version 1).²¹ RCTs were classified as having low risk of bias if none of the Cochrane domain-specific biases were rated as high risk of bias and three or fewer were rated as unclear risk; moderate if only one or none were rated as high risk of bias but four or more were rated as having an unclear risk; and high risk in all other situations.²² We assessed the quality of the comparison between each therapeutic class and placebo in the network using the Grading of Recommendation Assessment, Development and Evaluation (GRADE) recommendations for network meta-analysis (appendix p 7), and the Confidence in Network Meta-Analysis Software (CINeMA) for network risk of bias.²³ Finally, we rated the quality of evidence for each therapeutic class as very low, low, moderate, or high (appendix p 7).

Heterogeneity and network consistency

The I^2 statistic was used to assess heterogeneity between trials in the pairwise meta-analysis using the Higgins-Thompson categorisations (low heterogeneity <25%,

moderate 25% to <50%, high 50% to <75%).²⁴ To assess the consistency of the five network meta-analyses, we compared the model fitting statistics between consistent and inconsistent models. A consistent model was adopted for all outcomes (appendix p 7). We also did a node-splitting analysis to test for inconsistency and heterogeneity (appendix pp 8–11).²⁵

Sensitivity analyses and subgroup meta-regressions

We did three pre-planned sensitivity analyses: studies including only patients with secondary Raynaud's phenomenon; studies assessing severity only with the RCS; and exclusion of studies with a high risk of bias.

Meta-regressions were done to assess the potential influence of pre-determined variables of interest: latitude (given the strong influence of climate on Raynaud's phenomenon prevalence and severity), age, sex, duration of the disease, follow-up period, efficacy outcomes at baseline, study sample size, and study design (parallel vs crossover). Two unplanned additional meta-regressions were done to assess the influence of add-on status and smoking.

Role of the funding source

There was no funding source for this study.

Results

Our search yielded 58 double-blind RCTs,^{8,9,26–80} of which 33 were parallel and 25 were crossover studies (figure 1; appendix pp 12–17). The studies had been published between 1982 and 2019, and compared 15 classes of drugs, often against placebo (appendix p 18). Two trials were

unpublished: for one the results were available on ClinicalTrials.gov (NCT00822354); and for the other, the largest clinical trial testing the efficacy of a PDE5 inhibitor in secondary Raynaud's phenomenon (NCT01090492, n=243 patients), we reanalysed the individual patient data provided by the sponsor.

Study sample sizes ranged from eight to 308 patients. The main characteristics of included studies are presented in the appendix (pp 19–25). Overall, 3867 patients were included, among whom 3540 (91.5%) had secondary Raynaud's phenomenon and 3162 (81.8%) were women. The median study follow-up was 6 weeks (IQR 4–12). Among efficacy outcomes, the frequency of Raynaud's phenomenon attacks was available for 41 (70.7%) RCTs and 2193 (57.8%) patients, severity of attacks was available for 45 (77.6%) RCTs and 3503 (92.3%) patients, and duration of attacks was available for 23 (39.7%) RCTs and 1416 (37.3%) patients. Tolerability was reported in 55 (94.8%) RCTs and 3535 (93.2%) patients and acceptability was reported 56 (96.6%) RCTs and 3698 (97.5%) patients (appendix pp 25–28). The baseline characteristics of patients included in trials by drug class are summarised in the table.

Results of pairwise meta-analyses of each drug class versus placebo are presented in the appendix (pp 29–31). Most of the comparisons showed little or no heterogeneity. Moderate heterogeneity was found for three out of the 51 comparisons versus placebo (α -adrenoceptor antagonists and oral prostacyclin receptor agonists for the frequency outcome; and intravenous prostacyclin analogues for severity).

Graphical representations of the network of comparisons for each efficacy outcome are shown in figure 2 and the appendix (pp 32–34). There was at least one placebo-controlled trial for all drug classes except SSRIs. Global heterogeneity and between-study variance were low to moderate for all outcomes: I^2 ranged from 0% (for attack severity and duration) to 41% (for tolerability), and τ^2 ranged from 0.0 (for attack severity and duration) to 0.23 (for tolerability; appendix p 6). Direct and indirect evidence was consistent for all outcomes.

PDE5 inhibitors were the only class statistically more effective than placebo for all three efficacy outcomes (figure 3), with a mean difference of -0.36 (95% CrI -0.69 to -0.04) for frequency, -0.34 (-0.66 to -0.03) for severity, and -3.42 (-6.62 to -0.29) for duration of attacks (low to moderate level of evidence). CCBs were also superior to placebo for reducing the frequency (-0.35 [-0.67 to -0.02]) and severity (-0.84 [-1.25 to -0.45]) of attacks, with a low level of evidence. Besides CCBs and PDE5 inhibitors, two classes of drugs were superior to placebo for reducing the severity of Raynaud's phenomenon (although with a low to very low level of evidence): SSRIs (-1.54 [-2.68 to -0.41]) and oral prostacyclin receptor agonists (-0.48 [-0.80 to -0.16]; figure 3).

PDE5 inhibitors ranked best in terms of reducing the frequency and duration of attacks (on the basis of the

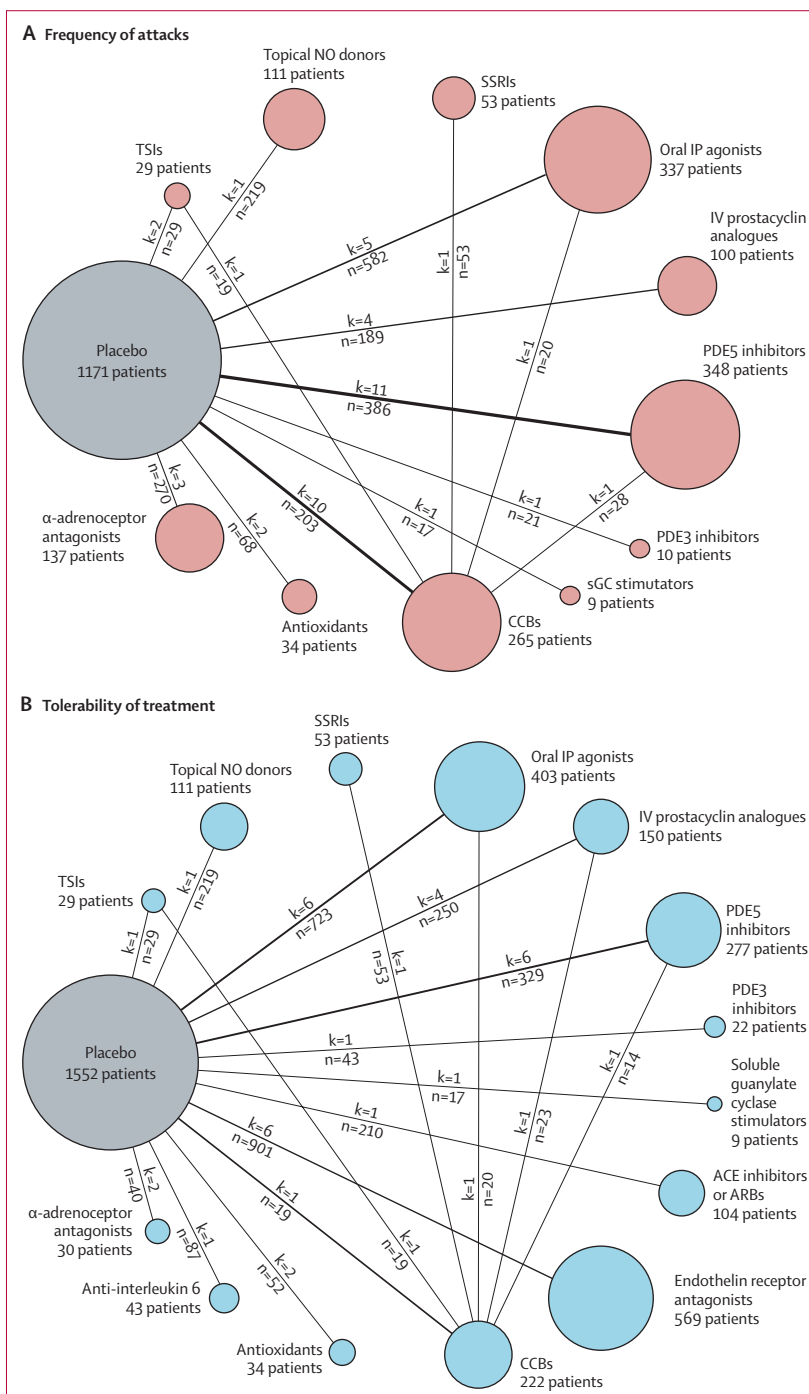


Figure 2: Network of included trials for attack frequency and drug tolerability outcomes
 The thickness of lines between nodes is proportional to the number of trials comparing the treatments. Nodes size is proportional to the number of patients in each treatment group. k indicates the number of studies and n indicates the number of patients in each comparison. ACE=angiotensin-converting enzyme. ARBs=angiotensin receptor blockers. CCBs=calcium-channel blockers. IP=prostacyclin receptor. IV=intravenous. NO=nitric oxide. PDE3=phosphodiesterase 3. PDE5=phosphodiesterase 5. sGC=soluble guanylate-cyclase. SSRIs=selective serotonin-reuptake inhibitors. TsIs=thromboxane synthase inhibitors.

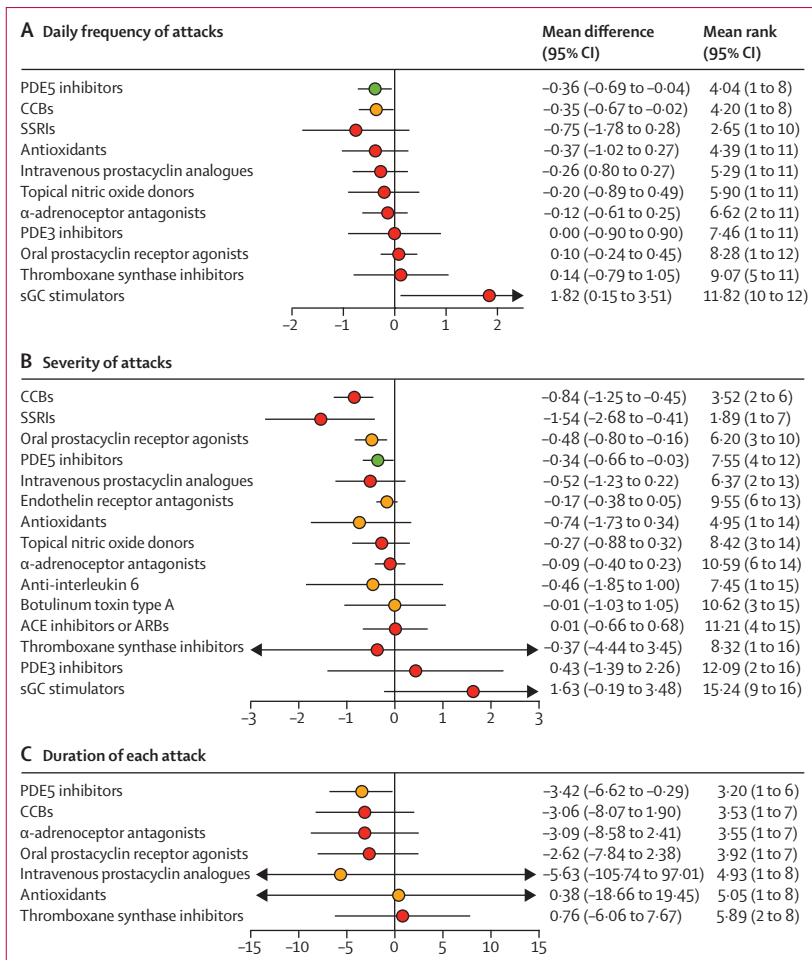


Figure 3: Forest plots of network meta-analysis results for efficacy versus placebo
 Drug classes are hierarchised according to the lower boundary of the mean rank 95% CrI. The colour depends of the average level of evidence according to GRADE (red=very low; orange=low; green=moderate). ACE=angiotensin-converting enzyme. ARBs=angiotensin receptor blockers. CCBs=calcium-channel blockers. CrI=credibility interval. PDE3=phosphodiesterase 3. PDE5=phosphodiesterase 5. sGC=soluble guanylate-cyclase. SSRIs=selective serotonin-reuptake inhibitors.

upper limit of the 95% CrI for the mean rank), whereas CCBs ranked best for reducing severity. However, considering the breadth of the mean rank 95% CrI, these results do not seem to be clinically meaningful.

PDE5 inhibitors had lower acceptability (IRR 2.61 [95% CrI 1.48–4.78]) and tolerability (3.30 [1.49–7.55]) than did placebo, as did oral prostacyclin receptor agonists (1.81 [1.08–3.15] for acceptability; 2.56 [1.27–5.25] for tolerability). CCBs also showed significantly worse tolerability (3.13 [1.33–7.04]) than that of placebo (appendix pp 35–36).

PDE5 inhibitors and oral prostacyclin receptor agonists had lower acceptability and tolerability than did placebo, and CCBs also showed significantly worse tolerability than that of placebo.

League tables with all comparisons from the network meta-analyses, for efficacy and safety outcomes, are presented in the appendix (pp 37–41).

The risk–benefit profiles of the different drug classes (along with the level of evidence) are summarised in figure 4. PDE5 inhibitors and CCBs showed highly similar profiles.

The overall risk of bias was judged to be low in 22 (38%), high in ten (17%), and unclear in 26 (45%) RCTs. High or unclear Cochrane domain-specific bias was lowest for participant and personnel blinding (9%), and highest for incomplete outcome data (50%; appendix p 42). The risk of bias for each RCT is summarised in the appendix (p 43). More than half of the studies were sponsored by one or more pharmaceutical companies. Risk of bias for pairwise comparisons with placebo for efficacy outcomes are shown in the appendix (pp 44–46). Network plots for the three efficacy outcomes show the risk of bias for all direct comparisons (appendix pp 47–49). The highest levels of evidence were found for PDE5 inhibitors, whereas studies on SSRIs, antioxidants, alpha-adrenoceptor antagonists, and ACE inhibitors or angiotensin receptor blockers provided very low levels of evidence (appendix pp 50–58).

Meta-regressions were consistent for the effect magnitude and direction for all efficacy outcomes (appendix pp 59–60). Older age was significantly correlated with a reduction in drug efficacy for severity. Sponsorship of studies by commercial companies, use of the treatment as an add-on therapy, smoking, and sample size did not significantly affect the results. Latitude, sex, disease duration, and follow-up period were not significantly associated with variations in effect size. Meta-regressions on baseline frequency, severity, and duration of attacks showed slight but non-significantly positive correlations between higher baseline values and greater efficacy (appendix pp 61–63). As post-hoc sensitivity analyses, we adjusted the results on baseline values (appendix pp 61–63). Notably, these analyses showed that PDE5 inhibitors and CCBs did not significantly lower the frequency of Raynaud's phenomenon in patients with fewer than five attacks per day.

Results of pre-planned subgroup and sensitivity analyses are presented in the appendix (pp 64–65). The most notable changes concern CCBs, for which the effect size was smaller when analyses were restricted to only patients with secondary Raynaud's phenomenon, or to high-quality trials. We added an unplanned sensitivity analysis to consider intravenous iloprost and other intravenous prostacyclin analogues separately (appendix p 66), but this restriction did not modify the main results. Trial sequential analyses revealed that the optimal information size was reached only by CCB for severity; and the results remained significant after a threshold adjustment for frequency. Results for PDE5 inhibitors remained significant after threshold adjustment for all three outcomes (appendix p 67).

Discussion

This systematic review and network meta-analyses provide a comprehensive synthesis of currently available data

from randomised studies of pharmacological treatments for secondary Raynaud's phenomenon. CCBs and PDE5 inhibitors both significantly decrease the frequency and severity of attacks in secondary Raynaud's phenomenon patients, with similar but small effect sizes. Notably, PDE5 inhibitors were the only drugs to significantly decrease the mean duration of Raynaud's phenomenon attacks, which is in line with previous observations.^{6,61} The level of evidence is stronger for PDE5 inhibitors than for CCBs. However, CCBs had greater acceptability than that of PDE5 inhibitors.

Although these results support current recommendations about the use of CCBs or PDE5 inhibitors as first line treatments for secondary Raynaud's phenomenon,⁴ they challenge the clinical relevance of these interventions. Indeed, the improvements in mean severity on a ten-point scale were only -0.84 (95% CrI -1.25 to -0.45) for CCBs and -0.34 (-0.66 to -0.03) for PDE5 inhibitors, both of which are far below the minimal clinically important difference for RCS in this population (about 1.5 points).¹² In addition, results from our meta-regressions suggest that patients with fewer than five attacks per day or with severity below four on a ten-point scale are not expected to have a significant benefit from these treatments. However, CrIs were large, indicating the need for more data.

There was no evidence that intravenous prostacyclin analogues are superior to placebo, whether intravenous iloprost is considered separately or with other analogues. This result disagrees with a previous meta-analysis that found, for iloprost versus placebo, a pooled mean difference of -0.69 (95% CI -1.12 to -0.26) for the severity score and no significant effect on other outcomes.⁷ As the same studies were included in both meta-analyses, this discrepancy could be explained by differences in the methodological approach: we used post-treatment data and used baseline measurements as a covariate, whereas the meta-analysis by Pope and colleagues⁷ used changes from baseline, which is no longer recommended.⁸¹ The network approach also provides additional information, which might influence the final results.

Other drug classes, such as SSRIs, oral prostacyclin receptor agonists, and antioxidants, showed superiority over placebo in terms of reducing the severity of Raynaud's phenomenon, but the level of evidence was low or very low, and this outcome was less robust. Heterogeneity in the effects of these treatments on the different outcomes raises concern about their actual efficacies. Current evidence is insufficient to support the use of the SSRI fluoxetine, which was added as a grade C recommendation in the 2016 update from EULAR.⁴ Overall, pharmacological interventions had only a modest effect (in the case of PDE5 inhibitors and CCBs) or failed to show any efficacy. Several trials have shown a strong placebo effect on frequency, severity, and duration of Raynaud's phenomenon attacks that might mitigate active treatment efficacy.^{8,9,42} Whether this placebo effect is related to a physiological improvement in cold tolerance or to behavioural changes during the trial

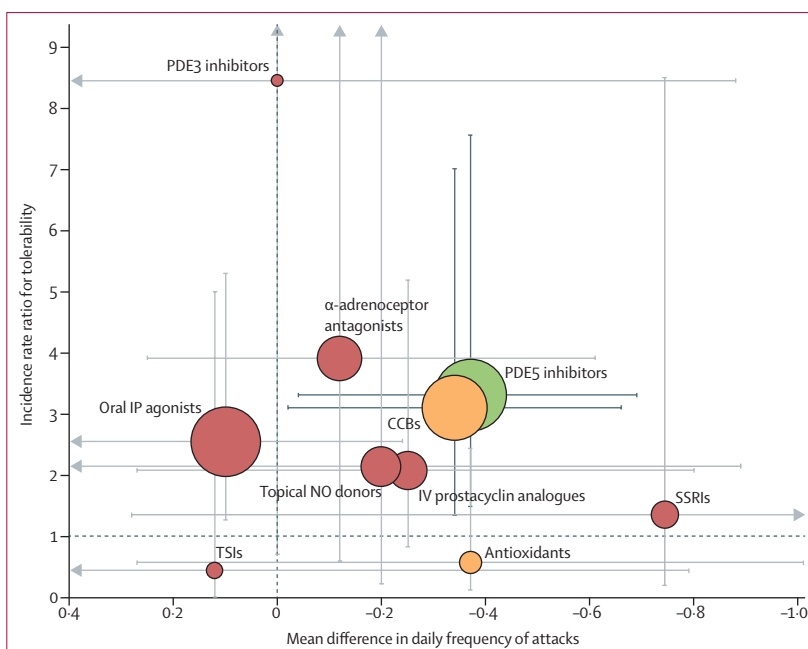


Figure 4: Risk-benefit profile of drug classes investigated

Network meta-analysis results showing the efficacy of each drug for reducing daily frequency of attacks of Raynaud's phenomenon (mean difference summary relative to placebo) versus the incidence rate ratio for tolerability. Bars indicate 95% credibility intervals. Node size is proportional to the number of patients in each drug class. The colour depends of the average level of evidence according to GRADE (red=very low; orange=low; green=moderate). CCBs=calcium-channel blockers. IP=prostacyclin receptor. IV=intravenous. NO=nitric oxide. PDE3=phosphodiesterase 3. PDE5=phosphodiesterase 5. SSRIs=selective serotonin-reuptake inhibitors. TSIs=thromboxane synthase inhibitors.

remains to be further explored.⁸² Moreover, several projects are ongoing to develop more robust outcomes in the field.⁸³⁻⁸⁵ Some patient characteristics might also be important determinants of treatment efficacy. Surprisingly, age was the only significant modifier of treatment effect in this meta-analysis. Indeed, no significant effect of CCBs or PDE5 inhibitors was found in patients older than 52 years (appendix p 61). This difference might be related to disease duration, with progressive structural vascular damage occurring over time, although disease duration did not significantly affect our results, probably because of a lack of power in the analysis. This finding deserves to be further explored through individual-patient meta-analyses.

Although several patient characteristics might influence treatment effects, they are difficult to identify in the context of a rare condition, and especially in secondary Raynaud's phenomenon considering the large within-patient and between-patient variability. To address this issue, we proposed the evaluation treatments in Raynaud's phenomenon using an individualised approach, by conducting N-of-1 trials.⁶¹ The strength of this approach is to estimate the treatment efficacy and safety for each patient, and to consider the individual patient's preference.

Our systematic review has several limitations. Many comparisons were judged as being of low or very low quality according to the GRADE framework, which restricts the validity of our results. Indeed, several small trials with poor methodology were the unique representatives

in the network of drug classes such as thromboxane synthase inhibitors, SSRIs, or antioxidants.

Secondly, there was a large degree of uncertainty, with only a small number of trials for many of the comparisons, and the available evidence might be insufficient to draw firm conclusions. In addition, our analyses combined intention-to-treat and per-protocol data; in a few cases, we were not able to clearly distinguish the number of patients included in the final analysis from the number of patients initially randomised, and we downgraded the quality rating of these trials. Finally, our findings are limited by a potential bias due to selective reporting. Indeed, 63 trials were excluded because their outcomes of interest could not be included in the final analysis (eg, when results were expressed as dichotomous variables).

Our findings suggest that several trials should be planned to explore areas of uncertainty in the field: SSRIs versus placebo, endothelin receptor antagonists versus placebo using frequency of attacks as the efficacy outcome, or CCBs versus PDE5 inhibitors (eg, nifedipine vs sildenafil). Although the impetus for conducting new trials involving CCBs or PDE5 inhibitors versus placebo might be weak given the information already available on these drugs (meaning that pharmaceutical companies are unlikely to sponsor such trials) and the difficulties inherent in running clinical trials in Raynaud's phenomenon, this research is needed for several reasons. First, although PDE5 inhibitors rank as the best treatment for two out of three outcomes and have the highest level of evidence, they are not approved for use in Raynaud's phenomenon in several countries such as the USA, France, or the UK. Second, around 25% of study populations in CCB trials comprise patients with primary Raynaud's phenomenon, and sensitivity analyses restricting data to trials including only patients with secondary Raynaud's phenomenon might substantially influence the results. It is possible that CCBs are actually inferior to PDE5 inhibitors, but this question needs to be addressed in a head-to-head trial. This network meta-analysis could serve as a basis for planning such trial in the future through conditional trial design methods.⁸⁶

A strong heterogeneity among the scales and scores used to assess the severity of attacks (from a severity score graded on a three-point scale to the RCS, and the Scleroderma Health Assessment Questionnaire or visual analogue scale for pain) limits the validity and extrapolation of our results. Notably, in the subgroup meta-analysis restricted to trials that used RCS as the efficacy outcome, no drug class had proven efficacy over placebo.

Finally, clinical efficacy and safety were evaluated by drug class, rather than by individual drugs. Although this method substantially increased the power to detect treatment effects, it could present a problem, particularly for those classes in which data for different drugs were pooled, such as antioxidants. However, between-study heterogeneity within drug classes was low in the pairwise meta-analysis, suggesting little variability of treatment effects.

In conclusion, the findings of this network meta-analysis provide no evidence for recommending any treatment with certainty in secondary Raynaud's phenomenon, and the level of evidence is low. However, CCB and PDE5 inhibitors might still be relevant when a pharmacological treatment is indicated, especially in patients with severe Raynaud's phenomenon. Our findings emphasise the pressing need for the development of new therapeutic strategies for secondary Raynaud's phenomenon, including non-pharmacological interventions.

Contributors

CK, MR, and J-LC conceived and designed the study. CK, ML, and MR acquired the data. CK, MR, SBa, and J-LC analysed and interpreted the data. CK and MR drafted the manuscript. CK, MR, ML, SBa, SBL, ALH, YA, MM-C, LT, and J-LC critically revised the manuscript. All authors approved the final manuscript.

Declaration of interests

MR has received research grants from United Therapeutics for other studies. J-LC has received research grants from Pfizer, United Therapeutics, Topadur Pharma, and Bioprojet for other studies. ALH has consultancy relationships with Boehringer-Ingelheim, Gesynta, and Camurus, has received research funding from Actelion and Gesynta, and has received speaker's fees from Actelion. MM-C has consultancy relationships or has received grant or research support from Pfizer, Bristol-Myers Squibb, Actelion, UCB Pharma, Bayer, ChemomAb, Genentech/Roche, Inventiva, and Lilly. YA has consultancy relationships with or has received grant or research support from Actelion, Pharmaceuticals US, Bayer AG, Bristol-Myers Squibb, Inventiva, Medac, Pfizer, Roche Pharmaceuticals, Genentech and Biogen IDEC, Sanofi-Aventis Pharmaceuticals, and Servier. All other authors declare no competing interests.

Data sharing

On publication of this Article, the full dataset will be made freely available online through the Open Science Framework.

Acknowledgments

We thank Pfizer for providing access to the individual data of the trial NCT01090492 testing the efficacy of a PDE5 inhibitors (PF-00489791) in Raynaud's phenomenon. We also thank Michel Cucherat (Lyon University Hospital, Lyon, France) for relevant and helpful advice; Florian Naudet and Clement Palpacuer (Rennes University Hospital, Rennes, France) for the base code of the quality figure; Theophile Tiffet (Grenoble Alpes University Hospital, Grenoble, France) for his help in R coding; and Alison Foote (Grenoble Alpes University Hospital, Grenoble, France) for correction of English language usage. This work was supported by the National Institute for Health Research Manchester Biomedical Research Centre.

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6. Drug repurposing in Raynaud's phenomenon through adverse event profile in the WHO pharmacovigilance database

Les résultats de la méta-analyse en réseau, présentée précédemment, mettent en lumière la faible efficacité globale de toutes les classes pharmacologiques actuellement utilisées dans le traitement du phénomène de Raynaud. Le besoin de nouvelles approches thérapeutiques est donc important. Le repositionnement de drogues est une méthode efficace pour identifier de nouveaux traitements dans une pathologie (112). Une multitude de méthodes ont été utilisées par le passé, une des plus populaires consiste à définir une « signature » de l'efficacité de traitements dans une pathologie donnée à partir de leur caractéristiques pharmacologiques, chimiques ou même par leur profil d'effet indésirables. Nous avons donc dans l'étude ci-dessous essayé d'identifier de nouvelles pistes thérapeutiques dans le phénomène de Raynaud à partir du profil d'effet indésirables des différentes drogues qui ont démontré leur efficacité.

Drug repurposing in Raynaud's phenomenon through adverse event signature matching in the WHO pharmacovigilance database

Running title: Drug repurposing in Raynaud's phenomenon

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Abstract (250 words)

Objective. Several pharmacological treatments are actually recommended for secondary Raynaud's phenomenon (RP) treatment. However, such treatments have modest efficacy and the extent to which this reduction is clinically meaningful is still uncertain. Thus, we aimed at generating repositioning hypotheses through adverse event signature matching in the WHO pharmacovigilance database.

Methods. We first defined an adverse event signature of the drugs recommended in secondary RP in the WHO pharmacovigilance database and we selected 14 adverse drug reaction (ADR) of interest. Then we selected all drugs associated with at least one case of erythromelalgia and data on the 14 ADR of interest were extracted. Lastly, we performed a hierarchical cluster analysis to identify unknown drugs with ADR similarities with vasodilatory drugs.

Results. A total of 179 drugs associated with 860,334 adverse events were extracted from the WHO pharmacovigilance database. After the hierarchical cluster analysis, we selected 6 clusters. The cluster 3 contained 7 drugs whose 5 are recommended in secondary RP or pertain to the same drug class: epoprostenol, nifedipine, nicardipine, lacidipine, israpidine and 2 others_alemtuzumab and fumaric acid potentially of interest.

Conclusion. Our study suggests that fumaric acid could be tested in the treatment of secondary RP. Experimental studies and clinical trials are further needed to evaluate this efficacy.

Keywords : Raynaud's phenomenon; drug repurposing.

INTRODUCTION

Raynaud's phenomenon (RP) is an exaggerated vascular response to cold, humidity or emotional stress, which typically manifest by an abrupt color changes of the finger's skin extremity.(1) While primary RP is the most frequent form (80–90%), RP may also be secondary to various connective tissue diseases (such as systemic sclerosis (SSc), systemic lupus erythematosus, vasculitis, etc.), vascular diseases or drugs.(2) Usually benign when primary, RP can provoke digital ischemia and digital ulcers when secondary. (1) Treatment includes patient education and general measures (such as cold exposure prevention) to prevent crises onset and includes pharmacological interventions such as calcium channel blockers or phosphodiesterase inhibitors 1.(1) Intravenous iloprost can be considered in RP secondary to SSc when oral therapies have failed. (3) However, such treatments have modest efficacy and the extent to which this reduction is clinically meaningful is still uncertain.(4,5) Moreover, all recently tested pharmacological treatments such as endothelin receptor antagonists, oral prostacyclin analogs / non-prostanoid IP-receptor agonists failed to demonstrate an efficacy, leaving place for improvement (6,7)

A way to efficiently identify new drugs, targets and pathways potentially of interest for a given disease is drug repurposing.(8) This strategy is an attractive option to lower overall development costs and shorter development timelines compared to a new drug development. Several strategies have been tested and developed for this purpose such as computational or experimental approaches. (8) One of the most popular one, signature matching, is based on the comparison of the unique characteristics or 'signature' of a drug against that of another drug. The signature could be derived from chemical structures, pharmacological affinity profile or adverse event profile. Indeed, every drug has a relatively unique adverse effect profile that could be used as a proxy for its therapeutic

properties through related mechanisms of action.(9) Several studies used this approach to generate drug repurposing hypothesis through multiple databases PharmGKB, DrugBank, Pubmed...(9–11) One of the largest databases of adverse event spontaneously reported worldwide is the WHO pharmacovigilance database which contains several millions of reports associated with suspected drugs. Using this database, our objective was to define an adverse events signature of efficient drugs in RP, and to generate repositioning hypotheses through hierarchical cluster analysis.

PATIENTS AND METHODS

The WHO pharmacovigilance database, VigiBase®.

At the time of the data extraction, VigiBase contained more than 19 million of reports of suspected adverse drug reactions (ADRs), submitted, since 1968, by a network of 134 countries, members of the WHO Programme for International Drug Monitoring.(12) VigiBase provides safety reports with patient information such as gender, age, medical history, country; suspected and concomitant drugs taken with chronological information, as well as drug indication and dosage; a description of the adverse effect with its severity and outcomes. The Uppsala Monitoring Centre research team developed a method (the Bayesian neural network) to identify pharmacovigilance disproportionality signals, i.e. an unexpected disproportionate association between a drug and an adverse event.(13) A pharmacovigilance disproportionality signal is deemed significant if the lower boundary of the 95% credibility interval of the Information Component (the disproportionality metric from the Bayesian neural network method) was superior to 0.(13)

Identification of an adverse event signature related to an efficacy in Raynaud's phenomenon.

We extracted all adverse events associated with a significant pharmacovigilance disproportionality signals related to nifedipine, sildenafil and iloprost in the WHO pharmacovigilance database. Then based on the pharmacological mechanism of action of nifedipine, sildenafil and iloprost, we selected all significant pharmacovigilance disproportionality signals related to their beneficial vasodilator and antiaggregant action in RP; through discussion among senior expert in vascular pharmacology, microcirculation and Raynaud's phenomenon (JLC,CK,MR).

We retained 14 variables: erythromelalgia, blood pressure decreased, blood pressure systolic decreased, dizziness, epistaxis, headache, hot flush, hypotension, nasal congestion, orthostatic hypotension, feeling hot, flushing, syncope and vasodilatation.

Redundant adverse events procuring analogous information were merged: blood pressure decreased-blood pressure systolic decreased-orthostatic hypotension-hypotension and feeling hot-flushing-hot flush.

Drug candidates selection.

When induced by a vasodilator drug erythromelalgia could be considered as a syndrome produced by the opposite vascular pathophysiological mechanism of RP, through digital skin vasorelaxation and blood flow increase. Indeed, erythromelalgia has been associated with pivotal drugs used in Raynaud's phenomenon.(14,15) We thereof considered erythromelalgia as a mandatory ADR and extracted all drugs with at least one erythromelalgia report in the WHO pharmacovigilance

database. We then excluded all vaccines, drug indicated in erythromelalgia treatment (Acetylsalicylic acid, venlafaxine, amitriptyline, gabapentin, pregabalin, glucocorticoids, lidocaine, mexiletine) and drugs known to induce Raynaud's phenomenon such as beta-adrenoreceptor blockers, sympathomimetics, stimulants, ergots alkaloids, dopaminergic agonists, bromocriptine, interferons, alkylating agents and ciclosporin. (16) For each drug, we extracted the number and proportion of each selected adverse event.

Cluster analysis

We performed a hierarchical cluster analysis to identify signature matching with other drugs potentially usable in RP. Cluster analysis was carried out from ascendant hierarchical clustering on the 14 selected variables using Ward' minimum variance method. Results were graphically represented in a dendrogram. We visually estimated the number of clusters. We assessed the stability and reproducibility of the clusters through calculation of the Jaccard coefficient. We conducted 500 iterations of the clustering process in randomly selected subsets to 50% of the original dataset, and estimated the cluster-wise stability with the Jaccard coefficient. A Jaccard similarity index > 0.5 indicates a stable and reproducible cluster(17).

The protocol of this study was pre-registered in Open Science Framework (Khoury, C. (2019) June 17). Drug repurposing in Raynaud's phenomenon in the WHO pharmacovigilance database. Retrieved from osf.io/prmak) and the data set of this study will be make freely available alongside to the publication of the results.

RESULTS

A total of 179 drugs associated with 860,334 adverse events were extracted from the WHO pharmacovigilance database. We excluded vaccines, drugs used to treat erythromelalgia and associated with induction of Raynaud's phenomenon resulting in 148 drugs (Supplementary Table 1). Drug clustering dendrogram is represented in Figure 1. We visually selected 6 clusters for analysis. Jaccard index pointed out a high cluster-wise stability for cluster 3 (0.90) and 6 (0.75) and a moderate stability for clusters 1 (0.66), 2 (0.52), 4 (0.59) and 5 (0.64). Three clusters contained drugs used in Raynaud's phenomenon, cluster 3, 4 and 5.

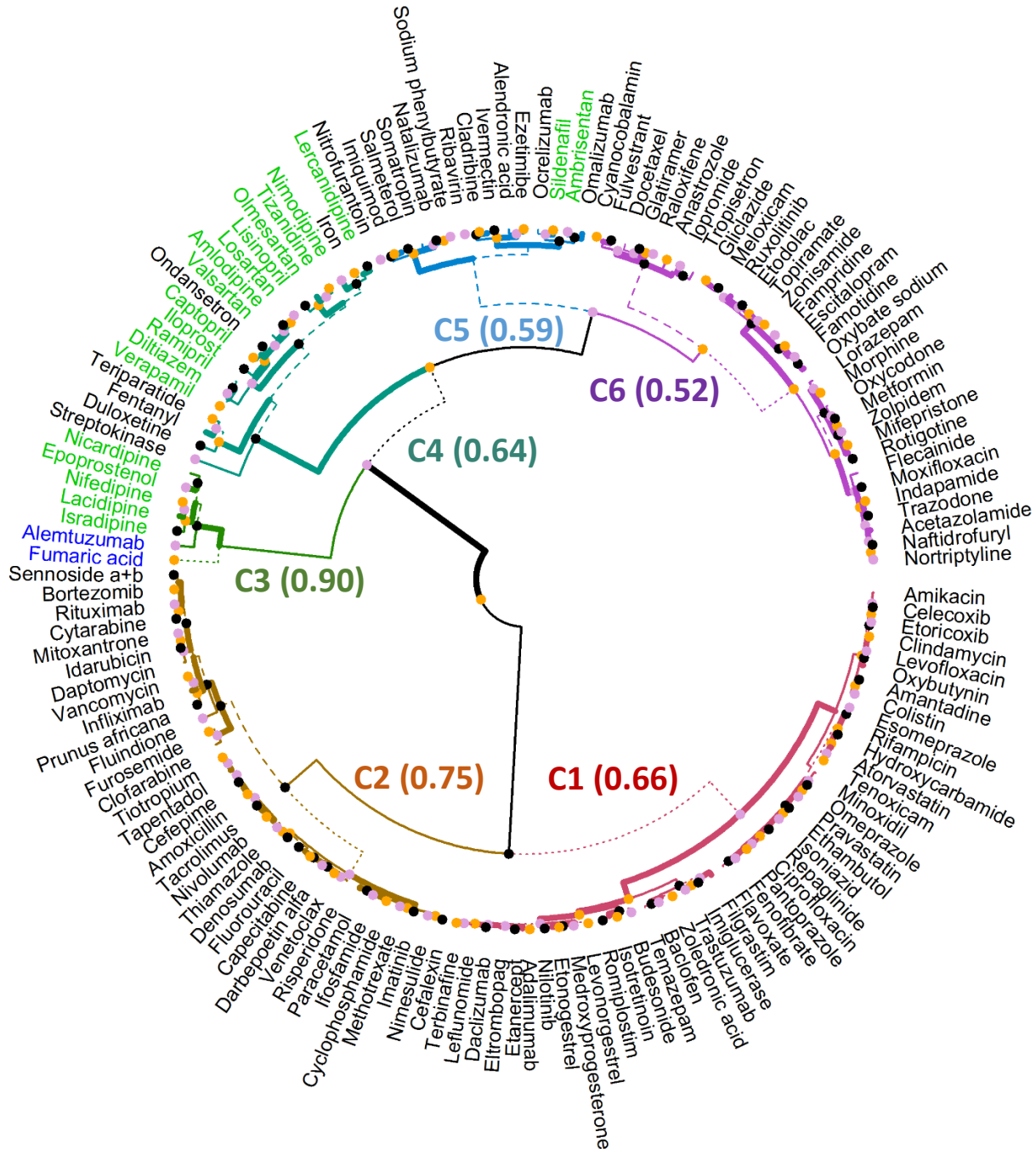


Figure 1. Dendrogram representing the results of the hierarchical cluster analysis. For each cluster the corresponding Jaccard index is reported. Drugs used in RP or pertaining to the same drug class are in green. Drugs potentially of interest are in blue.

The cluster 3 contained 7 drugs whose 5 are used in RP or pertain to the same drug class: epoprostenol, nifedipine, nicardipine, lacidipine, israpidine and 2 other_alemtuzumab and fumaric acid potentially of interest. Alemtuzumab displayed significant disproportionality signals of hypotension, flushing, epistaxis, headache and nasal congestion. Safety profile of fumaric acid was characterized by strong signals of flushing and headache.

The cluster 4 contained 20 drugs whom 16 are used in RP or pertain to the same drug classes and the cluster 5 contained only 2 drugs used in RP on 21.

DISCUSSION

To our knowledge this is the first repurposing study through signature matching in the WHO pharmacovigilance database and RP. We identified 2 drugs potentially of interest in secondary RP: alemtuzumab and fumaric acid. Indeed, those drugs pertain to a highly stable cluster alongside with 5 vasodilator drugs used in RP.

Fumaric acid esters, in particular dimethyl fumarate already approved for the treatment of multiple sclerosis, exert anti-inflammatory effects via targeting NF- κ B pathway and blocking YAP nuclear translocation and fibrotic responses in SSc fibroblasts.(18) Moreover, flushing is one of the most frequently described ADR of dimethyl fumarate. The adverse event is thought to be mediated by an activation of the G-protein-coupled receptor hydroxy-carboxylic acid receptor 2 inducing the synthesis of prostaglandins D₂ and E₂ by COX-1 in Langerhans cells and COX-2 in keratinocytes(19). A phase 1 randomized controlled trial comparison the efficacy of dimethyl

fumarate versus placebo is ongoing in SSc-PAH (NCT02981082). However, our results suggest that this drug could also have an interest in RP.

Alemtuzumab is a selective humanized monoclonal antibody directed against the CD52 antigen on T- and B-lymphocytes. Treatment with alemtuzumab produces a depletion of circulating B- and T-lymphocytes which have been implicated in the pathogenesis of systemic sclerosis(18). Indeed, accumulating evidence suggest that B cells are implicated in inflammation and skin fibrosis and T cells are linked to increased severity of skin and lung disease in SSc (18). A pilot phase 1/2 RCT clinical trial in SSc with alemtuzumab was withdrawn due to no patient enrollment (NCT01639573), moreover recent safety signals of autoimmune hepatitis, haemophagocytic lymphohistiocytosis, cardiovascular adverse events and neutropenia led to a restricted use of alemtuzumab in multiple sclerosis and an EMA benefit-risk review is ongoing. Moreover, the disproportionality signals of flushing and headache found with alemtuzumab seems to be related to infusion-associated reactions and not to pharmacological vasodilatory properties(20). An acceptable benefit-risk profile of alemtuzumab in secondary SSc seems therefore unlikely.

Beyond vasodilatory drugs, the potential efficacy of statins in SSc vascular dysfunction and RP has been highlighted (21) and is still under investigation (NCT02370784). However, in our study, atorvastatin was not associated with any of our selected ADR of interest. Thus, there is a risk that the expected benefit with statin may not be clinically significant.

The limitations of this study are firstly inherent to the nature of the database. Indeed, cases are spontaneously reported by physicians, pharmacist or event patients. Underreporting and selective reporting (e.g. due to medial alert, drug novelty, reporter qualification, ADR severity...) are well

described in pharmacovigilance, therefore distorting the known drug safety profiles. Moreover, ADR time to onset was not taken in account in this study. Although, almost all vasodilatory drugs were classified in two clusters underlying the safety profile similarity of such drugs in the WHO pharmacovigilance database. Lastly, in selecting only drugs for which at least 1 case of erythromelalgia was reported we probably excluded drugs potentially of interest.

CONCLUSION

To our knowledge this is the first repurposing study in the WHO pharmacovigilance database. Our study suggest that fumaric acid could be tested in the treatment of secondary RP. Experimental studies and clinical trials are further needed to evaluate this efficacy.

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7. Impact of global warming on Raynaud's phenomenon: an underestimated benefit

Si l'efficacité des traitements pharmacologiques reste modeste et si les nouvelles pistes thérapeutiques actuellement en cours d'évaluation échouent à apporter un bénéfice important aux patients atteints de phénomène de Raynaud, le réchauffement climatique apportera sans doute une réponse d'ici la fin du siècle...

Impact of global warming on Raynaud's phenomenon: an underestimated benefit

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Abstract

Objectives: The objective of the present study is to evaluate the impact of global warming on the worldwide prevalence and severity of Raynaud's phenomenon over the 21st century.

Design: We first estimated the correlation between average temperature and prevalence and severity of Raynaud's phenomenon. Then, we mapped the prevalence and the severity of Raynaud's phenomenon worldwide at Christmas 1999 using historical data and, using climate projections from the Inter-Sectoral Impact Model Intercomparison Project, we predicted the prevalence and severity of Raynaud's phenomenon at Christmas 2099 according to four greenhouse-gas emission scenarios.

Main outcome measures: Prevalence and daily frequency of Raynaud's phenomenon

Results: Our study shows that global warming may have a significant impact on the prevalence and the severity of Raynaud's phenomenon over the 21st century. However, as expected, this will greatly depend on the level of greenhouse-gas emissions.

Conclusions: We advise patients affected by Raynaud's phenomenon to welcome climate change. The solution will not come from industrial or academic pharmacologists. Instead, global warming will provide a significant therapeutic advance towards eradicating their disease.

WHAT IS ALREADY KNOWN ON THIS TOPIC

Prevalence and severity of Raynaud's phenomenon are known to be correlated with temperature.

WHAT THIS STUDY ADDS

Global warming may have a significant impact on the prevalence and the severity of Raynaud's phenomenon over the 21st century.

The most optimistic greenhouse gas scenario will only have a limited impact on the global prevalence and severity of Raynaud's phenomenon. However scenarios without greenhouse-gas emission reductions may largely improve the condition of patients suffering from Raynaud's phenomenon worldwide.

The projected impact of climate change on human health promises to be devastating. Large increases in morbidity and mortality are expected in association with a range of health outcomes, including heat-related illnesses, illnesses caused by poor air quality, undernutrition and selected vector-borne diseases in some locations [1]. However, studies are scarce regarding the benefits of global warming on health outcomes.

Raynaud's phenomenon is induced by excessive vasoconstriction of the peripheral microcirculation in response to environmental factors, essentially cold, but also stress or emotions [2]. Primary, or idiopathic, Raynaud's phenomenon is the most frequent form (80-90%), while in some cases Raynaud's phenomenon can be secondary to various autoimmune diseases (such as systemic sclerosis or systemic lupus erythematosus) or drugs [2]. The prevalence of Raynaud's phenomenon is estimated to be approximately 3 to 5% in the general population, with substantial variability according to climate and sex [3]. Most vasodilators currently used in Raynaud's, such as nifedipine or sildenafil, only have limited efficacy, below the minimal clinically important difference [4,5]. Moreover, most recent trials have failed to succeed, due to high heterogeneity and a significant placebo effect [6].

We hypothesize that global warming should not leave Raynaud's phenomenon as an unmet clinical need for too long. The objective of the present study is to evaluate the impact of global warming on the worldwide prevalence and severity of Raynaud's phenomenon over the 21st century.

Method

We first estimated the correlation between average temperature and the prevalence of Raynaud's phenomenon. The prevalence data were extracted from a systematic review of observational studies [7]. For each study we calculated the mean temperature during the winter preceding the publication of the study (from 1st November to 31 March) using historical climate data from the database developed by the Inter-Sectoral Impact Model Intercomparison Project (ISIMIP, <https://www.isimip.org/>).

We further predicted the impact of global warming on the severity of Raynaud's phenomenon, expressed as the average daily frequency of attacks, by using a model based on a Poisson regression including temperature (and other covariates), recently published by our team [8] (this model is available online, at https://datadryad.org/bitstream/handle/10255/dryad.196852/model_1.txt?sequence=1).

This model is derived from a series of n-of-1 trials containing more than 2000 days of exposition, with daily temperature measurements collected at the nearest weather station to the patient's home.

Finally, we mapped the prevalence and the severity of Raynaud's phenomenon worldwide at Christmas 1999 and, using climate projections from the ISIMIP, we predicted the prevalence and severity of Raynaud's phenomenon at Christmas 2099, according to four greenhouse-gas emission scenarios (Representative Concentration Pathway (RCP) 2.6, RCP4.5, RCP6.0, and RCP8.5) described in the Fifth Assessment Report of the United Nations Intergovernmental Panel on Climate Change [9]. The HadGEM2-ES model was used for the modelling scenario [10].

The RCPs represent the range of greenhouse-gas emission scenarios consistent with projections described in literature; they include a mitigation scenario (RCP2.6), two intermediate scenarios (RCP4.5 and RCP6.0), and one scenario with high greenhouse-gas emissions (RCP8.5).

Data analysis were performed with R version 3.3.0 [11] and map visualization with Panoply version 4.10.4 software [12].

No patients or members of the public were directly involved in this study. There are no plans to involve patients or the public in the dissemination of results.

Results

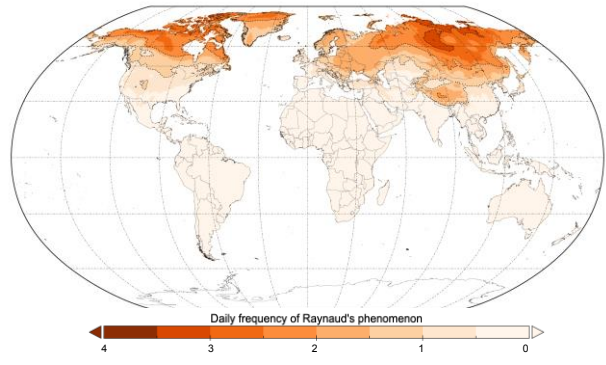
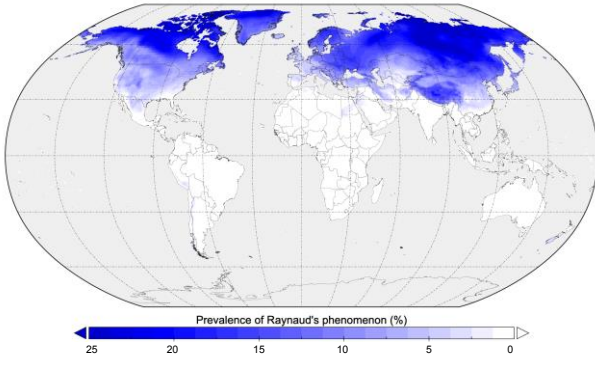
We found a high correlation between average temperature and the prevalence and severity of Raynaud's phenomenon ($p < 0.001$). According to these data, no Raynaud's phenomenon attack is expected to occur above an average temperature of 13°C, which is consistent with individual data collected in our series of N-of-1 trials [8]. Consequently, the prevalence of Raynaud's phenomenon in the general population is expected to decrease by 0.5% per degree Celsius increase. Furthermore, patients are expected to suffer from one less attack per week for each increase of 2.5 degrees Celsius.

The worldwide prevalence and severity of Raynaud's phenomenon at Christmas 1999 and the range of predictions based on four greenhouse-gas emission scenarios at Christmas 2099 are shown in Figure 1.

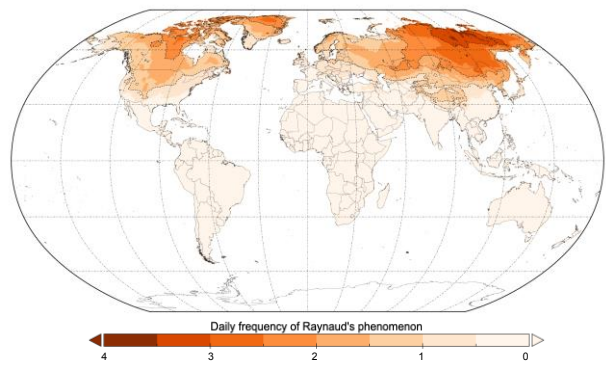
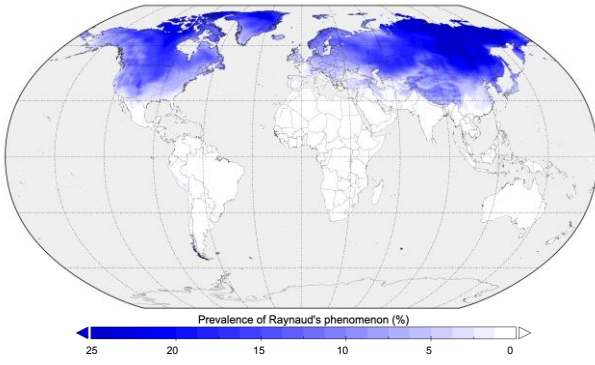
Prevalence of Raynaud's phenomenon

Severity of Raynaud's phenomenon

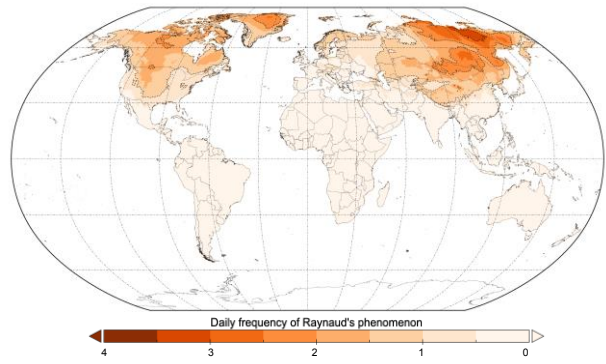
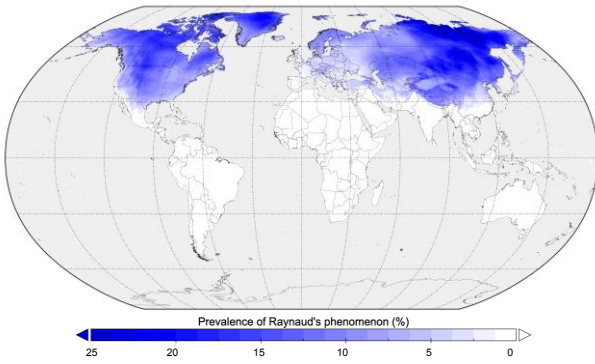
Christmas 1999



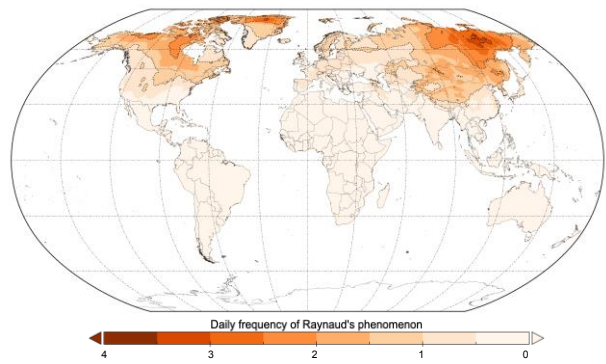
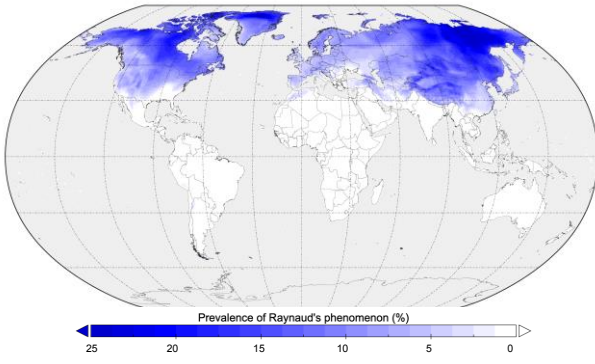
Christmas 2099 (RCP 2.6)



Christmas 2099 (RCP 4.5)



Christmas 2099 (RCP 6.0)



Christmas 2099 (RCP 8.5)

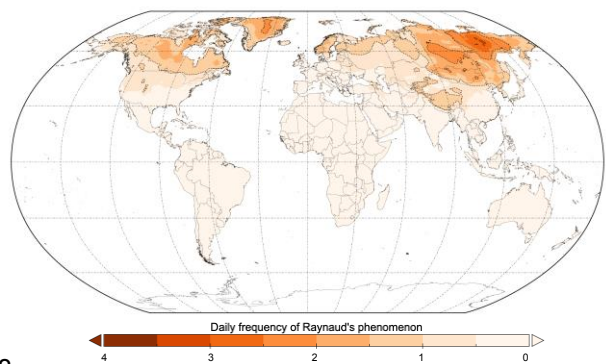
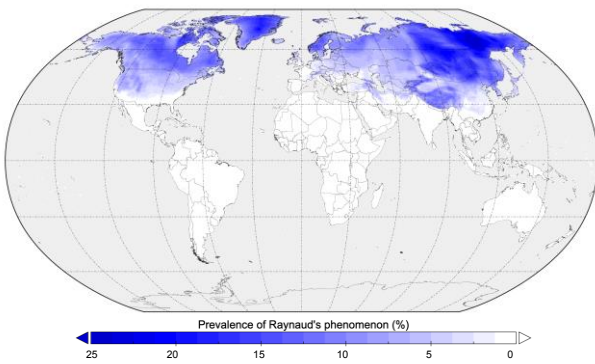


Figure 1. Prevalence and daily frequency of Raynaud's phenomenon during Christmas 1999 and Christmas 2099 according to four greenhouse gas emission scenarios (Representative Concentration Pathway (RCP) 2.6, RCP4.5, RCP6.0, and RCP8.5).

Discussion

Our study shows that global warming may have a significant impact on the prevalence and the severity of Raynaud's phenomenon over the 21st century. However, as expected, this will greatly depend on the level of greenhouse-gas emissions. The most optimistic greenhouse gas scenario (RCP 2.6), which aims at keeping global warming below 2°C above pre-industrial temperatures, only has a limited impact on the global prevalence and severity of Raynaud's phenomenon. Luckily, this scenario is becoming more and more unrealistic [13]. On the other hand, scenarios without greenhouse-gas emission reductions (predictions ranging between RCP6.0 and RCP8.5) may largely improve the condition of patients suffering from Raynaud's phenomenon. For example, people in western European countries could expect to be totally free of this painful and disabling condition in the event of the two higher gas-emission scenarios. Finally, patients in North America, Western Europe and Asia still suffering from Raynaud's phenomenon are not expected to suffer more than one or two crises over the Christmas period in 2099.

In this study we only used one modelling scenario, the HadGEM2-ES model, which is widely used for climate research [14,15], therefore uncertainty of our projections has not been evaluated but exist undoubtedly. The findings should thus be interpreted as potential impacts of climate change on Raynaud's phenomenon according to one hypothetical scenario and not as projections.

Conclusion

We advise patients affected by Raynaud's phenomenon to welcome climate change. The solution will not come from industrial or academic pharmacologists. Instead, global warming will provide a significant therapeutic advance towards eradicating their disease.

Contributors : CK, MR and JLC designed the study. CK conducted the study and performed the statistical analysis. MR and JLC supervised the statistical analysis. CK drafted the manuscript. MR and JLC supervised the writing. All authors contributed to the data interpretation and revised each draft for important intellectual content. All authors read and approved the final manuscript. All authors had full access to the data in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis. CK is the guarantor. The corresponding author (CK) attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted

Funding : this study was not funded

Competing interest: Dr. Khouri has nothing to disclose; Dr. Roustit reports grants from Bioprojet, grants from Pfizer France , grants from GIRCI Rhône-Alpes-Auvergne, grants from Association des Sclérodermiques de France , outside the submitted work; Dr. Cracowski reports grants from BIOPROJET, grants from Pfizer France , grants from GIRCI Rhône-Alpes-Auvergne, grants from Association des Sclérodermiques de France , outside the submitted work .

Ethical approval: not needed

The guarantor (CK) affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

Data sharing: No additional data are available.

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8. Conclusion et perspectives

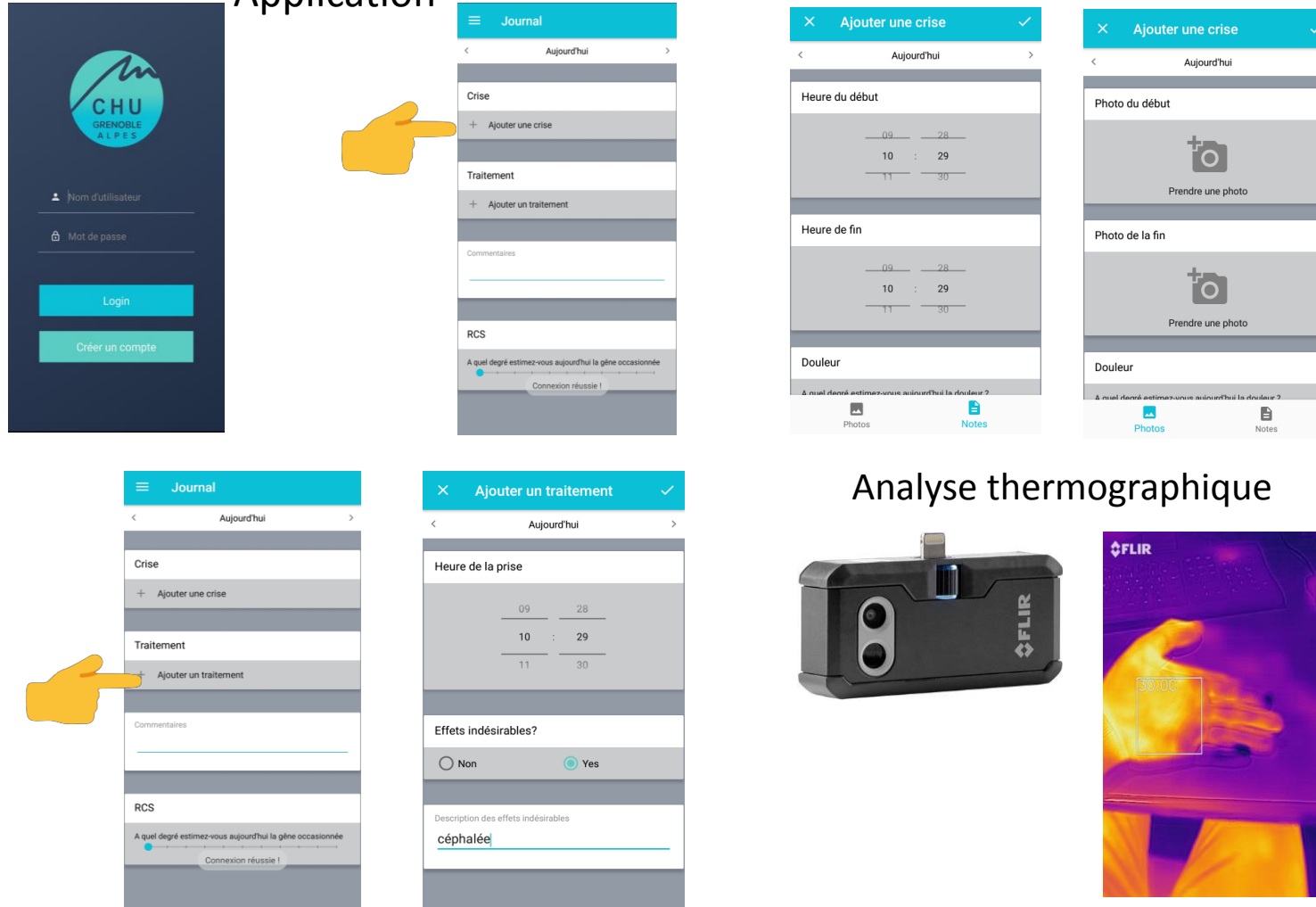
Les travaux que nous avons menés sur ce sujet nous ont permis d'identifier de nouvelles classes pharmacologiques à l'origine d'une induction ou aggravation du phénomène de Raynaud. Afin de compléter nos travaux et d'identifier de potentiels nouveaux signaux de pharmacovigilance nous sommes en train de réaliser une étude de disproportionnalité sur la base de pharmacovigilance de l'OMS. Celle-ci permettra de faire un état des lieux des notifications de phénomène de Raynaud potentiellement iatrogènes et de compléter la revue systématique de la littérature précédemment réalisée.

Les sources de données utilisables en pharmacovigilance pour l'étude des étiologies iatrogènes du phénomène de Raynaud sont malheureusement limitées. En effet, cet effet indésirable est très peu rapporté dans les essais cliniques et constitue très rarement un motif de consultation à l'hôpital en raison de son caractère fréquemment bénin. La réalisation d'études de pharmaco épidémiologies à partir de bases de données médico-administratives paraît donc peu adaptée. L'utilisation de données innovantes comme les réseaux sociaux ou forums pourrait être une piste intéressante à l'avenir.

Nous avons également mis en évidence dans ce travail le besoin important de nouvelles approches thérapeutiques dans le traitement du phénomène de Raynaud. Les thérapeutiques disponibles possèdent une efficacité médiocre et tous les essais cliniques récents ont échoué à démontrer une supériorité par rapport au placebo (8,34,113). L'une des raisons fréquemment évoquées pour expliquer l'échec des essais cliniques dans cette pathologie est l'important effet placebo. (34,113,114) L'étude des déterminants de l'effet placebo chez les patients atteints de phénomène de Raynaud paraît donc primordiale et fait l'objet d'un travail que nous réalisons actuellement. Au-delà de l'effet placebo, l'hétérogénéité dans la réponse aux traitements est également importante et des travaux afin d'identifier les caractéristiques des patients répondeurs et non répondeurs sont à mener. L'utilisation de méthode d'essais cliniques innovantes comme les essais de taille 1 (ou N-of-1) peut permettre d'identifier des patients répondeurs et son incrémentation en routine pourrait être une solution. Nous avons développé une application mobile, afin initialement de recueillir les critères de jugements dans les essais cliniques, qui pourrait à terme être utile dans le soin afin de quantifier la fréquence et sévérité des crises avant et après la mise en place d'un traitement pharmacologique (Figure 8).

Raynaud Monitoring

Application



Analyse thermographique

Figure 8. Captures d'écrans de l'application mobile Raynaud monitoring. Cette application permettra aux patients de noter l'heure de début et de fin de chaque crise ou de prendre des photos, de remplir l'échelle de gêne associée au Raynaud (RCS), de noter les effets indésirables éventuels ou la prise de traitements associés. Elle permettra également, à l'aide d'une caméra, d'enregistrer des données thermographiques pendant les crises. Toutes ces données sont transférées sur un serveur sécurisé du CHU de Grenoble.

L'échec des traitements pharmacologiques ouvre également la porte à l'exploration d'autres approches comme des approches nutritionnelles. Nous avons obtenu un financement afin de conduire un essai clinique comparant deux stratégies pharmaco-nutritionnelles dans le phénomène de Raynaud : le jus de betterave et la l-citrulline (NCT03749577). Le jus de betterave possède une forte concentration en nitrates qui seront, via un cycle entero-salivaire, biotransformés en NO. La citrulline est métabolisée en l-arginine, le précurseur de la NOS endothéliale. Ces suppléments vont donc provoquer une augmentation des concentrations de NO via des mécanismes différents. Cette étude de type N-of-1 débutera cet hiver. Afin d'étudier les déterminants de l'effet thérapeutique (et l'effet placebo), nous étudierons l'impact de la préférence des volontaires et du type de placebo (jus versus gélule). De plus, les patients hiérarchiseront les critères de jugements en fonction de ce qui leur paraît être le plus pertinent (sévérité, durée ou fréquence) ainsi que le seuil d'efficacité à partir duquel ils seraient prêts à prendre le traitement. A la fin de l'essai la probabilité d'efficacité individuelle pourra être calculée pour chaque patient selon ses propres critères. Le design de l'essai est schématisé Figure 9.

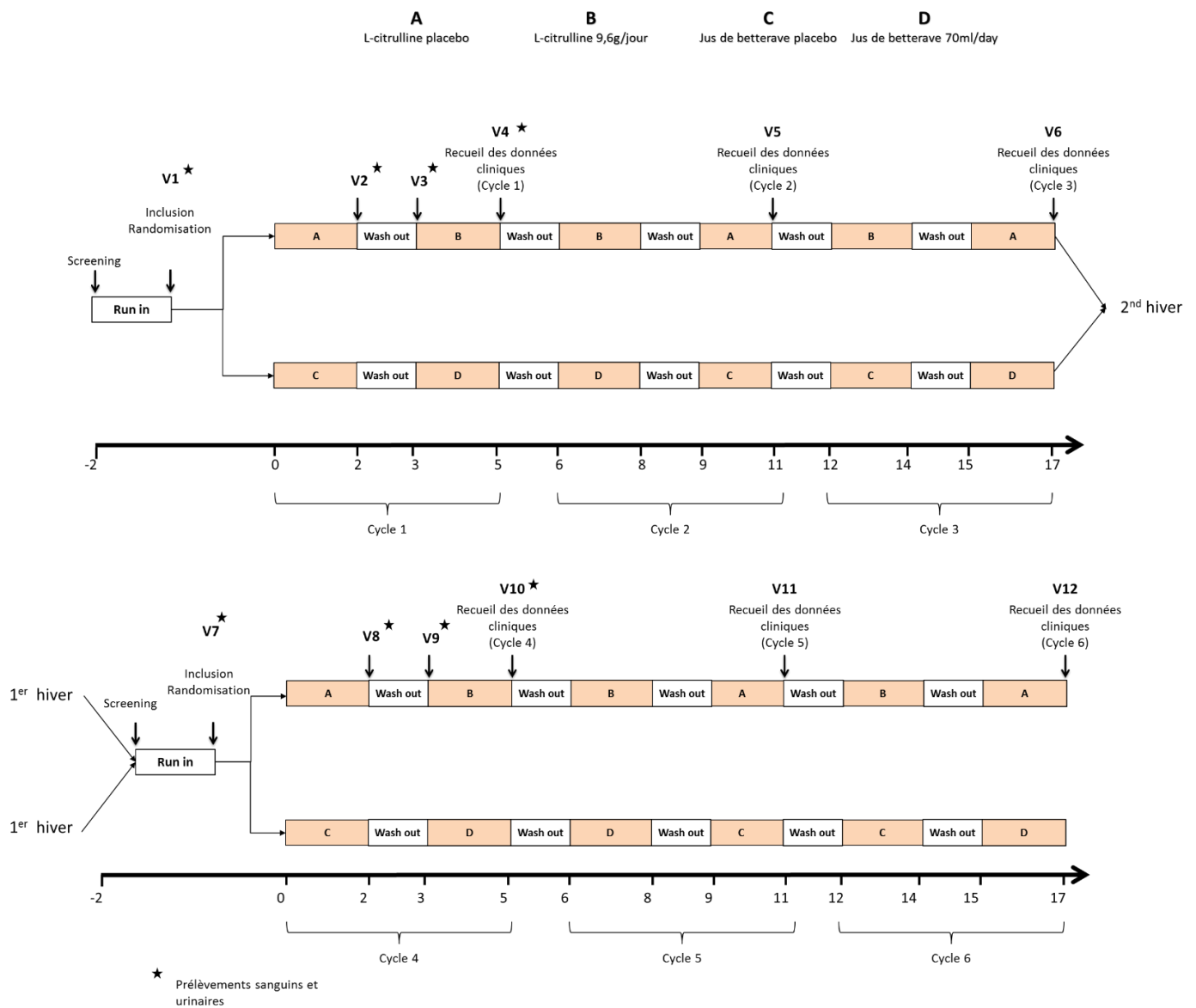


Figure 9. Flow chart de l'étude NIVOSE (NCT03749577). Chaque hiver les volontaires seront randomisés dans un des 2 bras de l'étude (jus de betterave vs placebo ou l-citrulline vs placebo). Chaque bras comprend 3 cycles de deux périodes (traitement actif ou placebo) randomisées séparées d'une semaine de wash out.

PARTIE 3. ÉTUDES SUR L'HYPERTENSION ARTERIELLE PULMONAIRE

1. Comparative safety of drugs targeting the nitric oxide pathway in pulmonary hypertension: a mixed approach combining a meta-analysis of clinical trials and a disproportionality analysis from the WHO pharmacovigilance database.

Dans ce travail nous avons comparé le profil d'effets indésirables des différents médicaments qui agissent sur la voie du monoxyde d'azote utilisés dans le traitement de l'HTAP. Ce travail fait suite à l'autorisation de mise sur le marché du riociguat (activateur de la guanylate cyclase soluble) et à son positionnement récent en première ligne du traitement de l'HTAP et de l'hypertension pulmonaire secondaire à une maladie thrombo-embolique (30). L'objectif de ce travail était d'identifier des syndromes d'effets indésirables communs et, au contraire, spécifiques à chacun de ces médicaments ; et donc des situations dans lesquelles un bénéfice pourrait être attendu par le switch entre ces classes en cas d'effets indésirables. Dans cette étude nous avons utilisé à la fois des données d'essais cliniques et de notification spontanée.

Comparative Safety of Drugs Targeting the Nitric Oxide Pathway in Pulmonary Hypertension



A Mixed Approach Combining a Meta-Analysis of Clinical Trials and a Disproportionality Analysis From the World Health Organization Pharmacovigilance Database

Charles Khouri, PharmD; Marion Lepelley, PharmD; Matthieu Roustit, PharmD; François Montastruc, MD; Marc Humbert, MD; and Jean-Luc Cracowski, MD

BACKGROUND: Recent guidelines recommend riociguat, a soluble guanylate cyclase (sGC) stimulator, and the type 5 phosphodiesterase inhibitor (PDE5i) tadalafil or sildenafil as treatments for pulmonary arterial hypertension. We compared the safety profiles of sildenafil, tadalafil, and riociguat in pulmonary hypertension.

METHODS: We combined two approaches. First, we performed a meta-analysis of safety data extracted from randomized controlled trials. Second, we conducted a disproportionality analysis of data from Vigibase, the World Health Organization's global database of individual case safety reports, to compare the safety profiles with real-life data.

RESULTS: In the meta-analysis, a significant difference between the three drugs was only detected for gastrointestinal disorders, in disfavor of riociguat ($P < .01$ for interaction). In the disproportionality analysis, the use of riociguat was associated with fewer reports of visual disorders but increased reporting of gastrointestinal, hemorrhagic, and musculoskeletal disorders compared with sildenafil and tadalafil. Pharmacovigilance signals of hearing/vestibular disorders were heterogeneous: vestibular disorders (dizziness) were reported more frequently for riociguat, whereas hearing disorders (deafness) were reported less frequently compared with PDE5is.

CONCLUSIONS: The safety profiles of PDE5is and sGC stimulators significantly differ in pulmonary hypertension. Accordingly, there is a safety rationale in switching between PDE5is and sGC stimulators because of their different side effects.

TRIAL REGISTRY: PROSPERO; No.: CRD42016051986; URL: <https://www.crd.york.ac.uk/prospéro/>. CHEST 2018; 154(1):136-147

KEY WORDS: adverse event; meta-analysis; pharmacovigilance; phosphodiesterase 5 inhibitors; soluble guanylate cyclase stimulators

ABBREVIATIONS: ADE = adverse drug event; ADR = adverse drug reaction; cGMP = cyclic guanosine monophosphate; GRADE = Grading of Recommendation Assessment, Development and Evaluation; ICSR = individual case safety report; NNH = number needed to harm; NO = nitric oxide; PAH = pulmonary arterial hypertension; PDE5i = phosphodiesterase-5 inhibitor; PDE6 = phosphodiesterase-6; PH = pulmonary hypertension; PRR = proportional reporting ratio;

RCT = randomized controlled trial; sGC = soluble guanylate cyclase; WHO = World Health Organization

AFFILIATIONS: From the Pharmacovigilance Unit (Drs Khouri and Lepelley), Grenoble Alpes University Hospital, Grenoble; the Clinical Pharmacology Department (Drs Khouri, Roustit, and Cracowski), Grenoble Alpes University Hospital, INSERM CIC1406, Grenoble; the Univ. Grenoble Alpes (Drs Khouri, Roustit, and Cracowski), UMR

Pulmonary hypertension (PH) refers to diseases characterized by a mean pulmonary artery pressure > 25 mm Hg.¹ Currently, three main pathophysiologic pathways are targeted in the management of type 1 PH (pulmonary arterial hypertension [PAH]): the prostacyclin, endothelin, and nitric oxide (NO) pathways.^{1,2} In the latter, phosphodiesterase-5 inhibitors (PDE5is) decrease the degradation of cyclic guanosine monophosphate (cGMP), which is responsible for vasodilation. PDE5is (sildenafil and tadalafil) have been approved for over a decade for PAH.³⁻⁶ More recently, riociguat, a soluble guanylate cyclase (sGC) stimulator which increases the production of cGMP, has been approved to treat PAH and type 4 PH (chronic thromboembolic PH). Currently, the European Society of Cardiology/European Respiratory Society guidelines⁷ recommend riociguat, tadalafil, or sildenafil for New York Heart Association functional class II and III PAH. Given that these drugs target the same pathway and cannot be combined,^{1,8} thorough comparison of their respective safety profiles may guide clinicians in choosing the most appropriate one.⁹

Methods

Study Design

Following a literature review to define the various categories of ADEs, we performed a meta-analysis and a disproportionality analysis using VigiBase (Fig 1).

Classification of ADEs

From the literature review we defined nine ADE categories from safety profiles of drugs targeting the NO pathway^{9,13-17}: cardiac arrhythmias; ischemic heart disease; visual, musculoskeletal, hearing/vestibular, and gastrointestinal disorders; edema; hemorrhages; and vasodilation-related disorders. These categories were coded according to the Medical Dictionary for Regulatory Activities classification as were

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This study has been presented at the French Congress of Pharmacology and Therapeutics, April 19-21, 2017, Rouen, France.

FUNDING/SUPPORT: The authors have reported to *CHEST* that no funding was received for this study.

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DOI: <https://doi.org/10.1016/j.chest.2017.12.008>

Assessment of drug safety is complex and may require mixing methods and approaches beyond clinical trials to get a precise overview of the safety profile of a drug or a therapeutic class. Meta-analyses of safety data from randomized controlled trials (RCTs) provide precise quantification of adverse drug events (ADEs) collected in a standardized way but on limited and selected populations. Contrariwise, pharmacovigilance databases are based on spontaneous reporting of adverse drug reactions (ADRs) in the general population, allowing detection of associations between the reporting of an ADR and a drug (a pharmacovigilance signal). The strength of this association may be used as a proxy of the risk of an ADR.¹⁰⁻¹²

Therefore, we compared the safety profile of sildenafil, tadalafil, and riociguat in PH by combining these two approaches. First, we performed a meta-analysis of safety data extracted from RCTs. Second, we conducted a disproportionality analysis using the World Health Organization's (WHO's) global individual case safety report (ICSR) database, VigiBase, to compare ADRs in real life.

ADEs extracted from studies included in the meta-analysis and ADRs from VigiBase (e-Appendix 1).

Meta-Analysis

The meta-analysis was conducted following a predefined protocol (registered on PROSPERO as CRD42016051986) and is reported according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses recommendations.

Search Strategy: We searched MEDLINE, ClinicalTrials.gov, the Cochrane Library, and the reference lists of all included studies, from 1966 to January 2016. See e-Appendix 2 for details of the search strategy.

Eligibility Criteria: We included only RCTs assessing the efficacy of sildenafil, tadalafil, or riociguat on PH. Details of inclusion and exclusion criteria, the screening process, and data collection form are available in e-Appendix 3.

Data Extraction: For each published study included, we searched clinical trial registers for safety results. If not reported, we asked the authors for complete safety data.

The following data were collected for each study: study characteristics (author name, year of publication, total number of patients randomized, length of follow-up, and number of study sites), patient characteristics (age, sex, and PH etiology), intervention (treatment, dosage, add-on or not, and duration of treatment), and outcomes (the number of patients with at least one ADE was extracted, classified, and pooled according to the adverse event category).

Risk of Bias Assessment: Independent assessment of risk of bias was made according to the *Cochrane Handbook for Systematic Reviews of Interventions*¹⁸ and using the Grading of Recommendation Assessment, Development and Evaluation (GRADE) (e-Appendix 4).¹⁹

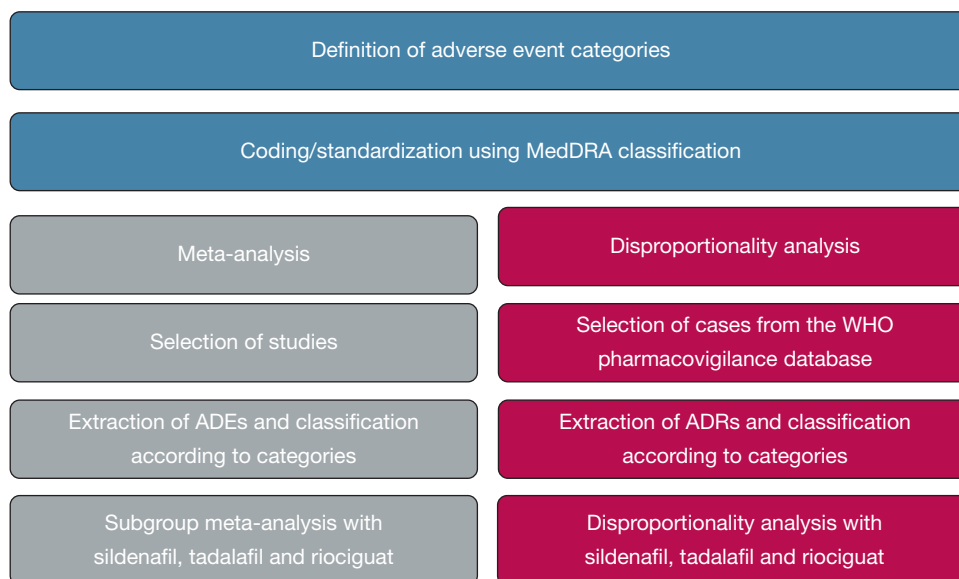


Figure 1 – Study design. ADE = adverse drug event; ADR = adverse drug reaction; MedDRA = Medical Dictionary for Regulatory Activities; WHO = World Health Organization.

Synthesis of Results and Statistical Analysis: We performed a direct meta-analysis using the DerSimonian-Laird random effect approach for each adverse event category.²⁰ Then subgroup analysis was performed by drug studied (riociguat, tadalafil, or sildenafil) in each adverse event subgroup. Subgroup difference was tested through testing the interaction among drug class subgroups, and $P < .05$ was considered significant. We used Q and I^2 statistics to assess heterogeneity, and a random effect model in case of substantial heterogeneity ($I^2 > 50\%$). When several arms were present in a study, we divided the control group population by the number of arms, as recommended by the Cochrane handbook.¹⁸ We computed the incidence rate of ADEs per patient-year for each study. Patient-years were estimated from the planned follow-up of each study. Incidence rate ratios and 95% CIs were used to compare adverse event incidence rates per patient-year for each drug vs their respective control groups. The number needed to harm (NNH) was calculated by the inverse of risk difference. When necessary for continuity correction, 0.5 was added to the numerator and denominator. R statistical software (version 3.2.3; R Foundation for Statistical Computing) was used for the meta-analysis.²¹

Disproportionality Analysis of Data From VigiBase

For the disproportionality analysis, we used the WHO international pharmacovigilance database (VigiBase), including all spontaneously reported cases of ADRs recorded between January 1, 1967, and August 25, 2016. This database contains > 13 million case reports from national pharmacovigilance centers from 130 countries, collected by the WHO Collaborating Centre for International Drug Monitoring (Uppsala, Sweden).

We extracted all cases associated with riociguat contained in VigiBase. Because sildenafil and tadalafil are also used as treatments of erectile dysfunction, we restricted our search to PH. We first extracted all cases associated with the brand names Revatio and Adcirca as indicated for PH. For cases associated with other brand names and with generic names, we restricted our search to a predefined list of indications (e-Appendix 5).

Disproportionality analysis was performed using the proportional reporting ratio (PRR) method that compares the rate of reporting of one effect among all reports for a given drug with the rate of reporting of the same effect among all reports for all drugs in the database.²² This allows for detecting associations between the reporting of an ADR and a drug (a pharmacovigilance signal).

The threshold for signal detection was defined as a PRR lower boundary 95% CI ≥ 1 and a number of cases ≥ 3 , according to the European Medicines Agency and the Pharmacoepidemiological Research on Outcomes of Therapeutics by a European Consortium recommendations.²³⁻²⁵ We considered that ADR categories differed significantly when CIs did not intersect. To minimize competition bias (bias affecting the disproportionality measure when an event of interest is strongly associated with another drug or class of drugs, or if an event is strongly associated with the drug of interest), we removed from the database all cases of PH and cardiac failure.²⁶ Another known bias is that PRR can vary over time.²⁷ To minimize such bias, we performed the disproportionality analysis at 3 years after drug marketing (2008 for sildenafil, 2012 for tadalafil, and 2016 for riociguat).

Results

Characteristics of Studies and Patients Included in the Meta-Analysis

Among 780 references identified and after removal of duplicates and screening of titles and abstracts, 165 reports had potential eligibility. After full-text screening,

13 RCTs were included in the quantitative analysis (Fig 2).

These studies enrolled 2,979 patients and reported 7,451 ADEs of interest. Characteristics of the included studies are summarized in Table 1.²⁸⁻⁴⁰ Placebo groups included 1,036 patients (308 patient-years), sildenafil included

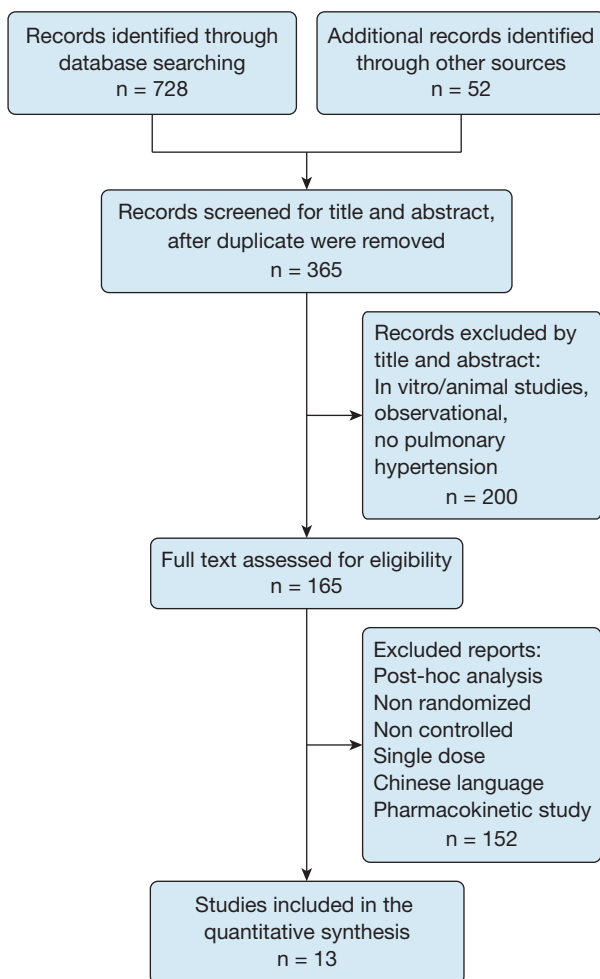


Figure 2 – Flowchart of the meta-analysis.

529 patients (132 patient-years), tadalafil included 745 patients (271 patient-years), and riociguat included 622 patients (167 patient-years).

Quality of Studies Included in the Meta-Analysis

Overall, the risk of bias was low: all 13 studies were double-blinded, eight (62%) provided sufficient information to assess allocation concealment, and nine (69%) reported adequate randomization. Incomplete outcome data were not detected, but only three study reported all ADEs^{30,31,36}; for other trials, the frequency threshold for reporting ADEs varied from 2% to 5% (Table 1). Two trials reported most frequent ADEs without specifying the threshold.^{29,34} Results from five studies were available on [ClinicalTrials.gov](https://clinicaltrials.gov) and these were used in the meta-analysis.^{30,37-40} One trial was only available on [ClinicalTrials.gov](https://clinicaltrials.gov)³³ and one author sent us complete safety data.³¹ Overall, six studies were considered as having moderate risk of bias, and seven

studies had low risk of bias. Results are summarized in Table 2.

We used GRADE recommendations to appraise the quality of the meta-analysis. Results are included in Figure 2 and details are provided in e-Table 1.

Characteristics of ICSRs Recorded in VigiBase

From the 13,734,630 ICSRs available in VigiBase at the time of extraction, we identified 6,642 safety reports for sildenafil, 3,420 reports for tadalafil, and 1,539 reports for riociguat. They accounted for 17,919 ADRs for sildenafil, 10,047 ADRs for tadalafil, and 8,569 ADRs for riociguat. Cumulative incidence of ADRs reported for sildenafil, tadalafil, and riociguat over time is shown in e-Table 2.

Comparison of the Meta-Analysis of RCTs and the Pharmacovigilance Disproportionality Analysis

The main results are presented in Figure 3. In the meta-analysis, considering the interaction tests, a significant difference between the three drugs was only detected for gastrointestinal disorders, in disfavor of riociguat ($P < .01$ for the interaction). The high rate of gastrointestinal disorders with riociguat (NNH = 0.7 patient-years), mostly nausea, diarrhea, and vomiting, was confirmed by an elevated PRR in the disproportionality analysis.

No association with cardiac arrhythmia and ischemic heart diseases was observed for any of the three drugs, and similarly no interaction was highlighted in the meta-analysis or the disproportionality analysis.

In the meta-analysis, sildenafil was significantly associated with an increased rate of visual disorders (NNH = 6.0 patient-years), but the interaction test was not significant. However, a higher signal for visual disorders with both PDE5is compared with riociguat was confirmed by the disproportionality analysis. Importantly, no retinal disorder (chromatopsia) was reported with riociguat in VigiBase.

No significant interaction between the three drugs was observed in the meta-analysis for hearing/vestibular disorders and hemorrhages. In contrast, disproportionality analysis revealed a higher signal with riociguat compared with PDE5is. Hemorrhages were essentially epistaxis, hemoptysis, and gastrointestinal bleeding. Hearing/vestibular disorder signals were heterogeneous: vestibular disorders (dizziness) were reported more frequently for riociguat, whereas hearing disorders (deafness) were reported less frequently compared with PDE5is. Considering

TABLE 1] Characteristics of Included Studies With Patient Baseline Characteristics and Treatments

Study Characteristics		Patients Characteristics				Treatment Characteristics				ADEs Reporting
Source/Year	Design	No.	Mean Age (y)	Female Sex (%)	Causes of Pulmonary Hypertension (%)	Active Drug	Daily Dose	Follow-Up (wk)	Comparator	
Galiè et al ²⁸ /2005	RCT parallel	278	48.7	75	Pulmonary arterial hypertension (type 1): Idiopathic (63%) Connective tissue disease associated (30%) Other (7%)	Sildenafil	20, 40, or 80 mg tid	12	Placebo	> 3%
Lewis et al ²⁹ /2007	RCT parallel	34	58	15	Pulmonary hypertension because of left-sided heart disease (type 2): Ischemic heart disease (50%) Nonischemic heart disease (50%)	Sildenafil	25 mg tid	12	Placebo	Most frequent
Simonneau et al ³⁰ /2008	RCT parallel	267	47.7	80	Pulmonary arterial hypertension (type 1): Idiopathic (79%) Connective tissue disease associated (21%)	Sildenafil (add on)	80 mg tid	16	Placebo and epoprostenol	All
Blanco et al ³¹ /2013	RCT parallel	63	65.5	8	Pulmonary hypertension because of lung disease (type 3): COPD (100%)	Sildenafil	20 mg tid	12	Placebo	All
Idiopathic Pulmonary Fibrosis Clinical Research Network et al ³² /2010	RCT parallel	180	69	17	Pulmonary hypertension because of lung disease (type 3): Lung fibrosis (100%)	Sildenafil	20 mg tid	12	Placebo	All serious ADEs Other > 5%
Pfizer ³³ /2013	RCT parallel	103	56	76	Pulmonary arterial hypertension (type 1)	Sildenafil (add on)	20 mg tid	12	Placebo and bosentan	All serious ADEs Other > 5%

(Continued)

TABLE 1] (Continued)

Study Characteristics		Patients Characteristics				Treatment Characteristics				ADEs Reporting
Source/Year	Design	No.	Mean Age (y)	Female Sex (%)	Causes of Pulmonary Hypertension (%)	Active Drug	Daily Dose	Follow-Up (wk)	Comparator	
Goudie et al ³⁴ /2014	RCT parallel	120	69	32	Pulmonary hypertension because of lung disease (type 3): COPD (100%)	Tadalafil	10 mg/d	12	Placebo	Most frequent
Galiè et al ³⁵ /2009	RCT parallel	405	53.8	78	Pulmonary arterial hypertension (type 1): Idiopathic (61%) Connective tissue disease associated (23%) Atrial septal defect (8%) Drug induced (4%) Other (4%)	Tadalafil	2.5, 10, 20, or 40 mg/d	16	Placebo	> 3%
Zhuang et al ³⁶ /2014	RCT parallel	124	51.5	79	Pulmonary arterial hypertension (type 1): Idiopathic (63%) Connective tissue disease associated (22%) Atrial septal defect (6%) Drug induced (9%)	Tadalafil (add on)	40 mg/d	16	Placebo and ambrisentan	All
Galiè et al ³⁷ /2015	RCT parallel	500	54.3	78	Pulmonary arterial hypertension (type 1): Idiopathic (53%) Heritable (3%) Connective tissue disease associated (37%) Drug induced (3%) Congenital heart disease (2%) HIV associated (2%)	Tadalafil (add on)	40 mg/d	24	Placebo and ambrisentan	All serious ADEs Other > 5%

(Continued)

TABLE 1] (Continued)

Study Characteristics		Patients Characteristics				Treatment Characteristics				ADEs Reporting
Source/Year	Design	No.	Mean Age (y)	Female Sex (%)	Causes of Pulmonary Hypertension (%)	Active Drug	Daily Dose	Follow-Up (wk)	Comparator	
Bonderman et al ³⁸ /2013	RCT parallel	201	58.1	14	Pulmonary hypertension because of left-sided heart disease (type 2): Ischemic cardiomyopathy (45%) Nonischemic cardiomyopathy (54%) Unknown (2%)	Riociguat	0.5, 1, or 2 mg tid	16	Placebo	All serious ADEs Other > 2%
Ghofrani et al ³⁹ /2013	RCT parallel	443	51	79	Pulmonary arterial hypertension (type 1): Idiopathic (61%) Heritable (2%) Connective tissue disease associated (25%) Congenital (8%) Drug induced (1%) Portopulmonary hypertension associated (3%)	Riociguat	1.5 or 2.5 mg tid	12	Placebo	All serious ADEs Other > 2%
Ghofrani et al ⁴⁰ /2013	RCT parallel	261	59	66	Chronic thromboembolic pulmonary hypertension (type 4): Inoperable (72%) Postoperative (28%)	Riociguat	2.5 mg tid	16	Placebo	All serious ADEs Other > 2%

ADE = adverse drug event; RCT = randomized controlled trial.

TABLE 2] Risk of Bias Summary

Study/Year	Random Sequence Generation	Allocation Concealment	Blinding Participant/ Personnel	Blinding Outcome Assessment	Incomplete Outcome Data	Selective Reporting	Other Bias
Ghofrani et al/2013 ³⁹	-	-	-	-	-	-	-
Ghofrani et al/2013 ⁴⁰	-	-	-	-	-	-	-
Bonderman et al ³⁸ /2013	-	-	-	-	-	-	-
Galiè et al/2015 ³⁷	-	-	-	-	-	-	-
Zhuang et al ³⁶ /2014	?	?	-	-	-	-	-
Galiè et al/2005 ²⁸	?	-	-	-	-	-	-
Lewis et al ²⁹ /2007	?	?	-	-	-	+	-
Simonneau et al ³⁰ /2008	-	-	-	-	-	-	-
Blanco et al ³¹ /2013	-	-	-	-	-	-	-
Goudie et al ³⁴ /2014	-	-	-	-	-	+	-
Galiè et al/2009 ³⁵	?	?	-	-	-	-	-
Idiopathic Pulmonary Fibrosis Clinical Research Network et al ³² /2010	-	-	-	-	-	-	-
Pfizer et al ³³ /2013	?	?	-	-	-	-	-

- = low risk; + = high risk; ? = unclear risk.

vasodilation-related disorders, we observed no differences in the meta-analysis (NNH = 1.2 patient-years, NNH = 1.0 patient-years, and NNH = 1.8 patient-years for riociguat, sildenafil, and tadalafil, respectively) and weak differences between sildenafil and riociguat in the disproportionality analysis.

Among PDE5is a trend toward less hearing/vestibular and gastrointestinal disorders with tadalafil compared with sildenafil was observed in both analyses. A main discrepancy between the two methods was observed concerning musculoskeletal disorders: the meta-analysis showed that tadalafil was significantly associated with increased risk, whereas in contrast, disproportionality analysis showed a stronger signal for riociguat.

Separate meta-analyses are available in [e-Figure 1](#). Results of the disproportionality analysis over time are presented in [Figure 4](#); in our study the analysis was performed 3 years after commercialization for each drug. Details on ADEs/ADRs recorded in clinical trials and in the WHO pharmacovigilance database are presented in [e-Table 3](#). No association with edema was detected in the meta-analysis for any of the drugs, and results of the disproportionality analysis are subject to confusion bias (bias created by a variable that influences both the dependent variable and independent variable causing a spurious association) because of a high prevalence of edema in patients with PH ([e-Fig 2](#)).

Sensitivity Analysis

We performed sensitivity analyses to assess the robustness of our results. Low-quality studies ([Table 2](#)) and studies with noncategory 1 PH were excluded ([e-Fig 3](#), [Table 1](#)). Sensitivity analyses substantially modified the results only for hearing/vestibular disorders associated with riociguat, which became nonsignificant. Sensitivity results are presented in [e-Table 1](#) and are included in the GRADE evaluation.

Discussion

Using data from RCTs and VigiBase, we found that among PAH drugs targeting the NO pathway, the use of riociguat was associated with fewer visual and hearing disorders but more gastrointestinal, hemorrhagic, musculoskeletal, and vestibular disorders than sildenafil and tadalafil.

Visual disorders are well-known adverse events of PDE5is. They are caused by the localization of phosphodiesterase-5 on endothelial and smooth muscle cells of the retina and choroid vessels, but also by nonspecific effects on phosphodiesterase-6 (PDE6) located on photoreceptors (rods and cones).⁴¹ Sildenafil is the principal inducer of retinal disorders (eg, chromatopsia) because of its high affinity for PDE6.^{42,43} Although tadalafil has low affinity for PDE6, visual symptoms could be caused by the modulation of retinal

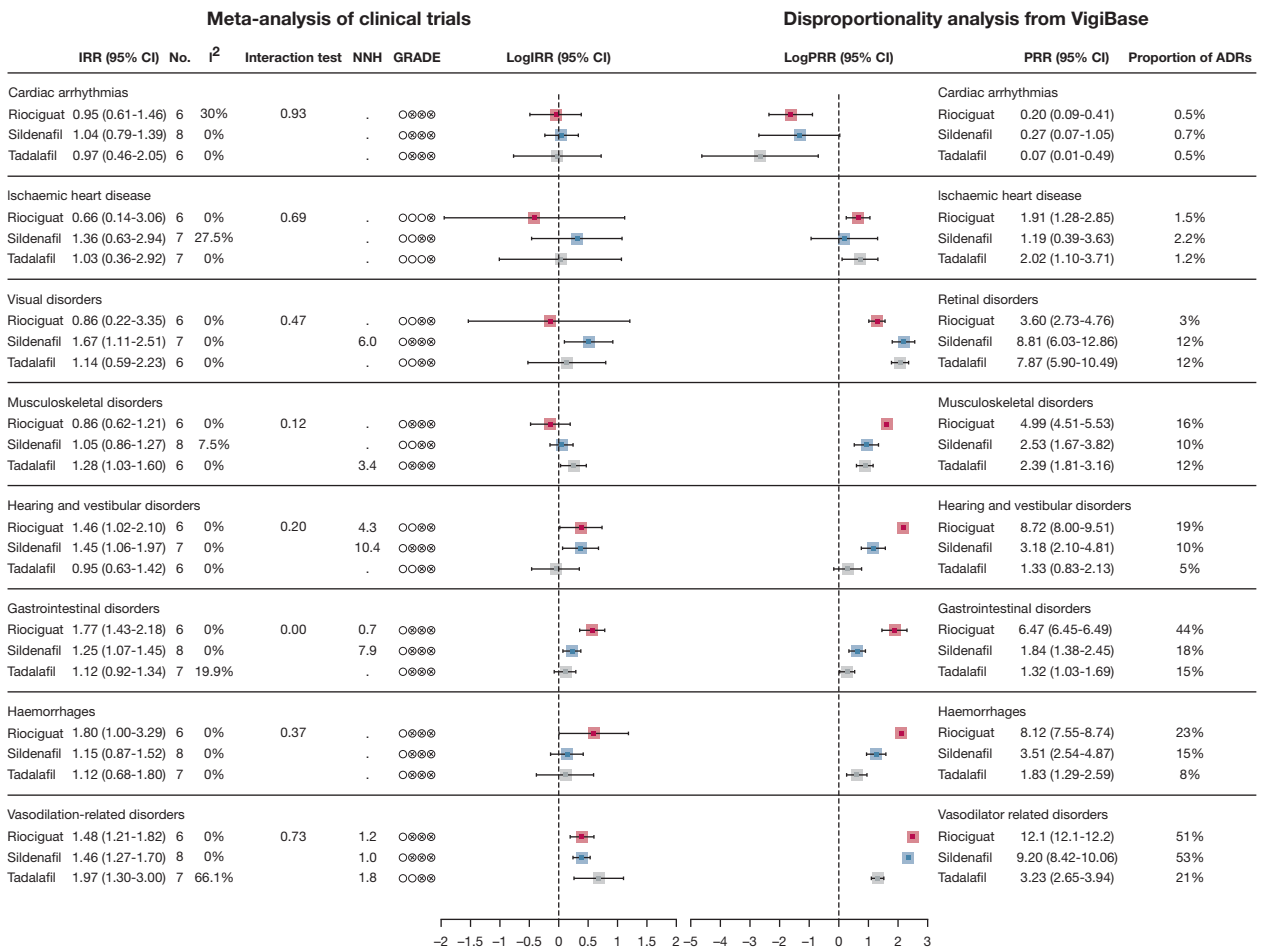


Figure 3 – Forest plot comparing the meta-analysis and the disproportionality analysis. GRADE quality of evidence includes moderate (○ ⊗ ⊗), low (○ ⊗ ⊗ ⊗), and very low (○ ○ ⊗ ⊗). GRADE = Grading of Recommendation Assessment, Development and Evaluation; I^2 = heterogeneity; IRR = incidence rate ratio; n = No. of studies in the subgroup; NNH = number needed to harm (patient-years); Proportion ADRs = proportion of adverse drug reactions of interest per overall number of adverse drug reactions in VigiBase for one given drug; PRR = proportional reported ratio. See Figure 1 legend for expansion of other abbreviation.

blood flow.^{41,43} No retinal disorder was reported with riociguat.

Musculoskeletal disorders are classically associated with PDE5is and are induced by nonspecific effects on muscular phosphodiesterase-11. The test for subgroup differences in the meta-analysis was nonsignificant, suggesting no differences among the three drugs. However, in the disproportionality analysis, although the effect size was similar between sildenafil and tadalafil, it was higher with riociguat. Although the presence of sGC at neuromuscular junctions could explain such disorders,⁴⁴ veracity of this higher rate of musculoskeletal disorders with riociguat remains to be further explored and confirmed.

Hearing and vestibular disorders are well described with PDE5is, and they are probably related to the accumulation of NO in cochlear and auditory nerves,

which induces oxidative stress and apoptosis.^{45,46} Our study suggests that tadalafil is less likely to be associated with such disorders than sildenafil. This result is concordant with epidemiologic studies, but the mechanism explaining this discrepancy remains to be elucidated.⁴⁷ Riociguat does not provoke local accumulation of NO, presumably explaining the lower rate of hearing disorders, and the pharmacologic signal was mostly represented by dizziness, which is not specific.

The NO-sGC-cGMP pathway is involved in gastrointestinal smooth muscle relaxation, peristalsis, and intestinal fluid secretion.⁴⁸⁻⁵¹ The higher frequency of gastrointestinal disorders associated with riociguat might be explained by the substantial expression of sGC in several types of gastrointestinal cells (eg, smooth muscle, interstitial cells).⁵⁰

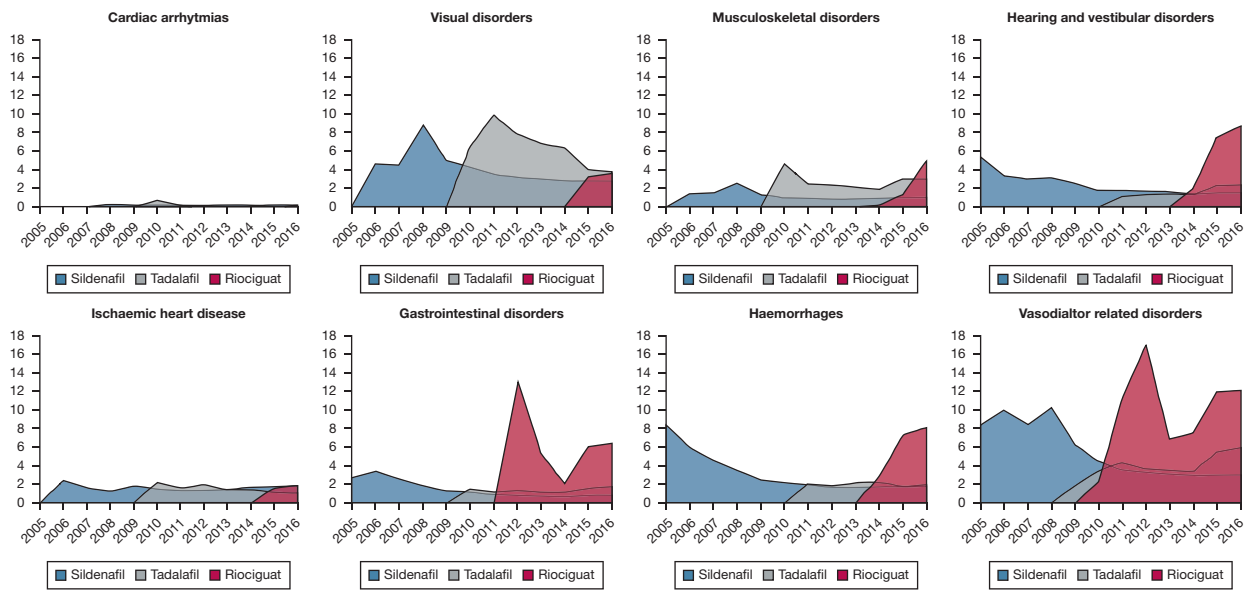


Figure 4 – Results of disproportionality analysis according to the time on the market.

The antiplatelet aggregation effect of NO donors is well described and is mediated by the NO-sGC-cGMP pathway.⁵² This effect is correlated to endogenous NO levels for the PDE5i but not for riociguat which activates sGC independently.⁵³ Given that NO levels are reduced in PH, the higher bleeding risk we found with riociguat may be related to increased activation of this pathway.⁹

In the pharmacovigilance disproportionality analysis, vasodilatation-related disorders seemed to be more frequently associated with riociguat and sildenafil than tadalafil. This suggests notification bias, in which underreporting for new drugs targeting the same pathways as existing drugs is a known risk factor. Indeed, tadalafil was marketed 4 years after sildenafil and physicians were aware of the safety profile of PDE5is, so they failed to signal adverse effects such as hypotension.

Our original approach mixes meta-analysis and pharmacovigilance methods to compare the safety profile of three drugs, both from clinical trials and postmarketing use. From clinical trials, we extracted almost twice as many ADEs from the trial databases compared with published articles (+85%) (e-Fig 4), highlighting the importance of searching such sources in a safety meta-analysis.⁵⁴ This allowed quantification of the risk of ADEs; however, unfortunately, they are not always completely or consistently reported. Indeed, we found discrepancies in reporting ADEs (Table 1), which might induce some bias. Moreover, RCTs are

underpowered to detect adverse events less frequent than the primary outcome. Finally, the selection of the maximal tolerated dose in run-in periods and the short follow-up duration of patients in RCTs (about 4 months per patient in our meta-analysis) reduces the incidence of some ADEs.

Analyzing the WHO pharmacovigilance database addresses these issues given that patients are not selected. On the other hand, notification may be selective and there is no control group. Although disproportionality analysis has been conceived to allow detecting signals, we used it in our study to compare strengths of associations between three drugs. The influence of length of time since marketing, media safety alerts, or selective notification makes interpretation of the results complex. In our study, the influence of time on the market on the PRR was striking (Fig 4), reflecting an important Weber effect in this drug class and highlighting the importance of taking into account such bias when comparing drugs.⁵⁵ However, despite these biases, a close correlation was found between relative risks and measure of disproportionality,¹⁰ and data-mining methods have proven their efficacy in pharmacovigilance.^{25,56} Moreover, we found a close similarity between adverse drug symptoms reported in RCTs and in VigiBase (e-Table 3). A further potential limitation is that riociguat, but not the PDE5i, is indicated for patients with thromboembolic PH that may differ from type 1 PAH.

Conclusions

This approach mixing a meta-analysis and a pharmacovigilance disproportionality analysis showed that safety profiles of PDE5is and sGC

stimulators significantly differ in PH. Accordingly there is a safety rationale in switching between PDE5is and sGC stimulators because of their different side effects.

Acknowledgments

Author contributions: C. K. had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis, including and especially any adverse effects. C. K. also assumes full responsibility for the integrity of the submission as a whole, from inception to published article. Study concept and design was conducted by C. K., M. L., and J.-L. C. Acquisition of data was done by C. K. and M. L. Analysis and interpretation of data was done by C. K., M. L. F. M., M. R., M. H., and J.-L. C. Drafting of the manuscript was completed by C. K. Critical revision of the manuscript for important intellectual content was completed by M. R., F. M., M. H., and J.-L. C. Approval of the final manuscript was completed by C. K., M. L., M. R., F. M., M. H., and J.-L. C.

Financial/nonfinancial disclosures: The authors have reported to CHEST the following: M. R. and J.-L. C. have received research grants from Pfizer, Actelion, GlaxoSmithKline, and Bioprojet for other studies. M. H. reports personal fees from Actelion Pharmaceuticals Ltd; personal fees from Bayer; personal fees from GSK; personal fees from Pfizer; personal fees from United Therapeutics, during the conduct of the study; and personal fees from Novartis, outside the submitted work. None declared (C. K., M. L., F. M.).

Other contributions: We thank Dr Blanco, MD, for sending us complete safety data of her study.³⁰ We thank Dr Camilla Westerberg, BSc Pharm, Research Pharmacist, Custom Search Services Team at the Uppsala Monitoring Centre Research Section for providing us the Vigibase data. We also thank Alison Foote, PhD (Grenoble Alpes University Hospital) for critically editing the manuscript. In Vigibase, the supplied data come from a variety of sources and countries. The likelihood of a causal relationship is not the same in all reports. The opinions and conclusions in this study are not necessarily those of the various centers of pharmacovigilance or of the World Health Organization.

Additional information: The e-Appendixes, e-Figures, and e-Tables can be found in the Supplemental Materials section of the online article.

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2. Pulmonary Arterial Hypertension associated with Protein Kinase Inhibitors: A pharmacovigilance-pharmacodynamic study.

Plusieurs cas d'HTAP ont été rapportés dans la littérature quelques mois après la mise sur le marché d'un inhibiteur de tyrosine kinase, le dasatinib. (115,116) Depuis de nombreux autres cas ont été rapportés avec d'autres inhibiteurs de protéine kinase ; notamment avec le bosutinib, le ruxolitinib ou le lorlatinib. (117–119) Nous avons réalisé une étude de « pharmacovigilance-pharmacodynamie », mixant une analyse de disproportionnalité et des données de pharmacodynamie afin de tenter de répondre à deux questions qui restaient en suspens :

- les HTAP sont-ils un effet indésirable de classe des inhibiteurs de protéine kinase ? Si non, quels inhibiteurs de protéine kinase sont concernés ?
- quel est le mécanisme physiopathologique de ces HTAP induits par les inhibiteurs de protéine kinase ?



Pulmonary arterial hypertension associated with protein kinase inhibitors: a pharmacovigilance–pharmacodynamic study

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Using the WHO pharmacovigilance database, PAH was found to be associated with dasatinib, bosutinib, ponatinib, ruxolitinib and nilotinib. The potential role of Src protein kinases and TEC in PAH induced by protein kinase inhibitors is further highlighted. <http://ow.ly/56sO30nMKMj>

Cite this article as: Cornet L, Khouri C, Roustit M, *et al.* Pulmonary arterial hypertension associated with protein kinase inhibitors: a pharmacovigilance–pharmacodynamic study. *Eur Respir J* 2019; 53: 1802472 [<https://doi.org/10.1183/13993003.02472-2018>].

ABSTRACT The pathophysiology of pulmonary arterial hypertension (PAH) induced by protein kinase inhibitors (PKIs) remains unclear. To gain knowledge into this rare and severe pathology we performed a study combining a pharmacovigilance approach and the pharmacodynamic properties of PKIs.

A disproportionality analysis on the World Health Organization pharmacovigilance database VigiBase using the reporting odds ratio (ROR) and 95% confidence interval was first performed. Then, we identified the most relevant cellular targets of interest through a systematic literature review and correlated the pharmacovigilance signals with the affinity for the different PKIs. We further performed a hierarchical cluster analysis to assess patterns of binding affinity.

A positive disproportionality signal was found for dasatinib, bosutinib, ponatinib, ruxolitinib and nilotinib. Five non-receptor protein kinases significantly correlate with disproportionality signals: c-Src ($r=0.79$, $p=0.00027$), c-Yes ($r=0.82$, $p=0.00015$), Lck ($r=0.81$, $p=0.00046$) and Lyn ($r=0.80$, $p=0.00036$), all belonging to the Src protein kinase family, and TEC ($r=0.85$, $p=0.00006$). Kinases of the bone morphogenetic protein signalling pathway also seem to play a role in the pathophysiology of PKI-induced PAH. Interestingly, the dasatinib affinity profile seems to be different from that of other PKIs in the cluster analysis.

The study highlights the potential role of the Src protein kinase family and TEC in PAH induced by PKIs. This approach combining pharmacovigilance and pharmacodynamics data allowed us to generate some hypotheses about the pathophysiology of the disease; however, the results have to be confirmed by further studies.

Introduction

Pulmonary hypertension is defined as an increase in mean pulmonary arterial pressure ≥ 25 mmHg assessed by right heart catheterisation [1]. The pathophysiology is characterised by an increased migration and proliferation of pulmonary arterial smooth muscle cells, leading to vascular remodelling [2]. The classification proposed by the European Society of Cardiology/European Respiratory Society guidelines defines five groups of different pathological features which characterise the diverse clinical pulmonary hypertension groups [1]. Group 1 relates to pulmonary arterial hypertension (PAH), a rare and life-threatening condition characterised by the remodelling of pulmonary arteries [3], and associated with various aetiologies. Indeed, PAH may be idiopathic, heritable, drug and toxin induced, or associated with conditions such as connective tissue disease, HIV infection, congenital heart disease or schistosomiasis, with worldwide heterogeneity.

Among drug-induced PAH, the multiple protein kinase inhibitor (PKI) dasatinib had been increasingly linked to PAH since 2009 [4, 5]. More recently, several cases have reported potential association with or deterioration of pre-existing PAH with other PKIs such as bosutinib, ponatinib and lapatinib [6–8].

Since these compounds inhibit multiple kinases, the identification of a target responsible for such a rare adverse event is challenging. We thus mined pharmacovigilance data mining with the pharmacodynamic properties of PKIs to gain knowledge into potential mechanisms underlying this rare and severe adverse event.

Methods

Study design

We first performed a disproportionality analysis from the World Health Organization (WHO) pharmacovigilance database VigiBase (www.who-umc.org/vigibase/vigibase). Disproportionality analyses are largely used by regulators to generate “pharmacovigilance signals” aiming at assessing putative links between drugs and adverse drug reactions (ADRs) [9]. Such methods compare the reporting proportion between a studied drug and all other drugs in the database for a given ADR. Several measures of disproportionality have been developed, but there is no recognised gold standard [10]. They do not provide risk quantification, but could be used as a proxy of the risk of an ADR when no other estimate is available (*i.e.* for extremely rare ADRs) [11–14]. In a second step we identified cellular targets of interest through a systematic literature review. Finally, we evaluated the association between the pharmacovigilance disproportionality signals and the affinity for different PKIs.

Pharmacovigilance database

VigiBase is the WHO global database of individual case safety reports (ICSRs). At the time of extraction, this database contained approximately 16 million reports of suspected adverse effects of medicines, from more than 150 countries, collected since 1968. VigiBase provides ICSRs with patient information such as sex, age, medical history and country; suspected and concomitant drugs taken with chronological information, as well as drug indication and dosage; and a description of the adverse effect with its severity and outcomes.

Selection of cases

We used the standardised high-level term “Pulmonary hypertension” of MedDRA (Medical Dictionary for Regulatory Activities) terminology to identify pulmonary hypertension cases from VigiBase. To select drug-induced type 1 PAH we excluded all ICSR of pulmonary hypertension associated with cardiac, pulmonary or thrombotic disorders, connective tissue diseases, HIV infection, congenital heart disease, or schistosomiasis. Details are available in supplementary appendix S1.

Then, ICSR containing drugs or toxins known to induce PAH (aminorex, fenfluramine, dexfenfluramine, benfluorex, amphetamines (dexamfetamine), phentermine and mazindol) were also excluded [15].

Selection of PKIs

To select PKIs with a reasonable level of information to calculate accurate reporting odds ratios (RORs), we included in the analysis only PKIs with more than 100 suspect ICSR reported in VigiBase between January 1, 2002 and December 31, 2017 [16]. We therefore selected 28 drugs: afatinib, alectinib, axitinib, bosutinib, cabozantinib, ceritinib, cobimetinib, crizotinib, dabrafenib, dasatinib, erlotinib, gefitinib,

This article has supplementary material available from erj.ersjournals.com

Received: Dec 31 2018 | Accepted after revision: Feb 17 2019

ibrutinib, lapatinib, lenvatinib, lestaurinib, osimertinib, nilotinib, palbociclib, pazopanib, ponatinib, regorafenib, ruxolitinib, sorafenib, sunitinib, trametinib, vandetanib and vemurafenib.

To avoid confounding in the pharmacovigilance signal interpretations, nintedanib and imatinib were excluded *a priori* from the selection because of suspected protopathic and indication bias. Indeed, it is impossible to distinguish reports of PAH induced by pulmonary fibrosis in nintedanib-treated patients and drug inefficacy in imatinib-treated patients from adverse events [17, 18].

Identification of protein kinases involved in PAH and affinity between PKIs and these targets

Cellular targets of interest involved in PAH pathophysiology were identified through a systematic literature review in MEDLINE with the Medical Subject Headings (“Familial Primary Pulmonary Hypertension”[Mesh]) AND “Protein Kinases”[Mesh].

Affinity data for the targets of interest were extracted from the International Union of Basic and Clinical Pharmacology/British Pharmacological Society Guide to Pharmacology in 2018 [19].

Disproportionality analysis

We first performed a disproportionality analysis with the ROR method for each PKI of interest considered as suspect [20]. We compared the proportion of PAH reported for each PKI with the proportion of PAH associated with all other drugs used as non-cases. The cut-off for signal detection was defined as a ROR lower boundary 95% confidence interval ≥ 1 and three or more cases [21]. We also performed a temporal analysis to assess to the influence of media safety alerts on the reporting rate of PAH among reported adverse events, as previously described [22].

Statistical analyses

To assess the link between the identified cellular targets of interest and pharmacovigilance signals, we calculated the Pearson correlation coefficient (r) between the negative logarithm of the dissociation constant K_d (pK_d) and the ROR.

We hypothesised that the higher the affinity for the cellular target, the higher the “risk” of notification of suspected drug-induced PAH. In order to take into account the multiplicity of comparisons, the statistical significance threshold for all p -values was adapted using a Bonferroni correction [23].

Sensitivity analyses were performed to assess the robustness of the results: 1) excluding PKIs that had less than three cases of PAH; 2) standardising the time on the market for the different PKIs at 6 years after the US Food and Drug Administration approval date, corresponding to the time between dasatinib approval and the first published safety alert; and 3) performing the correlation using other affinity data, extracted from DAVIS *et al.* [24].

We further performed a hierarchical cluster analysis, through the hierarchical k -means clustering method, to assess the similarity among receptor binding affinity profiles of the included protein kinases [25].

Lastly, for the PKIs associated with a significant pharmacovigilance disproportionality signal we studied the influence of media safety alerts on the reporting rate of PAH. Moreover, as suggested by a reviewer, we performed a multinomial regression analysis to assess the influence of dose and duration of exposure on the outcomes of the PAH cases (recovered/not recovered/died)

Descriptive results are expressed as mean with standard deviation or median (interquartile range (IQR)).

All analyses were performed using R version 3.2.3 (www.r-project.org).

Results

Selection of cases

Up to December 31, 2017, a total of 286 834 ICSRs were related to the 22 selected PKIs. Among them, 733 cases of pulmonary hypertension were extracted. The exclusion of cases associated with other PAH aetiologies and concomitant drugs led to 442 ICSRs included in the final analysis (supplementary figure S1).

Description of PAH cases

Among the 442 cases of PAH, 193 were female (43.7%), 202 were male (45.7%) and sex was unknown for 47 (10.6%); mean \pm SD age was 57.6 \pm 15.8 years. A pleural effusion was associated with PAH in 75 cases (17.0%). The median (IQR) delay between PAH and PKI introduction was 23 (6.3–41.3) months (data available for 206 ICSRs), with substantial heterogeneity: 2.9 (1.7–12.8) months for bosutinib, 27.9 (11.5–45.0) months for dasatinib, 11.7 (2.6–22.0) months for nilotinib, 10.7 (8.1–11.4) months for ponatinib and 12.0 (3.9–49.1) months for ruxolitinib.

Identification of protein kinases involved in PAH

35 protein kinases involved in PAH pathophysiology were identified through the literature review (supplementary figure S2): ALK1/5, AMPKa1/2, BMPR1/2, B-Raf, c-Yes, DDR1, EIF2K4, ERBB1, FAK, FGFR1/2, HER2, IGF1R, JAK1/2, JNK1/2, KIT, Lck, Lyn, HGF, PDGFR α/β , PKG, RAF1, ROCK2, Src, TEC, TIE2 and VEGFR1/2/3. Full definitions/aliases and the most relevant references about the target of interest are reported in supplementary appendix S2.

Disproportionality analysis

Among the 28 selected PKIs, at least one PAH case was reported for 22. A positive disproportionality signal was found for dasatinib, bosutinib, ponatinib, ruxolitinib and nilotinib, with a ROR of 28.64 (95% CI 25.53–31.93), 13.43 (95% CI 8.65–20.87), 3.88 (95% CI 1.86–7.46), 3.71 (95% CI 2.44–5.65) and 3.39 (95% CI 2.43–4.73), respectively. RORs are represented in figure 1. Results of the sensitivity analysis (standardising on time on the market) were consistent with the main analysis, except for nilotinib which became nonsignificant. Results are presented in supplementary appendix S3 and supplementary figure S3.

Drug dosages were available for 295 cases and are represented in figure 2. Among the 170 PAH cases associated with dasatinib, only two were reported with a dosage higher than recommended. No correlation was found between PKI dosage, duration of exposure and outcome severity (data not shown).

Correlation analysis

Among the 22 PKIs identified in VigiBase, affinity data for the target of interest were available for 16 [19]. Five protein kinases were significantly correlated with disproportionality signals: c-Src ($r=0.79$, $p=0.00027$), c-Yes ($r=0.82$, $p=0.00015$), Lck ($r=0.81$, $p=0.00046$), Lyn ($r=0.80$, $p=0.00036$) and TEC ($r=0.85$, $p=0.00006$). The proportion of variance (r^2) explained by the model was 0.72, 0.67, 0.64, 0.64 and 0.72 for c-Src, c-Yes, Lck, Lyn and TEC, respectively. The results of the correlation analysis for each target classified according to its main cellular function are presented in figure 3.

Results for c-Yes, c-Src and TEC remained significant in all three sensitivity analyses, while results for Lck and Lyn remained significant in two of them. Furthermore, two other targets became significantly associated with disproportionality signals in the sensitivity analysis excluding PKI with less than three PAH cases: ALK1 ($r=0.9$) and ALK5 ($r=0.98$). Results are presented in supplementary appendix S4.

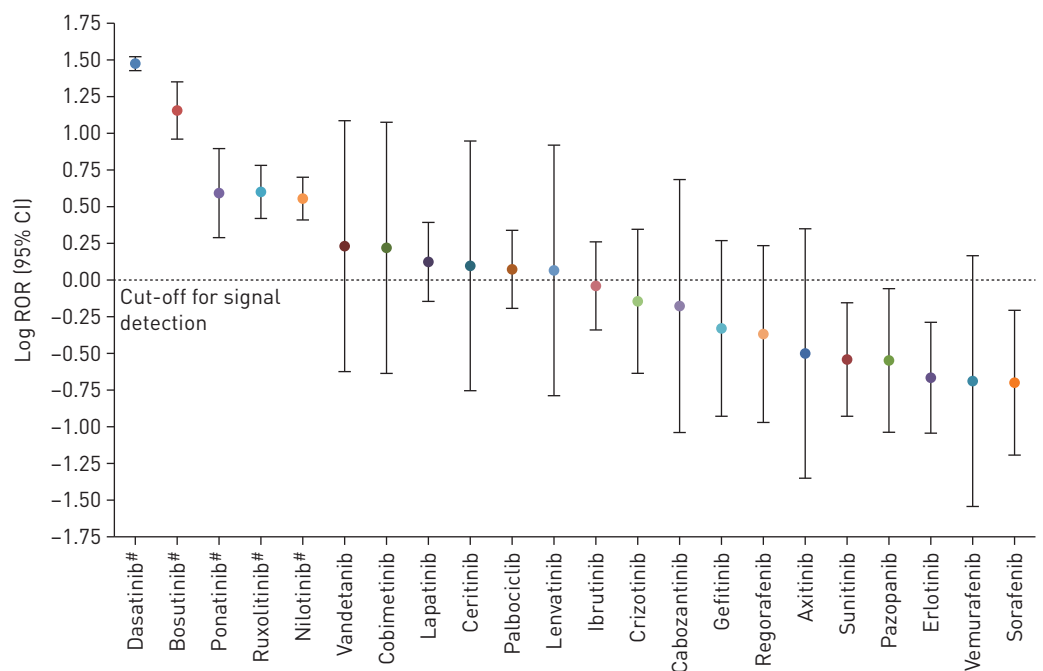


FIGURE 1 Forest plot of the reporting odds ratio (ROR) values of protein kinase inhibitor (PKI)-related pulmonary arterial hypertension. #: PKI associated with positive disproportionality signal.

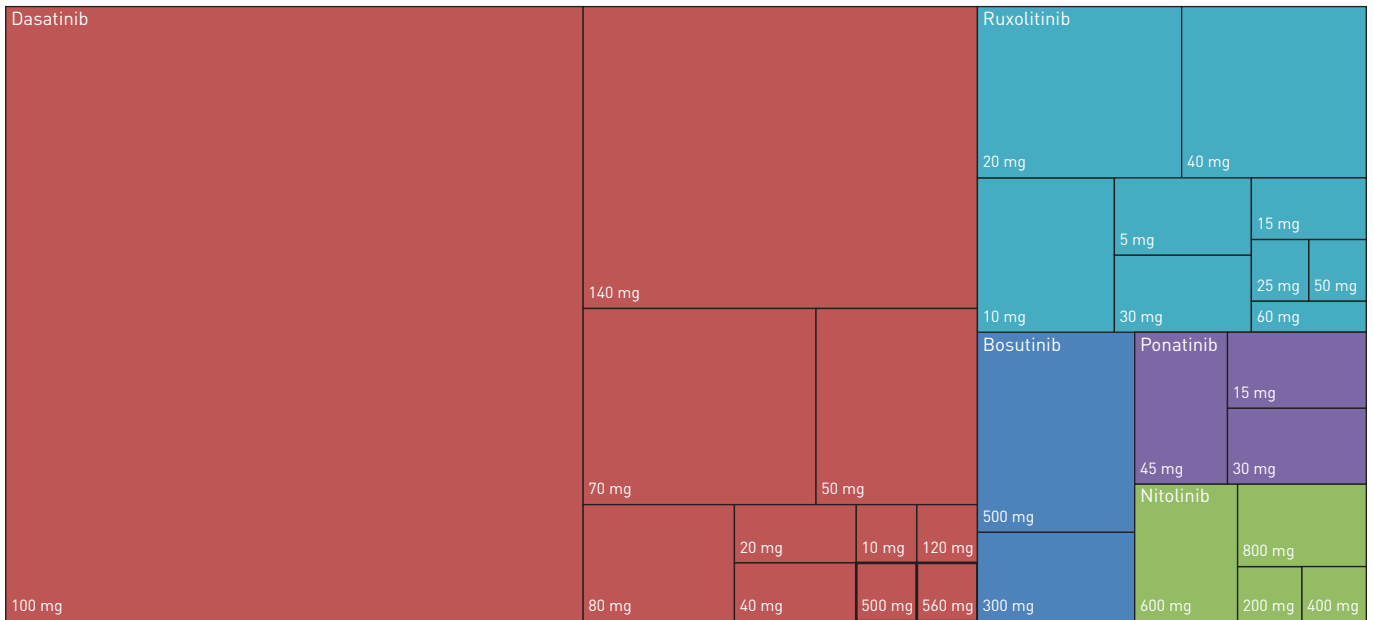


FIGURE 2 Treemap of daily drug dosages for the five most reported protein kinase inhibitors: dasatinib (n=170), bosutinib (n=13), ruxolitinib (n=36), nilotinib (n=9) and ponatinib (n=10). The area of the rectangles is proportional to the number of reported cases for each dosage/drug combination. Higher than recommended dosages of dasatinib (500 and 560 mg) are indicated.

Cluster analysis

We performed a hierarchical clustering based on the affinity data of each PKI. Results are presented in figure 4, which represents the degree of PKI affinity for the identified protein kinase involved in PAH. The dasatinib affinity profile differs from that of bosutinib, ruxolitinib and nilotinib.

Time-trend analysis

We studied the association between PAH reports and media safety alerts by a temporal analysis of the annual proportion of PAH reports for 1000 reported adverse events for each PKI with a significant pharmacovigilance disproportionality signal. Notably, an important increase in the rate of notification for dasatinib and bosutinib can be seen after first media alert. Results are presented in figure 5.

Discussion

To the best of our knowledge, this is the first pharmacovigilance analysis assessing the reporting risk of PAH associated with PKI use. Among more than 16 million ADRs reported in the WHO

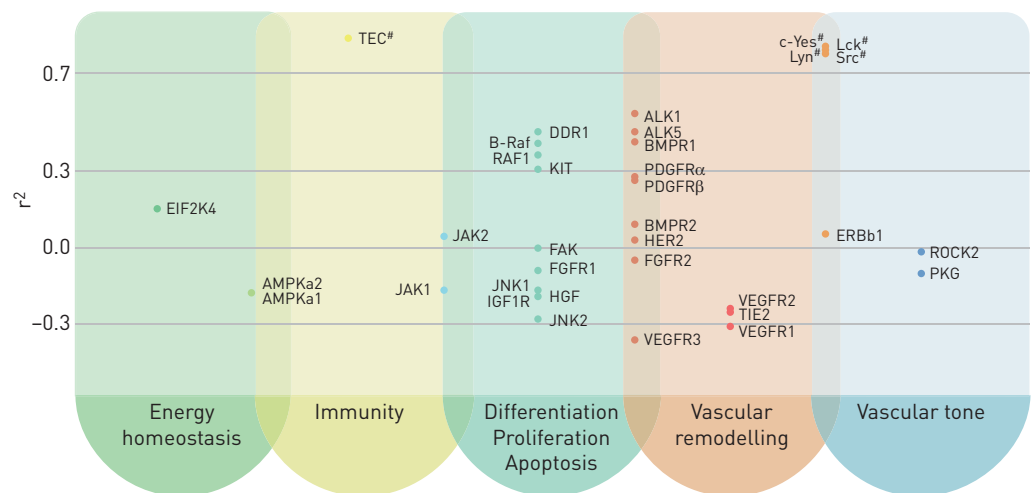


FIGURE 3 Manhattan plot synthesizing the correlation analysis. Pearson correlation coefficients (r^2) of each target classified according to its main cellular function. Full definitions/aliases are reported in supplementary appendix S2. #: significant correlation in the initial analysis.

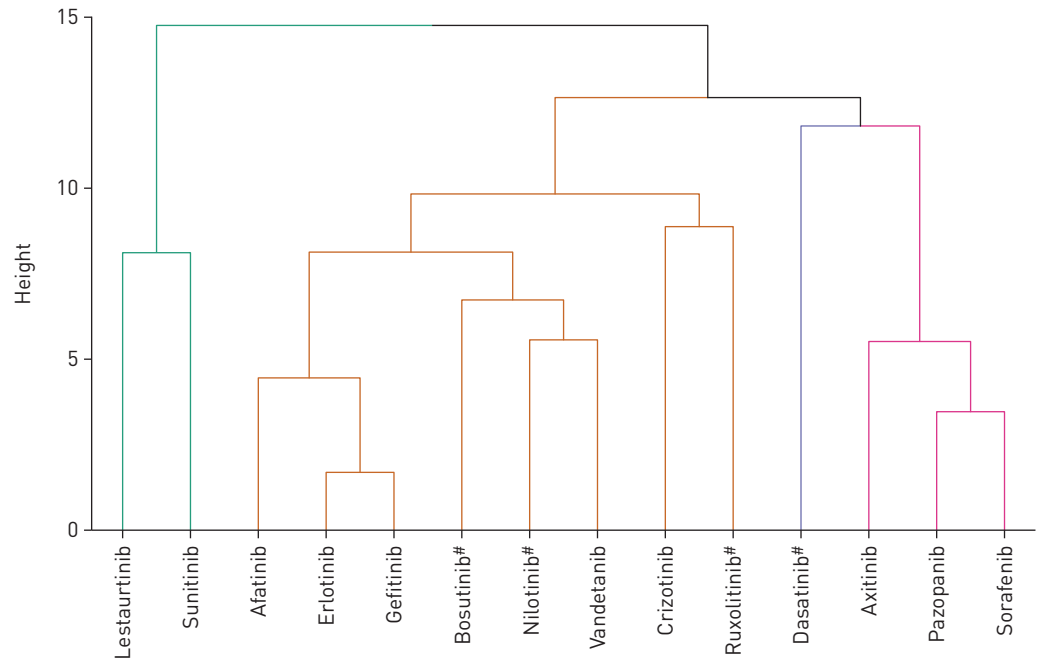


FIGURE 4 Cluster dendrogram of protein kinase inhibitors (PKIs) based on their affinity profile. #: PKI with a significant pharmacovigilance disproportionality signal.

pharmacovigilance database Vigibase at the date of the extraction, 286834 ICSRs were associated the 28 selected PKIs, including 442 PAH cases. Disproportionality analysis showed that dasatinib, bosutinib, ponatinib, ruxolitinib and nilotinib displayed a significant pharmacovigilance signal. Those results are consistent with the literature, with dasatinib being the most widely implicated PKI in induction or aggravation of PAH [5, 20–23]. More recently, bosutinib, ponatinib and ruxolitinib were also linked to PAH [6, 26]. Results for nilotinib seem less robust because the pharmacovigilance disproportionality signal disappeared in the sensitivity analysis and high dosages were used for a third of the cases. Moreover, well-documented case reports are still lacking in the literature for nilotinib. The pharmacovigilance signal found for ruxolitinib could also be questioned because ruxolitinib is prescribed in the treatment of polycythaemia vera and essential thrombocythaemia, which are recognised causes of pulmonary hypertension. Otherwise, a published case series suggested that lapatinib, a PKI used in breast cancer with human epidermal growth factor receptor mutations, might also cause PAH, but only one of the six

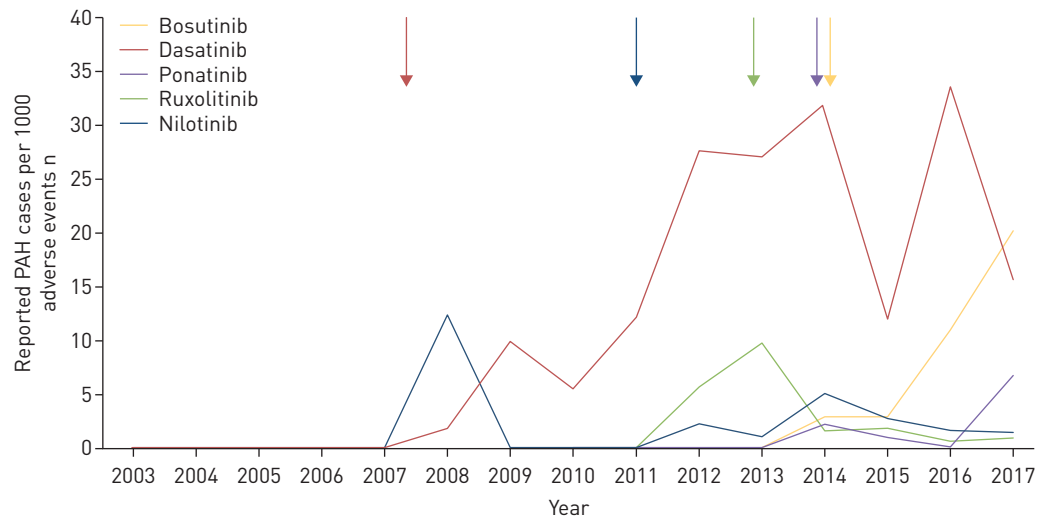


FIGURE 5 Proportion of reported pulmonary arterial hypertension (PAH) cases per 1000 reported adverse events per year for the five protein kinase inhibitors with a significant disproportionality signal. The arrows indicate the first published case reports in MEDLINE for each drug.

patients presented in this case series had right heart catheterisation confirming pre-capillary PAH [27]. In our study, lapatinib showed a weak, nonsignificant disproportionality signal with a ROR of 1.13 (95% CI 0.61–2.10). Although not included in our study because of a lack of reported ICSRs in VigiBase, lorlatinib has recently been linked to PAH [7]. Further studies are needed to confirm these first reports.

The correlation analysis showed that c-Src, c-Yes, Lck, Lyn and TEC were highly correlated to PAH reporting risk. The Src tyrosine kinase family contains nine members: three of them (Src, Fyn and Yes) are ubiquitously distributed and six (Blk, Yrk, Fgr, Hck, Lck and Lyn) are variously expressed depending on the tissue. Src tyrosine kinases are crucial for TWIK-related acid sensitive potassium 1 (TASK-1) potassium channel functioning, acting as a cofactor [28]. Mimicking hypoxia conditions, inhibition of Src kinases decreases TASK-1 activity resulting in an intracellular calcium level increase, and thus enhancing vasoconstriction and vascular remodelling [28]. However, these findings have to be balanced by the dasatinib dosage studied, which corresponded to 500 times the clinical dose. Beyond inhibition of such protein kinases, dasatinib might induce apoptosis and endothelial cell dysfunction through an increase of mitochondrial reactive oxygen species (ROS) that is independent from Src family kinase inhibition [29]. However, there are no significant changes in pulmonary haemodynamic parameters in rats treated daily with high doses of dasatinib (10 times the clinical doses) for 4 weeks [29]. Given the probable influence of PKI dosage on the onset of PAH, secondary targets may also have an important contribution that should be further elucidated in pre-clinical research [30–32].

TEC and Lyn have been linked to pleural effusions through an immune-mediated mechanism and could represent a common signalling pathway explaining the high proportion of such disorders in PKI-related PAH cases [14, 33]. Consistent with the high incidence of dasatinib-induced pleural effusion, rats treated with high doses of dasatinib developed pleural effusion following a period of at least 5 weeks, supporting a direct link between high doses of dasatinib and the development of pleural effusion [34]. Interestingly, this work highlights that high circulating levels of dasatinib alter pulmonary endothelial permeability in a ROS-dependent manner *in vitro* and *in vivo*, leading to pleural effusion.

Members of the bone morphogenetic protein signalling pathway showed heterogeneous results in our study. Although ALK1, ALK5 and BMPR1 showed a positive correlation in the main or sensitivity analysis, BMPR2, the primary cause of heritable PAH, did not show any correlation in our study. The bone morphogenetic protein signalling pathway is involved in cell proliferation, mitochondrial dysfunction and inflammation [18]. Mutation of *BMPR2*, the gene coding for the BMPR2 receptor, accounts for 70–80% of heritable PAH; furthermore, BMPR2 concentration has also been shown to be reduced in lung tissue from patients with PAH [35]. However, estimates indicate that only ~20% of individuals with a known genetic mutation in BMPR2 will develop PAH during their life, thus *BMPR2* mutation is required but is not sufficient alone for phenotypic expression and increases an individual's chance of developing PAH [18, 36]. Interestingly, CARUSO *et al.* [37] recently showed that BMPR2 reduction, through the microRNA miR-124, leads to the mitochondrial Warburg phenotype and may explain the mitochondrial increased ROS found by GUIGNABERT *et al.* [29].

The absence of an association between platelet-derived growth factor and vascular endothelial growth factor protein kinases reinforces the fact that vascular remodelling is not a major component of PAH induced by PKIs, which is consistent with the observations of PAH reversal upon PKI discontinuation. Despite this, there is some evidence suggesting that irreversible PAH should occur through ROS generation [26, 38].

Genetic mutations are considered to be permissive of disease and require additional epigenetic, inflammatory or environmental factors for the development of PAH in individuals with those mutations [39]. Similarly, and based on *in vitro* and *in vivo* findings, PKIs increase the risk of developing PAH but require a comparable genetic, epigenetic or environmental “second hit”, which remains to be identified [29]. According to published case series, a higher proportion of males may develop PKI-induced PAH, while the incidence of PAH is fourfold higher in females than in males in the general population [18]. It is known that males have a worse prognosis mainly because of a maladaptive response of the right ventricle to PAH; we thus cannot exclude a participation of hormones and sex in triggering PAH [40].

In the cluster analysis, we tried to identify a PKI family specifically involved in PAH. The results are mainly in accordance with the literature and consistent with the chemical structure of PKIs [41]. Interestingly, the dasatinib affinity profile for protein kinases involved in PAH seems unique among the drug class. However, PKIs such as vandetanib or crizotinib, which share a similar affinity profile to that of bosutinib, nilotinib and ruxolitinib, but which are used in solid-organ malignancies, are not associated with the reporting of PAH (figure 4). This observation may help to elucidate the role of the underlying haematological disease in the genesis of PAH beyond inhibition of protein kinases.

Given that pharmacovigilance notifications are based on a spontaneous reporting system, the number and proportion of cases reported for a medicinal product may vary depending on many factors, such as media

safety alerts, time since marketing or selective notification. Thus, the exact population exposed to a given drug is unknown. Illustrating this variability, the time-trend analysis showed a large increase in the rate of reporting after the first case series and case report publications. However, despite these biases, a correlation between relative risks and a measure of disproportionality was found [11]. Moreover, while we retrieved all cases for selection in this study we cannot exclude that instances of spurious PAH were included; indeed, only two cases reported abnormal right heart catheterisation results. Unfortunately, the medications introduced after the onset of the adverse event are not fulfilled in the database to avoid spurious pharmacovigilance signals, thus they could not be used for case selection. In two cases (one with dasatinib and one with ruxolitinib) a previous exposure to interferons was found, but the link with PAH onset was not considered strong enough to be excluded. Furthermore, new onset and aggravation of PAH were considered similar. Unfortunately, our study of comedications, associated pathologies and drug dosages was limited by the high rate of missing data in the ICSRs reported in Vigibase. This reinforces the importance of reporting all suspected ADRs on pharmacovigilance systems in order to improve their efficiency [42].

In the present pharmacovigilance–pharmacodynamic analysis, we assumed that PAH was caused by a single protein kinase and we did not account for co-inhibition of multiple protein kinases. However, we tried to address this limitation by performing a cluster analysis to identify at-risk groups of PKIs. Lastly, our study was not able to detect inhibition/activation of non-protein kinase cellular targets (e.g. proteasomes, G protein-coupled receptors, voltage-gated ion channels or ligand-gated ion channels). Therefore, the role of other targets in the pathogenesis of PKI-induced PAH cannot be ruled out.

Conclusions

To the best of our knowledge, this is the first pharmacovigilance analysis to investigate the risk of PAH associated with PKIs. The disproportionality analysis showed that dasatinib, as well as bosutinib, ponatinib, ruxolitinib and nilotinib, had a significant disproportionality signal. This study highlights potential the roles of Src protein kinases family and TEC in PAH induced by PKIs. Overall, this study contributes to a better understanding of PAH induced by PKIs and to identifying potential targets of interest that need to be further explored.

Conflict of interest: L. Cornet has nothing to disclose. C. Khouri has nothing to disclose. M. Roustit reports grants from United Therapeutics outside the submitted work. C. Guignabert has nothing to disclose. M-C. Chaumais reports nonfinancial support from Bayer and personal fees from Actelion, outside the submitted work. M. Humbert reports personal fees from Actelion, Bayer, GSK, Merck and United Therapeutics, during the conduct of the study. B. Revol has nothing to disclose. F. Despas has nothing to disclose. D. Montani reports grants and personal fees from Actelion and Bayer, and personal fees from BMS, GSK, MSD and Pfizer, outside the submitted work. J-L. Cracowski reports grants from Bioprojet and Topadur, outside the submitted work.

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3. Conclusion et perspectives

Ces travaux nous ont permis d'explorer et de comparer le profil d'effets indésirables de 3 traitements pour lesquels aucune différence en termes d'efficacité n'a été mise en évidence pour le moment. (120) Nous avons tenté de réaliser le même type d'étude sur les traitements agissant sur la voie de la prostacycline (PROSPERO 2018 CRD42018081269). Cependant la transparence et la qualité des essais cliniques sélectionnés étaient trop hétérogène pour pouvoir comparer les résultats ; essentiellement en raison du mélange d'anciens essais cliniques, sur l'époprostenol ou l'iloprost par exemple, à des essais plus récents comme avec le selexipag. Nous avons essayé d'obtenir les données individuelles de safety de ces essais, mais nos demandes ont été refusées par les laboratoires en question.

Nous avons également exploré le mécanisme d'action des HTAP induites par une classe médicamenteuse, les inhibiteurs de tyrosine kinases. Il reste dans ce domaine beaucoup de travaux à effectuer sur les mécanismes des autres étiologies iatrogènes, qui restent encore mal connues. De plus, une analyse de disproportionnalité à partir des données de Vigibase n'a jamais été réalisée. Par ailleurs, l'HTAP étant une pathologie dont la prise en charge est quasi-exclusivement hospitalière la détection et l'évaluation de signaux à partir bases de données médico-administratives paraît envisageable ; bien que la rareté de cette pathologie nécessite l'utilisation de bases très puissantes.

PARTIE 4. ÉTUDES SUR LES TROUBLES TROPHIQUES

1. Hierarchical evaluation of electrical stimulation protocols for chronic wound healing: an effect size meta-analysis.

L'amélioration de la cicatrisation d'ulcères cutanés est un champ de recherche fondamental au sein de notre équipe (<https://grenoblemicrocirculation.org/>). Nous utilisons notamment des méthodes d'iontophorèse afin de délivrer des médicaments directement au niveau de la lésion cutanée. (121–124) Nous utilisons donc le courant électrique pour augmenter la pénétration transcutanée de principes actifs vasodilatateurs. Ceci permet d'augmenter la concentration de médicaments au site de l'ulcère et de limiter les effets indésirables induits par des administrations systémiques de ces traitements. Plusieurs études suggèrent un effet propre du courant électrique sur la cicatrisation cutanée qui pourrait donc participer l'effet thérapeutique de l'iontophorèse. Afin d'étudier l'effet du courant électrique sur la cicatrisation cutanée et de comparer l'efficacité des différentes modalités d'électrostimulation nous avons réalisé cette méta-analyse.



SYSTEMATIC REVIEW

Hierarchical evaluation of electrical stimulation protocols for chronic wound healing: An effect size meta-analysis

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Manuscript received: December 13, 2016
Accepted in final form: September 7, 2017

DOI:10.1111/wrr.12594

ABSTRACT

Electrical stimulation (ES) has been tested for decades to improve chronic wound healing. However, uncertainty remains on the magnitude of the efficacy and on the best applicable protocol. We conducted an effect size meta-analysis to assess the overall efficacy of ES on wound healing, to compare the efficacy of the different modalities of electrical stimulation, and to determine whether efficacy differs depending on the wound etiology, size, and age of the chronic wound. Twenty-nine randomized clinical trials with 1,510 patients and 1,753 ulcers were selected. Overall efficacy of ES on wound healing was a 0.72 SMD (95% CI: 0.48, 1) corresponding to a moderate to large effect size. We found that unidirectional high voltage pulsed current (HVPC) with the active electrode over the wound was the best evidence-based protocol to improve wound healing with a 0.8 SMD (95% CI: 0.38, 1.21), while evaluation of the efficacy of direct current was limited by the small number of studies. ES was more effective on pressure ulcers compared to venous and diabetic ulcers, and efficacy tended to be inversely associated with the wound size and duration. This study confirms the overall efficacy of ES to enhance healing of chronic wounds and highlights the superiority of HVPC over other type of currents, which is more effective on pressure ulcers, and inversely associated with the wound size and duration. This will enable to standardize future ES practices.

Electrical stimulation has been tested for decades to improve chronic wound healing^{1,2} and available evidence now suggests possible efficacy. Recently, there has been renewed interest to determine the amplitude of the effect and the best modality and several systematic reviews³⁻⁶ and meta-analyses were published.⁷⁻⁹ However, while all of them concluded that this method improved chronic wound healing, results were conflicting and uncertainty remains regarding the best electrical stimulation protocol, and whether efficacy was similar across wound characteristics (underlying clinical condition, size, and age of the chronic wound).

Electrical stimulation protocols widely differed between studies: electrodes could be placed over or close to the wound; the electrical current could be either direct current (DC) with unidirectional continuous impulses that last more than 1 second, high or low voltage pulsed current (HVPC or LVPC) with dozen or hundred impulses per second or alternating current (AC) with bidirectional continuous impulses. In addition, frequency, intensity, duration, and amplitude of electrical stimulation vastly differed.

Some authors even used stochastic and random current with active electrodes placed on nervous pathways away from the wound, methods called frequency rhythmic electrical modulation system (FREMS) and transcutaneous electrical nerve stimulation (TENS). Beyond protocols that used conventional skin-contact ES apparatus, some studies also explored the effect of noncontact ES devices based on wireless micro current stimulation technology.¹⁰ However, such ES approach was considered too far away from the other to be included in this meta-analysis.

Another major issue is the heterogeneity in outcomes, which gives clues to the reason why previous meta-analyses failed to provide exhaustive synthesis of available information.^{8,9} Indeed, outcomes such as the number of completely healed ulcer, variation in wound size area, healing scores, or daily or weekly healing rates cannot be pooled directly, therefore, lowering the power of each single meta-analysis. To get around this data heterogeneity, we performed for the first time an effect size meta-analysis (standardized mean difference SMD) that allowed us to assess the overall efficacy of ES on chronic wound

healing and further compared the efficacy of the different modalities of electrical stimulation but also the respective effects of the wound etiology, size, and age of the chronic wound.

METHODS

We followed the preferred reporting items for systematic reviews and meta-analyses (PRISMA) for the reporting of our study.¹¹

Search strategy

Medline, Embase, clinicaltrial.gov, the Cochrane Library were searched from 1966 to January 2016 with following terms: “electric*” AND “wound OR ulcer.” We also searched on google scholar website (scholar.google.fr). The reference list of pertinent review articles and eligible studies were also retrieved.

Eligibility criteria

We included randomized controlled trials, with more than 10 ulcers in each group, comparing electrical stimulation to standard wound care and/or sham stimulation. Reasons for study exclusion were nonparallel design, use of electromagnetic fields, acute wounds, pediatric population, and nonhuman studies. Two reviewers screened the titles and abstracts independently (CK and SK). A paired consensus process was used to select relevant citations. Disagreements between reviewers were discussed until consensus was achieved. Then, full-text articles were reviewed and assessed for eligibility. Paired consensus was repeated to confirm article eligibility. Any disagreement between the two reviewers was resolved through discussion.

Data extraction

A data collection form was prepared to extract all relevant information from the included studies. One author reviewed each article and extracted data (CK) then a second author checked accuracy of all extracted data (SK).

The following data were recorded for each study:

- General study characteristics: author name, year of publication, total number of patients randomized, number of treatment groups, length of follow-up.
- Patient characteristics: age, type of ulcer, initial ulcer area.
- Intervention: type of electrical stimulation, treatment duration and cumulative treatment duration, electrical stimulation protocol, type of comparator (control type), electrode polarity.
- Outcomes: all outcomes related to wound healing efficacy and safety.

Risk of bias assessment

Independent assessment of risk of bias was made by the same reviewers according to the Cochrane Handbook for Systemic Reviews of Interventions¹² and the guidelines of the Cochrane Consumers and Communication Review Group.¹³ The risk of bias was rated as low, unclear, or

high for the following items: random sequence generation; allocation sequence concealment; blinding (participants, personnel); blinding of outcome assessment; completeness of outcome data, selective outcome reporting; and other sources of bias.

The overall risk of bias for each trial was defined as high-risk if more than three high-risk criteria were met, moderate-risk if two to three high-risk criteria were met, and low-risk if one or zero high-risk criterion were met. Moreover, studies were deemed to be at the highest risk of bias if they are scored as at high or unclear risk of bias for either the sequence generation or allocation concealment domains.

Then, the same two reviewers appraised the quality and content of included studies using the Grading of Recommendation Assessment, Development, and Evaluation (GRADE) recommendations for meta-analysis.¹⁴ Randomized controlled trials begin as high quality evidence, but can be rated down because of risk of bias,¹⁵ imprecision,¹⁶ inconsistency,¹⁶ indirectness,¹⁷ and publication bias.¹⁸ Finally, we rated their quality as very low, low, moderate, or high.

Statistical analysis

To use all available data reported in included studies we calculated the SMD effect size and standard error for the primary outcome (continuous variable) of each study using the formula

$$\text{SMD} = \frac{(\text{Mc} - \text{Me})}{\text{SD}}$$

where Mc is the mean of the outcome measure in the control group, Me is the mean of the outcome measure in the experimental groups, and SD is the pooled standard deviation of the two groups.¹⁹ For categorical variables, we first calculated an odds ratio and its associated confidence interval and then followed Chinn’s method to convert these into SMD and standard deviations.²⁰ In some cases standard deviations (SD) were not reported. We first tried to contact authors, and in case of no response we used a conservative approach consisting in replacing the missing SD by the largest outcome-specific SD from the other included studies. When reports contained data from assessments taken at different time points, we prioritized the last one. An effect size of 0.8 was considered large, 0.5 moderate, and 0.2 small.²¹ We used R statistical software (version 3.2.4) to lead statistical analysis. The compute.es (Compute Effect Sizes. R package version 0.2-2. URL <http://cran.r-project.org/web/packages/compute.es>) and MAd (Meta-Analysis with Mean Differences. R package version 0.8-2. URL <http://cran.r-project.org/web/packages/MAd>) package were used to calculate SMD effect sizes. Then, the metafor package (Metafor. R package version 2.0-0. URL: <http://www.jstatsoft.org/v36/i03/>) was used to perform the meta-analysis and lead sensitivity, subgroup analysis and meta-regressions. Heterogeneity was assessed using Cochrane’s *Q* and *I*² statistics. DerSimonian–Laird random effects models were used in cases of statistical heterogeneity (*I*² ≥ 50% or a significant test for heterogeneity).

We explored sources of heterogeneity with six prespecified subgroups:

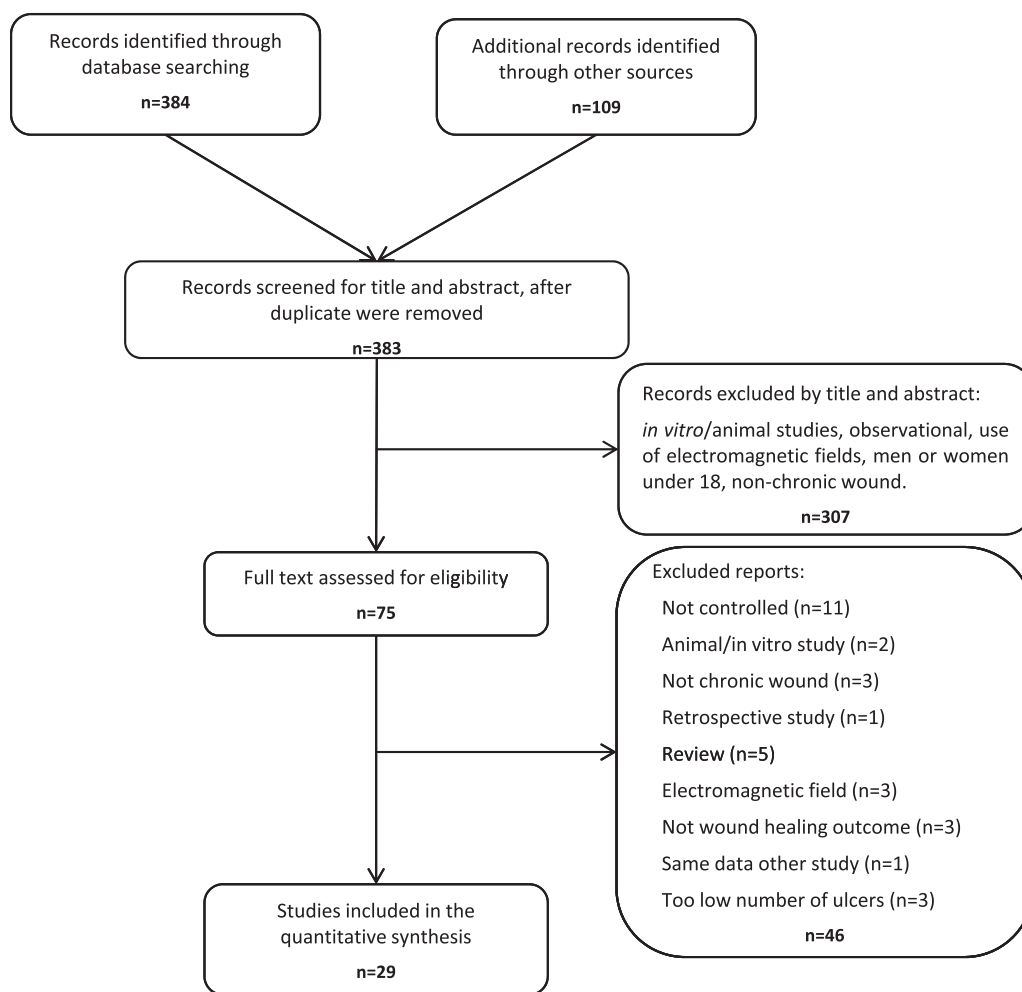


Figure 1. Flowchart.

- Electrode placement: active electrode over the wound or nearby the wound
- Type of electrical stimulation: unidirectional, bidirectional, or nervous stimulation.
- Type of electrical waveform (DC vs. HVPC vs. LVPC vs. AC)
- Electrode polarity (switch vs. nonswitch)
- Ulcer etiology (diabetic vs. venous vs. pressure)
- Type of outcome (NHU vs. WSA vs. score vs. WHR)

Meta-regressions were performed to take into account covariates of interest, that is, age, initial wound size and ulcer duration, cumulative duration treatment. For meaningful purpose, when meta-regressions were significant we categorized the variable and we added it into the subgroup analysis.

We carried out a sensitivity analysis by analyzing only low risk of bias studies. We considered the results as robust if the change was inferior to 20%.

Funnel plot asymmetry was explored using Egger’s regression test as recommended by Cochrane handbook for systemic reviews of interventions, with $p < 0.05$ suggesting

publication bias.¹² Trim and Fill analysis was used to assess number of missing studies.²²

RESULTS

Characteristics of studies and patients

Of 493 references identified, after removal of duplicates and titles and abstract screening, 75 reports proved potential eligibility. On full text screening 29 randomized controlled trials^{23–46} were included in the quantitative synthesis. (Figure 1)

All studies were randomized controlled trials (RCT) assessing the efficacy of electrical stimulation on wound healing. The meta-analysis included 1,510 patients and a total of 1,753 ulcers including mainly pressure (41%), diabetic (28%), or venous (24%) ulcers. Fifty-eight percent ($n = 1,012$) of these ulcers were treated with electrical stimulation, while in control groups 362 received sham stimulation (49%) and 379 received standard wound care (51%). The mean initial ulcer area was $11.9 \pm 8.5 \text{ cm}^2$ and

the mean ulcer duration before treatment was 10.3 ± 10.15 months. Detailed data are presented on Supporting Information Table S1.

Four studies assessing different electrical stimulation protocols were divided in the meta-analysis,^{24–26,31} leading to a total of 36 different studies included in the analysis. Active electrodes, that is, electrodes that stimulate the healing process, were placed over the wound with the passive electrode placed on intact distal skin in 17 studies, while both electrodes were placed nearby the wound or on distal nerve points in 18 studies. One study used both methods according to the area of the ulcer.³⁵ The type of current was direct ($n = 4$), low voltage pulsed ($n = 4$), high voltage pulsed ($n = 16$), or alternative current ($n = 11$). This data was missing for 1 study.⁴⁵ We also observed an important variability in ES stimulus parameters including waveform, intensity, polarity, treatment duration, and frequency. In particular, we reported a considerable diversity of total cumulated duration of ES protocols with a range from 6 to 728 hours. All available electrical stimulation parameters are summarized on Supporting Information Table S2.

Quality of included studies

The risk of bias among the included studies was highly heterogeneous. Among the 29 included studies, 14 (48%) supplied sufficient information to assess allocation concealment and 13 (45%) reported an adequate randomization process, 12 (41%) studies were double blinded and one was single blinded. Five presented incomplete outcome data (17%) and two presented selective reporting (7%). Notably, in one study the sham stimulation was shorter than the treatment duration (3 weeks vs. 4 weeks). Overall, 15 (52%) studies were considered of high risk of bias, 6 (21%) of moderate risk of bias and 8 (36%) of low risk of bias. Results are summarized on Supporting Information Figure S1.

We also performed a GRADE evaluation of all subgroups quality of evidence. Results are detailed on Supporting Information Table S3 and added with subgroup analysis.

Funnel plot asymmetry was detected by Egger's regression test ($p < 0.0001$) and Trim and Fill analysis ($p < 0.0001$). However, no missing studies were detected on the left side of the funnel plot. (Supporting Information Figure S2)

Primary outcome

Overall efficacy of electrostimulation on wound healing was $SMD = 0.72$ (95% CI: 0.48, 1.0, $I^2 = 78\%$; low quality of evidence) corresponding to a moderate to large effect size.¹⁹ Results are summarized on Figure 2.

Subgroup analysis

When studies were analyzed depending on electrode placement, effect size of electrode placed over or nearby the wound were $SMD = 0.84$ (95% CI: 0.48, 1.19, $I^2 = 78\%$; low quality of evidence) and $SMD = 0.63$ (95% CI: 0.31, 0.94, $I^2 = 79\%$; very low quality of evidence), respectively (Supporting Information Figures S3 and S4). In the "active electrode over the wound" group, we identified three

different unidirectional current profiles: DC, HVPC, and LVPC; and we showed that both pulsed protocols induced a significant improvement of wound healing with $SMD = 0.80$ (95% CI: 0.38, 1.21, $I^2 = 79\%$; moderate quality of evidence) and $SMD = 0.58$ (95% CI: 0.03, 1.13; moderate quality of evidence) for HVPC and LVPC, respectively. DC presented none significant effect with $SMD = 1.42$ (95% CI: -0.42 , 3.26, $I^2 = 92\%$; very low quality of evidence). In the "active electrode nearby the wound" group, similar results were observed with significant effect size only for HVPC $SMD = 0.60$ (95% CI: 0.16, 1.03, $I^2 = 0\%$; low quality of evidence). Nervous stimulation produced also a significant effect on wound healing with $SMD = 1.30$ (95% CI: 0.43, 2.18, $I^2 = 82\%$; low quality of evidence). Results are summarized on Figure 3.

When studies were analyzed depending on ulcer etiology, effect size was larger on pressure ulcer ($SMD = 1.00$ (95% CI: 0.54, 1.46, $I^2 = 84\%$), low quality of evidence) compared to venous ($SMD = 0.29$ (95% CI: 0.04, 0.55, $I^2 = 0\%$), high quality of evidence) and diabetic ulcers ($SMD = 0.67$ (95% CI: 0.21, 1.12, $I^2 = 73\%$), very low quality of evidence; interaction test, $p = 0.28$).

Results of subgroup analysis are summarized on Table 1.

Metaregressions

A trend of a faster healing rate with younger and smaller ulcers was observed on the overall meta-analysis ($p = 0.269$ and $p = 0.2$, respectively). However, metaregressions on duration of the wound and initial size area became significant in the more homogenous HVPC over the wound subgroup ($p = 0.047$ and $p = 0.018$, respectively; Supporting Information Figures S5 and S6).

Sensitivity analysis

When considering only low risk of bias studies overall effect was $SMD = 0.90$ (95% CI: 0.44, 1.37), $I^2 = 67\%$, $k = 7$ corresponding to a large effect size. Results are presented on Supporting Information Figure S7.

Safety

The safety of electrical stimulation protocol was evaluated in eight studies,^{23,28,32,34–36,42,47} among them five studies reported side effects. Adunsky et al.²³ reported two tingling sensation and two local irritations (DC). Feedar et al.²⁸ reported a tingling sensation on 20% of patients (LVPC). Houghton et al.³⁵ reported one skin irritation and one burn (HVPC). Jankovic et al.³⁶ described one burning sensation (FREMS). Lastly, Mulder et al.⁴² reported one skin irritation and seven tingling sensation (HVPC).

DISCUSSION

This study is the first effect-size meta-analysis pooling all available RCT data on electrical stimulation for wound healing. First of all, our results confirm the overall efficacy of electrical stimulation to enhance healing of chronic wounds. Second, when studies were analyzed according to the electrical current characteristics, we found that unidirectional HVPC with the active electrode over the wound was the best protocol to improve wound healing. Finally, electrical stimulation was more effective on pressure ulcers

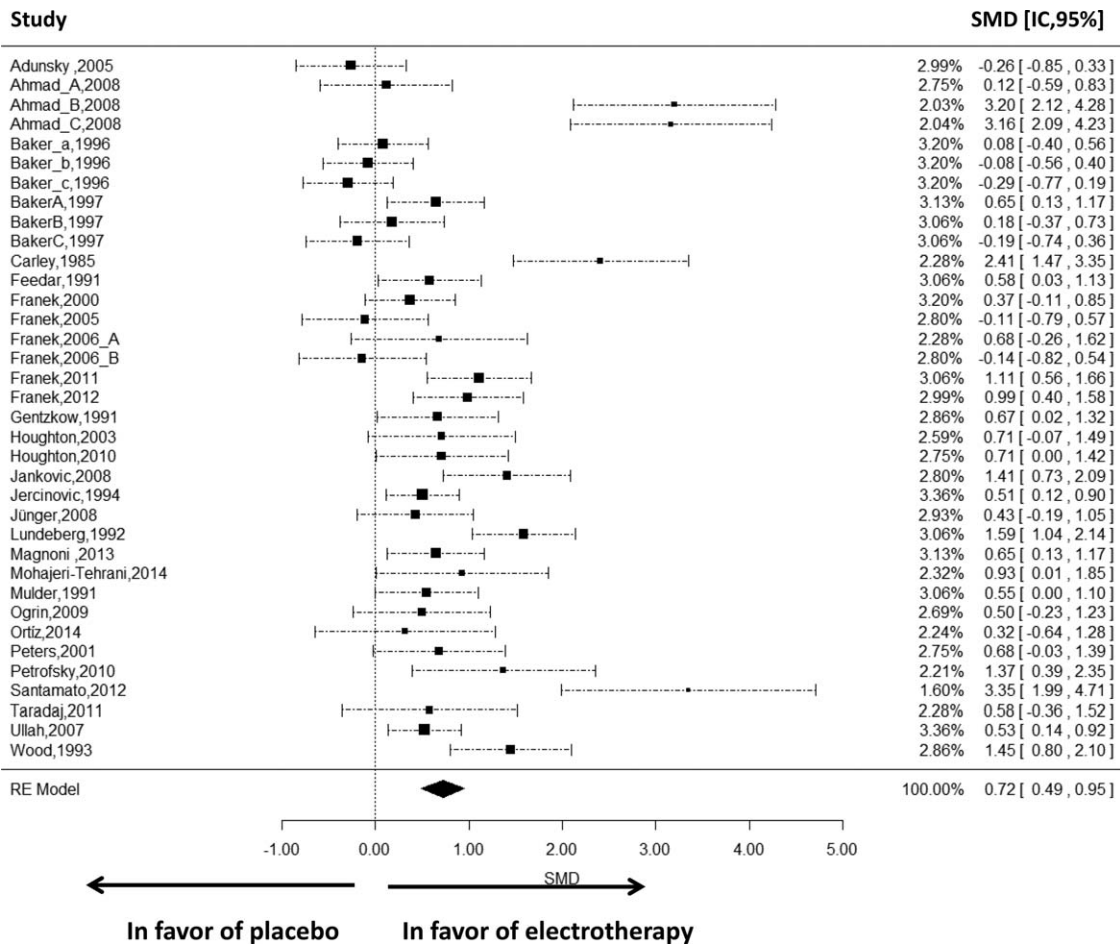


Figure 2. Forest plot, effect size estimates from SMD.

compared to venous and diabetic ulcers, and the efficacy was inversely associated with the size and duration of the wound.

The trends for superiority of unidirectional over bidirectional current is in accordance with a previous meta-analysis led by Koel and Houghton in 2014.⁷ This is meaningful when considering that application of external electrical field on unhealed wound will trigger and mimic the unidirectional current of injury. The current of injury is a physiological process appearing when a breach is created on the skin and in turn triggers a cascade of events, for example, directs endothelial cell, increases collagen production, and fibroblast proliferation.⁴⁸ It has also been shown that decreasing this current of injury by pharmacological agents significantly impaired healing.⁴⁹ Koel and Houghton in 2014.⁷ did not distinguish the placement of electrodes and the type of current. HVPC was superior on other currents whenever the electrodes were placed over or around the wound. This constant efficacy could be explained by the greater depth of penetration of this current.⁵⁰ Moreover, Guo and coworkers, studying cell migration when applying an electrical field, a phenomenon

called galvanotaxis, found that human dermal fibroblast migrated toward the anode with a rate proportional to the voltage applied.⁵¹

We found no significant efficacy for DC current SMD = 1.42 (95% CI: -0.42, 3.26, $I^2 = 92%$) however this conclusion relies on a limited number of studies ($n = 2$). Indeed, most of available studies are old and not controlled, and were, therefore, not included in our meta-analysis. In a previous meta-analysis,⁸ DC was suggested to significantly improve wound healing, however, this sensitive results was based on only three studies, one of which was excluded of our meta-analysis because of poor methodological quality.

Surprisingly, reversing the polarity of the active electrode does not seem to alter healing and is even associated with a better outcome SMD = 0.87 (95% CI: 0.47, 1.26, $I^2 = 80%$) vs. SMD = 0.67 (95% CI: 0.26, 1.07, $I^2 = 0%$). This is consistent with previous studies performed in animal models^{52,53} and might be explained by the specific benefits associated to each polarity: anodal currents possess a greater antibacterial effect,⁵⁴ attract fibroblast,⁵¹ and macrophages⁵⁵; whereas cathodal currents attract

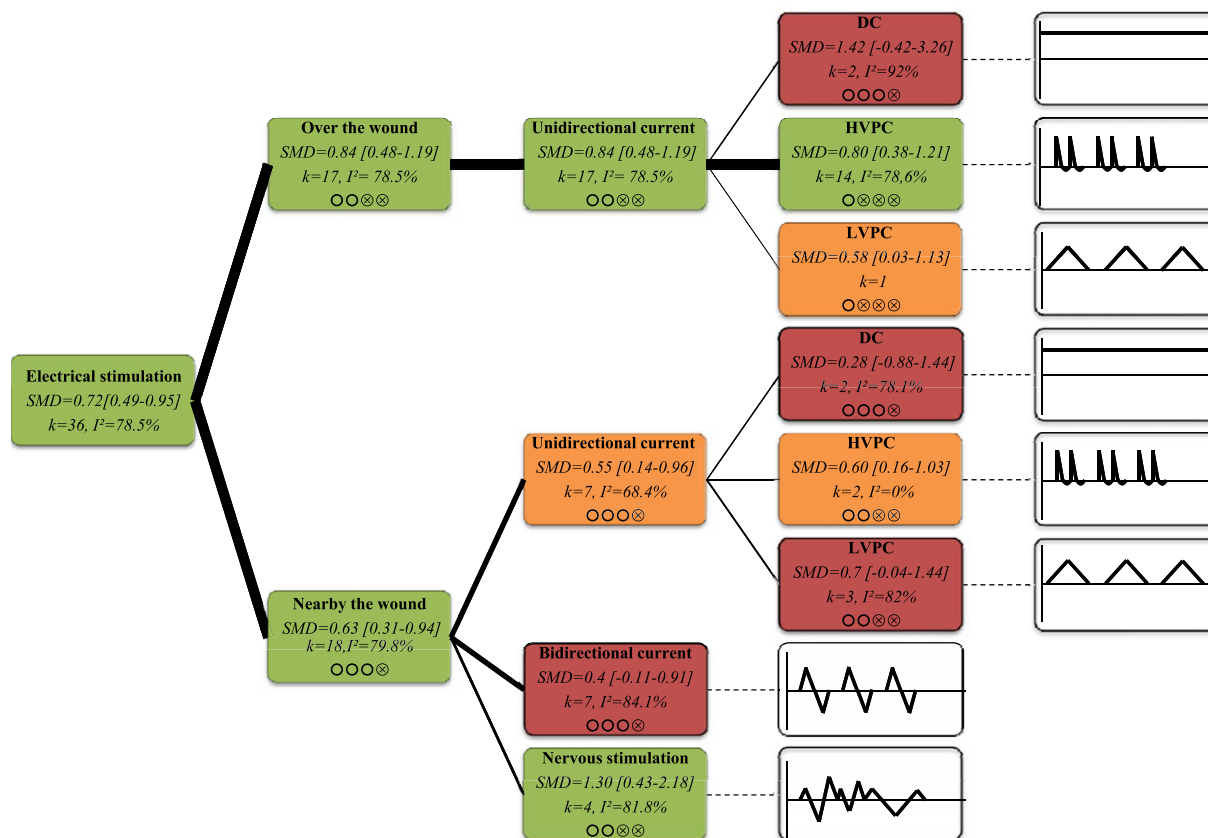


Figure 3. Hierarchical subgroup analysis and GRADE quality of evidence. Thickness of the lines is proportional to the number of trials that included the direct comparisons and color varies according to the significance of the inferior limit of the confidence interval (inferior to 0 red; inferior or equal to 0.2 orange; superior to 0.2 green). GRADE quality of evidence: ○○○○ MODERATE; ○○○○ LOW; ○○○○ VERY LOW. [Color figure can be viewed at wileyonlinelibrary.com]

keratinocytes,⁴⁸ epidermal cells,⁴⁹ neutrophils and lymphocytes,⁵⁶ and increase fibroblast proliferation.⁵⁷ Therefore, we may assume that periodically reversing electrode polarity would combine the advantages of both polarities. Of note an interesting RCT is ongoing comparing anodal and cathodal HVPC on peri wound blood flow and wound healing (ACTRN12615001281583).

Beyond specificities of each modality of exogenous electrostimulation, the observed benefit may be explained by a global current-induced effect that implies: (1) angiogenesis promotion^{58,59} and (2) blood flow and tissue oxygenation increases.⁶⁰ We could hypothesize that benefits of nervous stimulation (SMD = 1.30 (95% CI: 0.43–2.18), I²=82%) observed in our study are only mediated by these last mechanisms.⁶¹ Indeed, neurogenic vasodilation induced by TENS is a well-known phenomenon that was observed for decades and that is mechanistically consistent with a sympathetic blockade of C-fibers induced by a stimulation of A beta fibers.^{62,63}

We found that electrical stimulation is more efficient on pressure (SMD = 1.00 (95% CI: 0.54, 1.46)) than on diabetic ulcers (SMD = 0.67 (95% CI: 0.21, 1.12)) and on venous (SMD = 0.29 (95% CI: 0.04, 0.55)). This might be related to the involvement of neurovascular interaction in

the regulation of local blood flow that is more pronounced on small arteries and arterioles than veins.⁶⁴

We can hypothesize that in younger and smaller wounds, current of injury could be more easily triggered and these wounds better respond to electrical stimulation.

One of the main issues to synthesize knowledge on studies aimed at assessing efficacy of an intervention on wound healing is the use of a wide variety of outcomes. Indeed, studies included in this meta-analysis used four main types of primary endpoint: number of healed ulcer, variation in wound size area (area or reduction percentage), healing scores, or healing rate (daily or weekly). This heterogeneity led other authors to perform a meta-analysis for each endpoint and, therefore, interpretation of paradoxical results was extremely difficult.^{8,9} Using a common effect size to pool and aggregate studies gives the opportunity to get around this problem and allowed us to perform powerful subgroup analysis. When subgrouping studies by type of primary outcome we showed that WSA was the most sensitive endpoint and should preferably be used in studies assessing wound healing. Contrariwise, this study highlighted the difficulty to improve a solid criterion such as the number of healed ulcer. We also found that a standardized sham stimulation protocol overestimates the

Table 1. Results of subgroup analysis, interaction tests, and GRADE quality assessment

Subgroup	Number of studies	SMD	95% CI	Heterogeneity [†]	<i>p</i> [‡]	GRADE quality of evidence
Electrode placement						
Electrode over the wound	17	0.84	0.48 to 1.19	78.5%***	0.70	○○⊗⊗
Unidirectional current	17	0.84	0.48 to 1.19	78.5%***		○○⊗⊗
DC	2	1.42	-0.42 to 3.26	92%***	0.64	○○○⊗
HVPC	14	0.80	0.38 to 1.21	78.6%***		○⊗⊗⊗
LVPC	1	0.58	0.03 to 1.13	NA		○⊗⊗⊗
Electrode nearby the wound	18	0.63	0.31 to 0.94	78.9%***		○○○⊗
Bidirectional current	7	0.4	-0.11 to 0.91	84.1%***	0.14	○○○⊗
Nervous stimulation	4	1.30	0.43 to 2.18	81.2%***		○○⊗⊗
Unidirectional current	7	0.55	0.14 to 0.96	68.4%***		○○○⊗
DC	2	0.28	-0.88 to 1.44	78.1%*	0.73	○○○⊗
HVPC	2	0.6	0.16 to 1.03	0%		○○⊗⊗
LVPC	3	0.7	-0.04 to 1.44	82%***		○○⊗⊗
Electrode polarity						
Electrode switch	16	0.87	0.47 to 1.26	79.7%***	0.59	○⊗⊗⊗
Cathode only	3	0.67	0.26 to 1.07	0%		○⊗⊗⊗
Control type						
Sham stimulation	22	0.60	0.31 to 0.90	80.8%***	0.17	○○○⊗
SWC	14	0.92	0.57 to 1.26	70.8%***		○○⊗⊗
Ulcer etiology						
Pressure ulcer	11	1.00	0.54 to 1.46	84.1%***	0.28	○○⊗⊗
Venous ulcer	7	0.29	0.04 to 0.55	0%		⊗⊗⊗⊗
Diabetic ulcer	8	0.67	0.21 to 1,12	72.5%***		○○○⊗
Outcome						
NHU	8	0.38	-0.07 to 0.83	65.6%**	<0.01	○⊗⊗⊗
WSA	17	1.21	0.82 to 1.60	80.1%***		⊗⊗⊗⊗
Score	2	0.87	0.42 to 1.32	29.1%		○○○⊗
WHR	9	0.21	-0.02 to 0.45	49.9%*		○○○⊗

[†]Variance between studies as a proportion of the total variance; heterogeneity tested using the *I*² statistic. The *p*-values indicated in this column refer to whether the *Q* statistic is significant (the *I*² statistic does not include a test of significance).

**p*-Value of heterogeneity test inferior to 0.05.

***p*-Value of heterogeneity test inferior to 0.01.

****p*-Value of heterogeneity test inferior to 0.001.

[‡]*p*-Value of interaction with treatment group.

effect approximately from 30% compared with SWC. This suggests that the placebo component of the electrostimulation protocol is not negligible.

One of the main limitations of our study is that we were not able to explain the important heterogeneity in the meta-analysis despite the subgroup analysis. However, a wide variety of stimulus parameter should yet to be further studied (e.g., voltage, frequency, intensity application time, and etc.) and should contribute to the residual heterogeneity.

This study confirms the overall efficacy of electrical stimulation to enhance healing of chronic wounds and highlights the superiority of HVPC over other type of currents. This is an important result in the way of a future standardization of electrical stimulation practices. In addition, the effect of electrical stimulation is not similar

across all types of chronic wounds, being more effective on pressure ulcers, and inversely associated with the wound size and duration. Further studies are needed to find the best stimulus parameters of HVPC. However, given the non-negligible placebo component in ES we advocate for the use of a common primary endpoint (e.g., decrease of wound size area), sham stimulation and high-quality design trials.

ACKNOWLEDGMENTS

We thank Michel Cucherat for his advice on the methodology of this study.

Source of Funding: None.

Conflict of Interest: The authors state no conflict of interest.

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Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site.

2. SGLT-2 inhibitors and the risk of lower-limb amputation: Is this a class effect?

Les inhibiteurs du sodium-glucose co-transporter-2 sont une classe pharmacologique récente ayant démontré une efficacité sur la mortalité cardiovasculaire chez les patients atteints de diabète de type 2 (125). Cependant, un sur-risque d'amputation des membres inférieurs a été mis en évidence lors d'un large essai clinique portant sur la canagliflozine. (126) La physiopathologie de ces amputations est pour le moment inconnue mais probablement en lien avec l'induction de troubles trophiques cutanés. Par conséquent, la FDA a émis un « boxed warning » sur la canagliflozine alors que l'EMA a ajouté cet effet indésirable à tous les inhibiteurs du sodium-glucose co-transporter-2. Dans cette étude nous avons utilisé les données de notification spontanée de la base de pharmacovigilance de l'OMS afin de voir si ces notifications concernaient exclusivement la canagliflozine ou si les autres inhibiteurs de SGLT-2 étaient également concernés.

BRIEF REPORT

SGLT-2 inhibitors and the risk of lower-limb amputation: Is this a class effect?

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Inhibitors of the sodium-glucose co-transporter-2 (SGLT-2) are a novel class of glucose-lowering agents that show promising results. However, the use of canagliflozin has been associated with an increased risk of lower-limb amputation. Whether this risk concerns other SGLT-2 inhibitors is unclear, and our objective was to address this issue. We performed a disproportionality analysis using the WHO global database of individual case safety reports (VigiBase). Among the 8 293 886 reports available between January 2013 and December 2017, we identified 79 reports of lower-limb amputation that were associated with SGLT-2 inhibitors. Among all blood glucose lowering drugs, the proportional reporting ratio (PRR) was increased only for SGLT-2 inhibitors (5.55 [4.23, 7.29]). While we observed an expected signal for canagliflozin (7.09 [5.25, 9.57]), the PRR was also high for empagliflozin (4.96 [2.89, 8.50]) and, for toe amputations only, for dapagliflozin (2.62 [1.33, 5.14]). In conclusion, our results reveal a positive disproportionality signal for canagliflozin, and also for empagliflozin, and, for toe amputations only, for dapagliflozin. However, our analysis relies on a limited number of cases and is exposed to the biases inherent to pharmacovigilance studies. Further prospective data are therefore needed to better characterize the risk of amputations with different SGLT-2 inhibitors.

KEYWORDS

amputation, SGLT-2 inhibitor, type 2 diabetes

1 | INTRODUCTION

Inhibitors of the sodium-glucose co-transporter-2 (SGLT-2) are a novel class of glucose-lowering agents that show promising results concerning the reduction of cardiovascular risk in patients with type 2 diabetes.^{1,2} However, the recent CANVAS trial showed an increased risk of amputation in patients treated with canagliflozin.² As a result, the US Food and Drug Administration (FDA) now requires a “boxed warning” to be added to the canagliflozin drug label, while the European Medicines Agency (EMA) includes a warning in the prescribing information for all SGLT-2 inhibitors. Yet, retrospective analysis of adverse events from the EMPA-REG outcome trial, which was not designed to collect exhaustive information on amputations, did not reveal any increased risk of lower-limb amputation with empagliflozin.³ Recently, signal analysis from the FDA Adverse Event Reporting System (FAERS) and a large retrospective study have reached conflicting conclusions regarding the risk of amputation with

canagliflozin.^{4,5} Furthermore, a recent population-based cohort study showed that, for all SGLT-2 inhibitors, treatment initiation was associated with a higher risk of below-knee amputation.⁶ Because the majority of amputations in that study were observed with use of canagliflozin, whether the 2 other SGLT-2 inhibitors, empagliflozin and dapagliflozin, also increase the risk of amputation remains unclear. The aim of this work was to further address this question using an international pharmacovigilance database.

2 | METHODS

We conducted a disproportionality analysis using the WHO global database of individual case safety reports (ICSRs) (VigiBase), which includes spontaneously reported cases of adverse drug reactions (ADRs). Currently, 127 full member countries are involved in the WHO Programme for International Drug Monitoring and submit

ICSRs of suspected adverse drug events associated with medicinal products.

Data extraction was carried out using Vigilyze (Uppsala Monitoring Centre), for records between January 1, 2013 (labeling date of the first marketed SGLT-2 inhibitor, canagliflozin) and December 31, 2017. We extracted all ICSRs associated with the following preferred terms: "foot amputation," "leg amputation," "metatarsal excision" and "toe amputation" (grouped into "lower-limb amputation"). Drugs of interest were canagliflozin, dapagliflozin and empagliflozin, as suspect or concomitant drug.

Disproportionality analysis was performed using the proportional reporting ratio (PRR), which is the ratio of the rate of reporting of 1 effect among all reports for a given drug over the rate of reporting of the same effect among all reports for all drugs in the database.⁷ This allows detection of pharmacovigilance signals, that is, associations between the reporting of an ADR and a drug. To take into account the baseline risk of amputation related to diabetes, we performed the disproportionality analysis among blood glucose-lowering drugs (ATC class A10B), which include biguanides, sulfonylureas, thiazolidinediones, dipeptidyl peptidase-4 (DPP-4) inhibitors, glucagon-like peptide-1 (GLP-1) analogues and SGLT-2 inhibitors, but excludes insulin. If a report was associated with several glucose-lowering drugs it was counted in each drug class.

The cut-off for signal detection was defined as a PRR lower boundary 95% confidence interval greater than or equal to 1 and a number of cases (n) greater than or equal to 3, according to the definition of the European Medicines Agency.⁸ Because insulin use, which is

a surrogate for the severity of diabetes, is associated with increased risk of amputation, we performed a sensitivity analysis of ICSRs of lower-limb amputation that was not associated with concomitant insulin therapy. Other sensitivity analyses were carried out according to the level of amputation: toe amputations; major amputations ("leg amputation"); or all amputations (the preferred terms "amputation" and "limb amputation" were added to the main extraction terms). Time-trend analysis was conducted to detect a potential media bias.

3 | RESULTS

Among the 8 293 886 ICSRs reported in Vigibase since 2013, 369 543 were related to a blood glucose-lowering drug, and 31 495 to SGLT-2 inhibitors. We identified 79 reports of lower-limb amputation associated with SGLT-2 inhibitors (Table S1, Supporting Information). Among all glucose-lowering drug classes, the PRR for lower-limb amputations was above signal detection cut-off only for SGLT-2 inhibitors (PRR = 5.55 [4.23, 7.29]). Exclusion of ICSRs with concomitant insulin therapy yielded similar results (PRR = 6.02 [4.37, 8.30]) (Table 1).

Additional sensitivity analyses showed PRR above the signal detection threshold for toe amputations (PRR = 7.84 [5.65, 10.88]), major amputations (PRR = 2.86 [1.75, 4.69]) and all amputations (PRR = 5.95 [4.61, 7.67]) (Table 1).

Interestingly, while we observed an expected signal with canagliflozin for lower-limb amputations and in all sensitivity analyses, there was also a signal with empagliflozin, whatever the level of amputation

TABLE 1 Disproportionality analysis

	Reports of amputations (n) PRR (95% CI)					Total reports in Vigibase since January 1, 2013
	Lower limb	Toe	All ^a	Major	Lower limb, excluding insulin	
All glucose-lowering drugs (excluding insulin)	232	147	258	74	165	369 543
DPP-4 inhibitors	41 1.24 (0.88, 1.73)	28 1.36 (0.90, 2.05)	43 1.15 (0.83, 1.60)	12 1.12 (0.60, 2.07)	31 1.35 (0.91, 1.99)	54 615
GLP-1 analogues	15 0.37 (0.22, 0.62)	8 0.31 (0.15, 0.63)	16 0.35 (0.21, 0.59)	5 0.39 (0.16, 0.96)	8 0.7 (0.43, 1.15)	58 206
Thiazolidinediones	19 0.38 (0.24, 0.60)	7 0.21 (0.10, 0.45)	24 0.43 (0.29, 0.66)	12 0.82 (0.44, 1.52)	18 0.36 (0.21, 0.62)	70 664
Alpha glucosidase inhibitors	0	0	0	0	0	8142
Sulfonylureas	45 1.03 (0.75, 1.43)	26 0.92 (0.60, 1.41)	48 0.98 (0.72, 1.34)	14 1.00 (0.56, 1.79)	31 1.00 (0.68, 1.48)	69 827
Biguanides	98 1.12 (0.86, 1.46)	64 1.18 (0.85, 1.64)	104 1.04 (0.81, 1.33)	29 0.99 (0.62, 1.58)	62 0.98 (0.72, 1.35)	145 825
SGLT-2 inhibitors	79 5.55 (4.23, 7.29)	62 7.83 (5.64, 10.86)	92 5.95 (4.61, 7.67)	15 2.73 (1.55, 4.81)	57 6.02 (4.37, 8.30)	31 495
Canagliflozin	56 7.09 (5.25, 9.57)	42 8.91 (6.23, 12.74)	67 7.82 (5.92, 10.32)	11 3.89 (2.05, 7.38)	45 8.38 (5.95, 11.80)	15 873
Dapagliflozin	10 1.81 (0.96, 3.41)	9 2.62 (1.33, 5.14)	11 1.79 (0.98, 3.27)	0 NA	8 2.03 (1.00, 4.13)	8980
Empagliflozin	14 4.96 (2.89, 8.50)	12 6.86 (3.80, 12.37)	14 4.43 (2.59, 7.58)	2 NA	9 3.99 (2.04, 7.80)	4728

PRR is the ratio of the rate of reporting of 1 effect among all reports for a given drug over the rate of reporting of the same effect among all reports for all drugs in the database.⁷ If a report was associated with several glucose-lowering drugs it was counted in each drug class. Abbreviations: NA: not applicable, due to an insufficient number of ICSRs in the database. Results in bold refer to positive disproportionality signals.

^a The unspecified preferred terms "amputation" and "limb amputation" were added to the extraction.

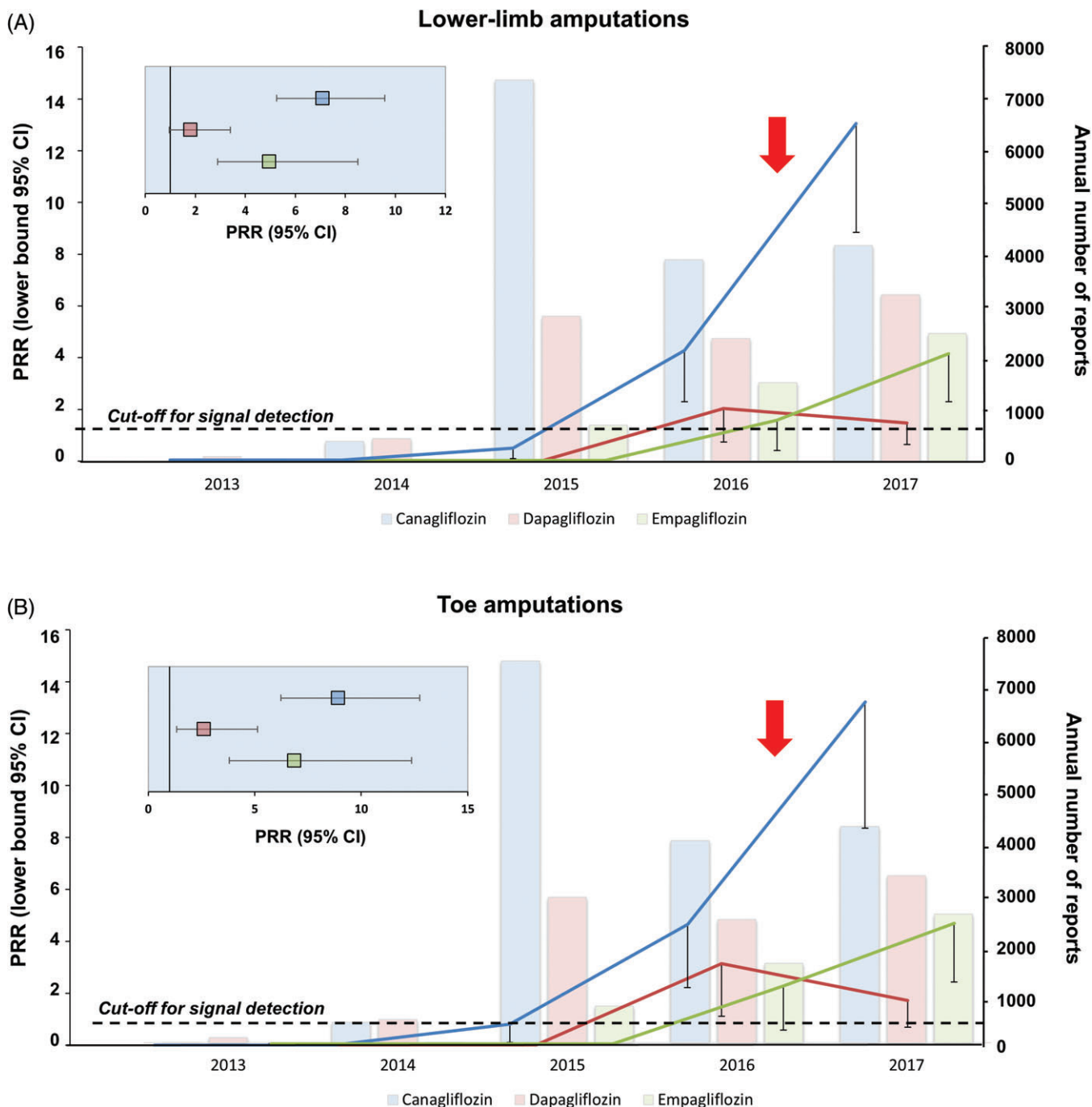


FIGURE 1 Annual number of reports and annual PRR (with lower boundary 95% confidence intervals) of lower-limb amputations (A) and toe amputations (B) for the different SGLT-2 inhibitors. In the blue boxes forest plots show the PRR (with 95% confidence intervals) for the entire study period. The red arrows symbolize the first FDA safety communication issued in May 2016

(Figure 1 and Table 1). We also observed a signal with dapagliflozin, but only for toe amputations. It is worth noting, however, that the PRR for major amputations could not be calculated for empagliflozin and dapagliflozin, because of an insufficient number of ICSRs in the database. After excluding ICSRs with concomitant insulin use, a signal was observed for lower-limb amputations with all SGLT-2 inhibitors (Table 1).

Despite the limited number of cases, there was no significant difference in age and insulin treatment between reports of SGLT-2 inhibitors and other glucose-lowering drugs. In contrast, there were more men among reports of SGLT-2 inhibitors (Table S2). Time-trend analysis shows a dramatic increase in PRR in 2017 for canagliflozin, reflecting a

possible media bias, while PRR for empagliflozin, dapagliflozin and other glucose-lowering drugs remained stable (Figure 1 and Figure S1).

4 | DISCUSSION

These data suggest that the risk of lower-limb amputation may not only concern canagliflozin, but also empagliflozin, and possibly dapagliflozin. Our findings differ from those reported in a previous disproportionality analysis from the FAERS database,⁴ but that analysis included no report for dapagliflozin and only 2 for empagliflozin. Nevertheless,

the limited number of reports found in our study remains a limitation. Another limitation of using spontaneous reporting is the lack of information for appropriate adjustment for potential confounders. Indeed, because of a high rate of missing data, we were not able to take into account comorbidities, previous history of amputation, concomitant medications (with the exception of insulin) or history of cardiovascular disease.

Pharmacovigilance analyses are exposed to bias that affects the disproportionality measure: time since marketing, media safety alerts or selective notification. Indeed, time-trend analysis revealed increased reporting after the first FDA safety communication was issued in 2016, after an interim analysis of the CANVAS trial. Although many ICSRs with canagliflozin were reported in the FAERS before publication of the CANVAS study results,⁴ media bias may have contributed to strengthening the signal in 2017. Yet, the impact of media safety alerts for canagliflozin on the signal for empagliflozin and dapagliflozin, and for other glucose-lowering drug classes, is not obvious as they have remained relatively stable over the past 2 years.

The reported signal for empagliflozin conflicts with a recent retrospective analysis of the EMPA-REG OUTCOME trial, which found no association between empagliflozin and the risk of lower-limb amputation.³ Similarly, pooled analysis from 13 placebo-controlled trials with dapagliflozin vs placebo did not show an increased risk of amputation.⁹ However, the duration of the trial was short (up to 24 weeks) and a limited number of amputations were observed.

The mechanisms underlying the risk of amputation remain largely unknown. However, there is no obvious pharmacological reason that would explain differences between the various SGLT-2 inhibitors. Volume depletion and reduced tissue perfusion, in patients with already impaired arteriolar reactivity, may contribute to tissue necrosis and amputation. A direct effect of SGLT-2 inhibitors on vascular function in small arteries, which is complex and depends on the vascular bed and on duration of treatment and health condition,¹⁰ cannot be excluded. However, to date, little is known about the effects of SGLT-2 inhibitors on the cutaneous microvascular function in humans.

In conclusion, our results reveal a positive disproportionality signal for canagliflozin, and also for empagliflozin, and, for toe amputations only, for dapagliflozin. However, our analysis relies on a limited number of cases and is exposed to the biases inherent to pharmacovigilance studies. Further prospective data are therefore needed to better characterize the risk of amputations with use of the different SGLT-2 inhibitors.

ACKNOWLEDGEMENTS

We thank Dr Alison Foote (Grenoble Alpes University Hospital) for critically editing the manuscript. We also thank the Uppsala Monitoring Center for providing us full access to Vigibase, and especially Dr Camilla Westerberg, Research Pharmacist, Custom Search Services Team at the Uppsala Monitoring Centre Research Section, for helping with specific data requests.

Disclaimer

The data in Vigibase come from a variety of sources and countries. The likelihood of a causal relationship is not the same in all reports.

The opinions and conclusions in this study are not necessarily those of the various centers of pharmacovigilance or of the WHO.

Conflict of interest

The authors declare no potential conflict of interests.

Author contributions

C.K. and M.R. designed the research. C.K. analyzed the data. All authors interpreted the data and wrote the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Khouri C, Cracowski J-L, Roustit M. SGLT-2 inhibitors and the risk of lower-limb amputation: Is this a class effect? *Diabetes Obes Metab*. 2018;1-4. <https://doi.org/10.1111/dom.13255>

3. Drug-induced skin ulcer: a combined disproportionality analysis using data from Medline and Vigibase.

Les étiologies iatrogènes d'ulcères cutanés sont très peu étudiées dans la littérature. Aucune étude de pharmacovigilance n'a jamais été réalisée et seule quelques revues non systématiques de la littérature existent (127,128). Nous avons donc réalisé deux analyses de disproportionnalité, en parallèle, sur la base de pharmacovigilance de l'OMS et sur les données de la littérature (PubMed) afin d'identifier de potentiels nouveaux signaux de pharmacovigilance.

Drug-induced skin ulcer: a combined disproportionality analysis using data from Medline and Vigibase

Short running title: Drug-induced skin ulcer

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Abstract (max 350 words)

Importance: Skin ulcers are a common negatively affecting the individual's quality of life, causing pain and social discomfort, and represent a substantial economic burden to healthcare systems. Numerous etiologies have been identified however little is known about iatrogenic skin ulcers.

Objective: We aimed to identify unknown drugs associated with the onset or the aggravation of skin ulcers.

Design: We performed a combined pharmacovigilance disproportionality analysis using data from VigiBase®, the World Health Organization (WHO) pharmacovigilance database, and from the literature to identify pharmacological classes suspected to induce skin ulcers.

Setting:

Participants: All reports of skin ulcers between inception and January, 31 2019 in the WHO pharmacovigilance database and Medline were included in the disproportionality analyses.

Main Outcome(s) and Measure(s): The information component (IC), a Bayesian neural network method, was used to identify pharmacovigilance disproportionality signals of drugs induced skin ulcers. A disproportionality signal was deemed significant if the lower boundary of the 95% credibility interval of the Information Component (IC025) was superior to 0.

Results: Through the analysis of 22,292 reports of skin ulcers in the WHO pharmacovigilance database and 835 articles in Medline we identified 25 drugs that may cause skin ulcers. Notably, our results identified new safety signals involving 6 protein kinase inhibitors (sorafenib, afatinib, ibrutinib, cabozantinib, lenvatinib and tofacitinib), 3 bisphosphonates (alendronic acid, pamidronic acid and zoledronic acid), leflunomide, interferons, rofecoxib and digoxin; and confirmed known association of skin ulcer with protein kinase inhibitors such as sunitinib,

erlotinib, ponatinib and pazopanib, bevacizumab, antimetabolites (methotrexate and hydroxycarbamide), mTOR inhibitors (everolimus and sirolimus), glucocorticoids and pentazocine.

Conclusions and Relevance:

In this study we identified several unknown drugs with a plausible pharmacological mechanism that may cause skin ulcers. Although confirmatory studies have to be performed clinicians should be aware of these potential adverse events.

Keywords

Drug safety, skin ulcer, pharmacovigilance

Key points (Question/Findings/Meaning) (75-100 words)

Question: What are the pharmacovigilance signals of iatrogenic skin ulcers in the WHO pharmacovigilance database and in the literature?

Finding: We retrieved all reports of drug related skin ulcers in the WHO pharmacovigilance database and in Medline. Overall, we found a possible association between skin ulcers and 26 drugs. Notably, our results identified new safety signals involving 6 protein kinase inhibitors, 3 bisphosphonates, leflunomide, interferons, rofecoxib and digoxin: and confirmed several known association of skin ulcer.

Meaning: Clinicians facing recalcitrant or recurrent skin ulcers should be aware of a potential over-risk of cutaneous ulceration induced by the drugs identified in this study.

Introduction

Skin ulcers are defined as a breakdown in the skin that may extend to involve the subcutaneous tissue or even to the level of muscle or bone. These lesions are common, particularly on the lower extremities which have an estimated prevalence of 1 to 2% among adults in United States (1). They negatively affect the individual's quality of life, causing pain and social discomfort, and represent a substantial economic burden to healthcare systems (2–4). According to the implicated pathogenesis, skin ulcers could be classified in 3 main groups: pressure ulcers, vascular ulcers (e.g. venous and arterial) and neuropathic ulcers (e.g. diabetic) (5). Furthermore, several other less frequent conditions could be implicated: infection, vasculitis, skin malignancies or ulcerating skin diseases such as pyoderma gangrenosum (6). Skin ulcerations have occasionally been reported in literature as adverse drug reaction (7), however no pharmacovigilance study have been performed to date and little is known about iatrogenic skin ulcers. We therefore performed a combined pharmacovigilance disproportionality analysis using data from VigiBase®, the World Health Organization (WHO) pharmacovigilance database, and from the literature to identify pharmacological classes suspected to induce skin ulcers.

Methods

1. Disproportionality analysis in the WHO pharmacovigilance database

VigiBase® is the WHO global database of individual case safety reports (ICSRs). This database contains over 18 million reports of suspected adverse drug reactions, submitted, by more than 130 country members of the WHO Programme for International Drug Monitoring.(8) A disproportionality analysis aims to identify signals of disproportionate reporting (SDR), i.e. an abnormal proportion of a drug-event pair compared to all other drug-event pairs contained in the database. Several methods of disproportionality exist, we used the Bayesian neural network method developed by Uppsala Monitoring Centre research team which displayed the best

sensitivity and specificity (9,10). A pharmacovigilance disproportionality signal was deemed significant if the lower boundary of the 95% credibility interval of the Information Component (IC025) was superior to 0 (11,12).

Disproportionality analysis in Vigibase was performed following a two-stage procedure in order to minimize false positive discover rate. We first extracted all drugs associated with a pharmacovigilance disproportionality signal of the non-specific preferred term “skin ulcer” (PT). Then we selected only drugs associated with at least a positive disproportionality signal for another skin ulcer preferred term: “decubitus ulcer”, “diabetic ulcer”, “ischemic skin ulcer” or “varicose ulceration”.

2. Systematic review and disproportionality analysis from Medline

A literature review of English and French language articles published until July 2019 was performed through Medline using the following search terms: ‘skin ulcer/chemically induced’. All references were retrieved by two independent authors (CK, DD). We selected all articles reporting the onset or aggravation of skin ulcer related to drug use. Non-human studies (i.e., animal studies), non-drug-related skin ulcer, mucous ulcers and ulcers linked to extravasation, pyoderma gangrenosum, auto-immune diseases were excluded. All remaining articles were classified according to the involved drug and we performed a disproportionality analysis using the same method as above described but using the data from Medline. Such method have already been performed by agencies and researches to improve drug safety signal detection. (13,14) To calculate the total number of adverse reactions associated with identified drugs we used the subheading Mesh terms “/adverse effects” associated with the drug name and “/chemically induced” associated with skin ulcer (14,15)

3. Expert review

Lastly, the relevance of all disproportionality signals was assessed through meeting among senior experts in dermatology, pharmacovigilance, vascular pharmacology, microcirculation

and skin ulcers (JLC,CK,SB,MR). All drugs considered to be potentially affected by protopathic or indication bias were excluded from the analysis.

Results

From the 18,578,159 ICSRs available at the time of the extraction (2019-01-31), we identified 17,672 reports of ‘skin ulcer’, 4,008 of ‘decubitus ulcer’, 460 of ‘diabetic ulcer’, 92 of ‘varicose ulceration’ and 60 of ‘ischaemic skin ulcer’ (Figure 1). 173 drugs were associated with a pharmacovigilance disproportionality signal of “skin ulcer”. Within those drugs, 52 displayed a disproportionality signal for at least another specific skin ulcer term.

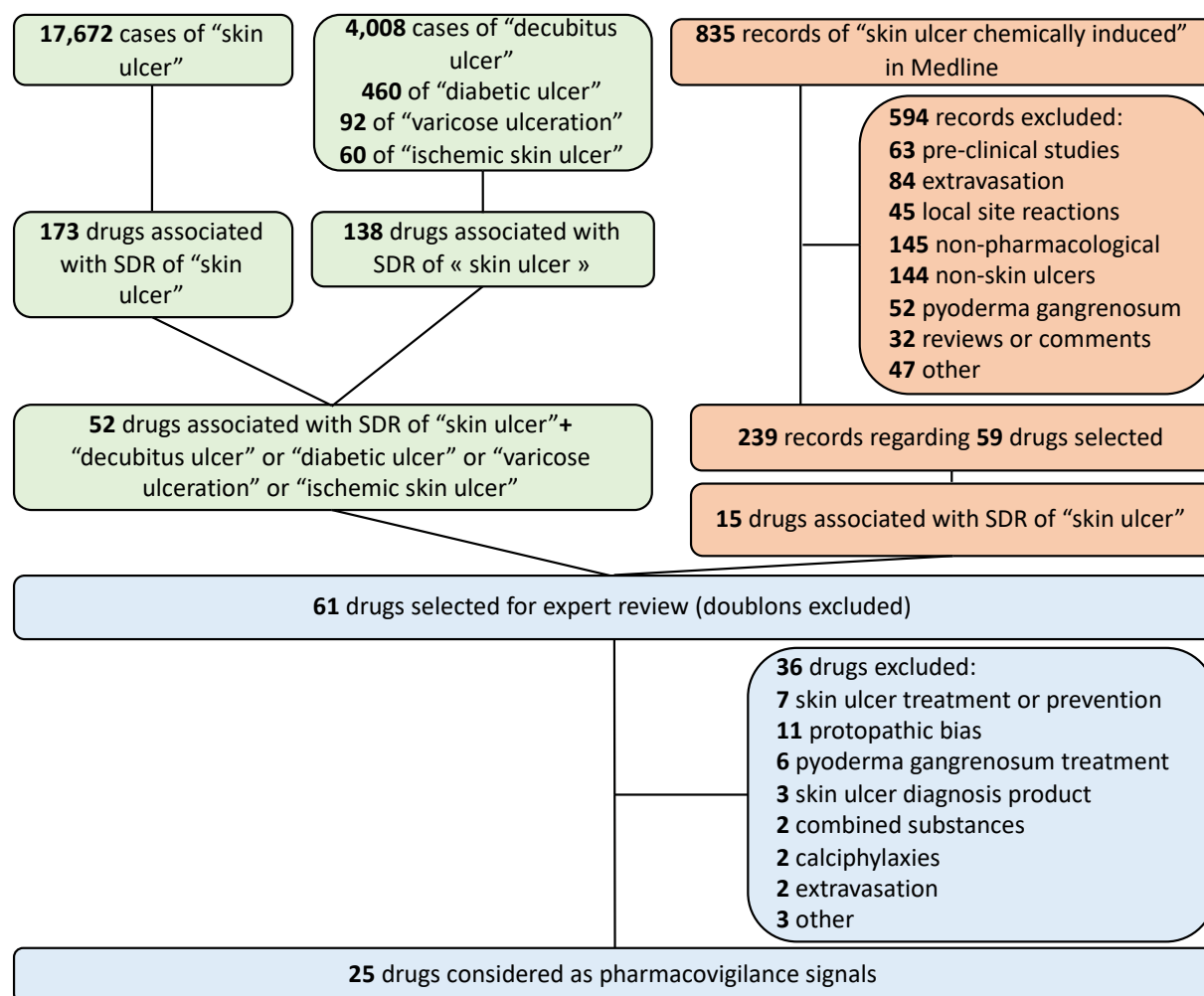


Figure 1. Study flow chart. The signal detection and assessment process is divided in 3 parts: green: disproportionality analysis in Vigibase; orange: disproportionality analysis in Medline; blue: expert review

Among 835 records of skin ulcer chemically induced identified in the literature after screening titles and abstracts, 241 reports regarding 59 drugs were selected. Main reasons for exclusion were pre-clinical studies (n=63), extravasation (n=84), hypersensitivity reactions (n=76), local site reaction (n=45), non-pharmacological substance (n=145), non-skin ulcers (n=144) and pyoderma gangrenosum (n=52). Among the 59 drugs, 15 displayed a disproportionality signal in Medline.

Through discussion among the expert group, we excluded 36 drugs because suspicion of protopathic, indication bias or combined substances (Supplementary Table 1). Finally, 25 drugs have been selected and could be considered as potential pharmacovigilance signals of skin ulcer (Table 1).

<i>Drug classes</i>	<i>Drugs</i>	<i>Vigibase disproportionality signal</i>	<i>Medline disproportionality signal</i>	<i>SPC</i>
<i>Protein kinase inhibitors</i>	Sorafenib	✓		
	Sunitinib	✓	✓	Pyoderma
	Afatinib	✓		
	Erlotinib	✓		Skin fissure
	Ibrutinib	✓		
	Cabozantinib	✓		
	Lenvatinib	✓		
	Pazopanib	✓	✓	Skin ulcer
	Tofacitinib	✓		
	Ponatinib	✓		Pain of skin
<i>mTOR inhibitors</i>	Everolimus	✓		Skin lesion
	Sirolimus	✓		
<i>Antimetabolites</i>	Methotrexate	✓	✓	Skin ulcer
	Hydroxycarbamide	✓	✓	Leg ulcer
	Leflunomide		✓	
<i>Bisphosphonates</i>	Alendronic acid	✓		
	Pamidronic acid	✓		
	Zoledronic acid	✓		
<i>VEGF inhibitors</i>	Bevacizumab	✓		Wound healing complications
<i>Interferons</i>	Interferon beta	✓	✓	
<i>Cardiac glycosides</i>	Digoxin	✓		

<i>Cox 2 inhibitors</i>	Rofecoxib	✓	
<i>Glucocorticoids</i>	Prednisone	✓	Impaires wound healing
<i>Potassium channel activators</i>	Nicorandil	✓	Skin ulcerations
<i>Opiate analgesics</i>	Pentazocine	✓	Ulceration of the skin

Table 1. Results of the disproportionality analyses. A ✓ is indicative of a disproportionality signal. Moreover, verbatim about skin ulcers, if any, retrieved from the summary product characteristics (SPC) are presented. Unknown drugs are in green

Discussion

To the best of our knowledge, this study is the first pharmacovigilance study aiming at identify iatrogenic etiology of skin ulcers. Using data from the WHO pharmacovigilance database and from the literature, we found a possible association between skin ulcers and 25 drugs. Notably, our results identified new safety signals involving 6 protein kinase inhibitors (PKI), 3 bisphosphonates, leflunomide, interferon beta, rofecoxib and digoxin: and confirmed known association of skin ulcer with PKI such as sunitinib, erlotinib, ponatinib and pazopanib, bevacizumab, antimetabolites, mTOR inhibitors, glucocorticoids and pentazocine.

Overall, four main mechanisms are supposed to be implicated in the pathogenesis of iatrogenic skin ulcers: angiogenesis inhibition, direct skin toxicity and diminishing skin perfusion through vasoconstriction or increased blood viscosity (Figure 2).

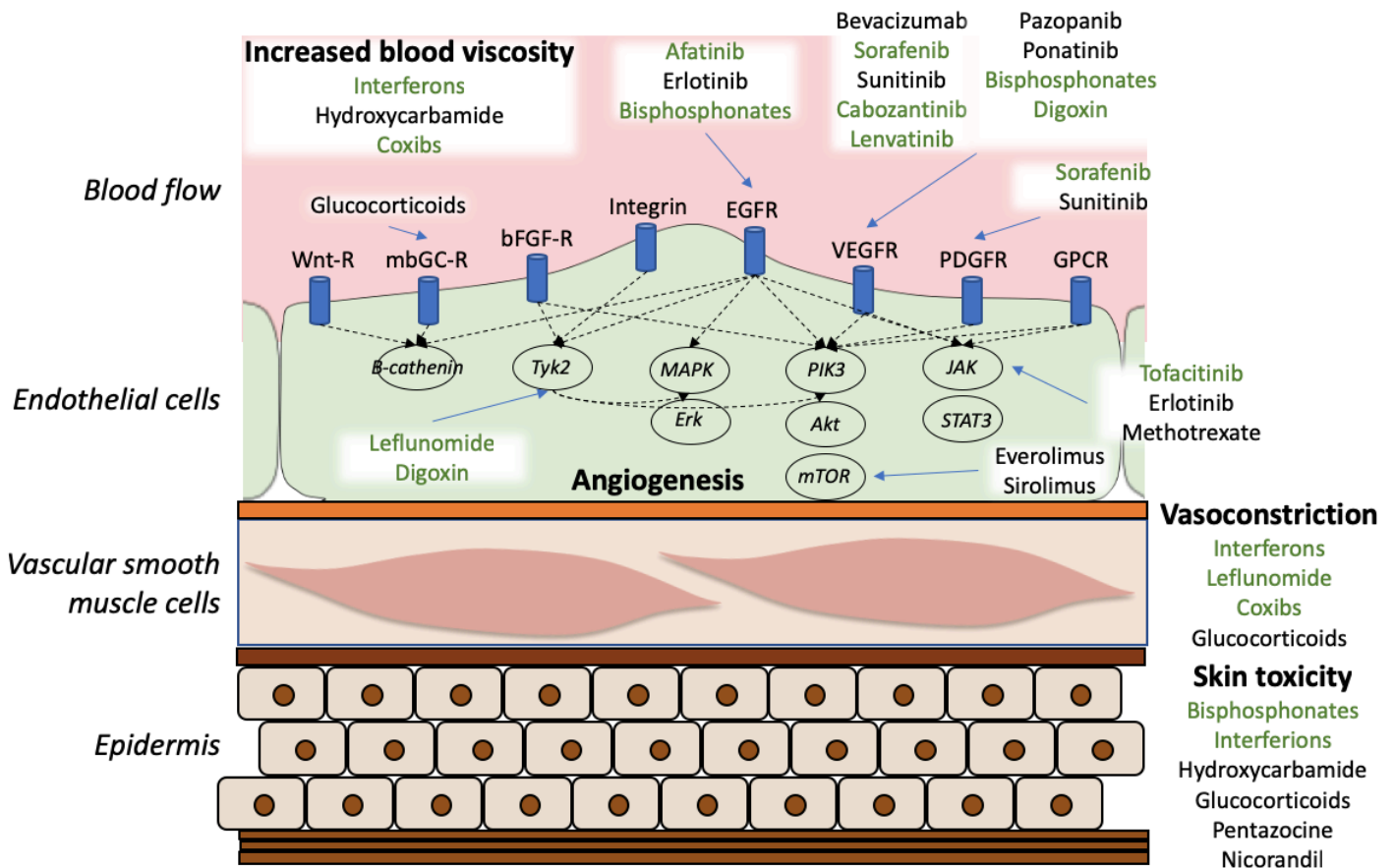


Figure 2. Schematic representation of the mechanisms of iatrogenic skin ulcers. Unknown drugs are in green. GPCR, G-protein-coupled receptors ; endothelial growth factor (EGF); fibroblast growth factor (FGF); platelet-derived growth factor (PDGF) ; vascular endothelial growth factor (VEGF); mbGC-R: membrane glucocorticoid receptor.

Angiogenesis is a key factor in the proliferation phase of wound healing, and besides, antiangiogenic agents are known to delay wound healing. (16,17) Moreover, it has been shown that growth factors such as VEGF are implicated in vascular homeostasis in adults, in the absence of any pathological insult. (18) Angiogenesis is a complex process regulated by several growth factors (VEGF, EGF, TGF, PDGF..), integrins, cytokines (IL-8) and chemokines (SDF-1). (19) In this pharmacovigilance study, most of the identified drugs displayed anti-angiogenic properties. The anti-VEGF, bevacizumab, is well known to delay wound healing and is estimated to increase the risk of wound healing complications from an OR of 2.32 (1.43, 3.75) against placebo, and cases reported the onset of skin ulceration during bevacizumab therapy

(20,21). All the identified PKI displayed anti-angiogenic properties: sorafenib, sunitinib through VEGF and PDGF inhibition; cabozantinib, levantinib, pazopanib and ponatinib through VEGF inhibition; afatinib and erlotinib through EGFR inhibition; ibrutinib and tofacitinib through JAK inhibition (and tyrosine kinase 2 for tofacitinib). Inhibition of mTOR (everolimus, sirolimus) also displayed anti-angiogenic effect through downregulating the expression of VEGF-A/VEGFR-2 and VEGF-C/VEGFR-3. (22)

The link between skin ulcer and bisphosphonates has never been suggested previously to our knowledge. Although some cases were associated with osteonecrosis of jaw, such adverse drug reaction seems pharmacologically conceivable. Indeed, a growing evidence is demonstrating that bisphosphonates, notably zoledronic acid, inhibit cell migration and angiogenesis through inhibition of the EGFR/Akt/PI3K pathway (23), VEGF (24) and induce fibroblast and vascular endothelial cell apoptosis (25,26). Moreover, a recent study suggested that bisphosphonates interfere with the supply of calcium causing basal layer epithelial cells dysfunction (27).

The mechanism underlying the risk skin ulcer induced by recombinant cytokines, such as interferons, remain unclear. However, main hypotheses suggested that interferons induce a local immune-mediated inflammatory response in the skin, have vasospastic, procoagulant, vasoconstrictor effects and cause microvascular abnormalities such as luminal occlusion especially by interleukin-1 production and endothelial cells proliferation. (28–36)

The pathophysiology of skin ulcer induced by antimetabolites is not well understood and is probably multifactorial. Some hypotheses suggested a direct cytotoxic effect of hydroxycarbamide on epidermal cells (especially on keratinocytes in the basal layer) (37–49) causing a dystrophy of keratinocytes (50,51) with an inhibition of collagen synthesis (46,47). Although the role of the drug is difficult to assess due to the underlying pathology, some studies identified macrocytic erythroblasts, inducing microcirculation impairment causing cutaneous anoxia and microthrombi formation (42,44,48,49,52–55). Furthermore, methotrexate showed

to provoke endothelial cell inhibition and apoptosis (56,57) and to inhibit JAK tyrosine kinases (58). Leflunomide has been shown to inhibit the Protein-tyrosine kinase 2-beta which is a signaling protein involved in cell migration and angiogenesis (60,61) and to inhibit the activity of cyclo-oxygenase-2 (62).

Digoxin is a well-known HIF alfa inhibitor and has been shown to reduce the production of growth factors, in particular VEGF (59,60). Recent data also indicate that digoxin inhibits endothelial focal adhesion kinase and angiogenesis (61).

The relationship between skin ulcer and coxibs is based on two main hypotheses: a selective COX-2 inhibition reducing prostacyclin (PGI₂) synthesis, a vasodilator and a platelet aggregation inhibitor (62). Furthermore, they reduce the PGE₂ and VEGF release from keratinocytes (63). It has also been demonstrated that PGE₂ and PGI₂ are implicated in angiogenesis through VEGF pathway activation (64).

The mechanism linking skin ulcer and glucocorticoids seems related to their antiproliferative effect on keratinocytes, melanocytes and fibroblasts, thus decreasing collagen synthesis and cause cutaneous atrophy (65). Moreover, recent studies suggest a non-genomic mechanism of glucocorticoids through a membranous glucocorticoid receptor resulting in the inhibition of the Wnt signaling pathway and keratinocyte migration (66). Lastly, it has been recently proposed that glucocorticoids inhibits PGE₂ production through COX2 inhibition this producing dermal vasoconstriction (67).

Skin ulcers associated with nicorandil are well described and probably induced by a direct toxic effect of nicotinic acid on epithelial cells (68,69). The increased risk of skin ulcer was found to be of Hazard Ratio 1.85 (95% CI 1.27-2.69) compared to non-users (69). Pentazocine is also associated to skin ulcer genesis since 1979 probably through direct local inflammation and microthombi formations (70).

Pharmacovigilance databases are constituted of adverse drug reactions spontaneously reported by physicians, healthcare professionals or even patients. Therefore several limitations of disproportionality analyses need to be acknowledge. Indeed selective reporting of adverse drugs reactions and the lacking of clinical data to verify such reactions could provoke misclassification of the reported symptoms (71). In addition, the onset of skin ulcer could be multifactorial and the causality with drug exposure could be difficult to assess given the high rate of missing clinical data. Moreover, the results of disproportionality analyses may be influenced by the extent of use of the drug, media coverage, the severity of the reactions and competition with other drugs and adverse events. Despite these bias we identified several drugs known to induce skin ulcers, which could be considered as positive controls. Moreover, our disproportionality analysis of the data from Vigibase followed a two-stage procedure, a conservative method allowing a greater specificity by suppressing a maximum of false positives, but probably leading also to a loss of sensitivity. Lastly, performing disproportionality analyses in the literature and in a pharmacovigilance database seems to be a complementary approach. Indeed, we identified well described drugs in the literature that were masked in WHO pharmacovigilance database.

Conclusion

In this study we several unknown drugs that may cause skin ulcer: tyrosine kinase inhibitors, bisphosphonates, leflunomide, interferon beta, digoxin or cox 2 inhibitors with a plausible pharmacological mechanism. Although confirmatory studies have to be performed clinicians should be aware of these potential adverse events.

Acknowledgment

The thanks Vigibase for giving us access to the data. The supplied data from VigiBase come from a variety of sources. The likelihood of a causal relationship is not the same in all reports. The information does not represent the opinion of the UMC or the World Health Organization.

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4. Conclusion et perspectives

L'identification de médicaments susceptibles d'entraîner un risque accru de développer des ulcères cutanés est encore actuellement peu investiguée. Cependant, c'est une thématique d'importance puisque l'impact en santé publique de ces effets indésirables pourrait être fort. En effet, les ulcères cutanés sont une pathologie relativement fréquente entraînant une forte morbidité et des coûts pour la société très importants. (5–7)

Nous avons identifié dans nos travaux plusieurs médicaments d'intérêts, notamment les inhibiteurs de cyclooxygénase-2, les bisphosphonates et certains inhibiteurs de tyrosine kinase. La confirmation et l'évaluation du sur-risque d'apparition d'ulcère cutané avec ces classes thérapeutiques reste à réaliser. Les ulcères cutanés peuvent être identifiés dans les bases médico-administratives via les hospitalisations ou les remboursements de pansements ; des études de pharmaco-épidémiologie peuvent donc être envisagées pour évaluer ces signaux. Nous sommes actuellement en train de d'effectuer une étude sur l'Echantillon Généraliste des Bénéficiaires qui a pour objectif d'évaluer le risque d'ulcères cutanés avec les inhibiteurs de cyclooxygénase-2. Ce schéma d'étude pourra ensuite être transposé à d'autres classes médicamenteuses comme les bisphosphonates.

De plus, au sein du laboratoire de recherche HP2 notre équipe a développé des modèles animaux présentant un retard de cicatrisation cutanée afin initialement d'étudier et d'évaluer l'efficacité de traitements pharmacologiques sur la cicatrisation d'ulcères chroniques. Nous envisageons d'utiliser ces modèles afin d'explorer le mécanisme ulcérogène des médicaments dont nous aurons confirmé l'implication.

PARTIE 5. ÉTUDES MÉTHODOLOGIQUES

1. Impact of pharmacovigilance disproportionality analysis study design on the correlation with drug-related risks.

De nombreuses méthodes ont été développées afin de minimiser les biais affectant les analyses de disproportionnalité. (73,78) Ces méthodes comprennent l'exclusion de médicaments ou effets indésirables rentrant en compétition avec l'EI étudié, la prise en compte de biais induits par la médiatisation de certains EI, un changement de groupes comparateurs...L'impact de ces différentes méthodes sur la taille d'effet des résultats n'a jamais été étudiée de manière systématique.

Par ailleurs, plusieurs études ont récemment utilisé à la fois des données de pharmacodynamie et des résultats d'analyses de disproportionnalité afin d'émettre des hypothèses physiopathologiques à propos d'effets indésirables. (5–8) L'analyse de disproportionnalité est alors utilisée comme approximation du risque d'effet indésirable lorsque ce risque est inconnu et difficilement estimable par d'autres méthodes. L'étude de la corrélation entre les analyses de disproportionnalités et un risque d'effet indésirable estimé par d'autres méthodes comme des méta-analyses est très peu étudié. De plus, l'impact des différents designs sur ces corrélations n'est pas connu.

Impact of pharmacovigilance disproportionality analysis study design on the correlation with drug-related risks.

Running title:

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Funding source

None

Conflict of interest statement

The authors have stated explicitly that there are no conflicts of interest in connection with this article

Key words: Disproportionality analysis, vibration of effect, pharmacovigilance

Abstract

Aims

Disproportionality analyses (DA) are widely used for safety signal detection in pharmacovigilance spontaneous reporting systems databases. While such analyses are not intended to provide risk quantification, several studies have recently suggested a correlation between the measures of disproportionality and drug-related risks. In this context, we aimed at testing the influence of methodological choices on the results (vibration of effect) and on the correlation with the risk of adverse drug reactions (ADRs).

Methods

We extracted ADR Odds Ratios (ORs) from meta-analyses used as reference and calculated corresponding Reporting Odds Ratios (RORs) from Vigibase. We tested five DA designs and 2 correlation methods. We calculated the performances of each design, of the median ROR across designs, and correlated RORs to ORs. We also calculated the relative bias and agreement of ROR compared to OR and adapted the measures of vibration of effect to the DA.

Results

We selected 5 meta-analyses which displayed a panel of 13 ADRs. A significant correlation for 7 out of the 13 ADRs studied in the primary analysis was found. None of the methods for ROR calculation (time, region or population standardization, drug considered concomitant) systematically improved the correlation results. Whereas correlation was found between OR and ROR agreement was poor. According to Bland and Altman method, mean differences between OR and ROR ranged from 37.6 for time standardization to -0.1 in restricting the analysis to a therapeutic area.

Conclusions

This study provides further evidence that effect sizes obtained from meta-analyses and from disproportionality analyses to assess drug related ADR risks sometimes correlate. However, there is large vibration of effect of disproportionality analyses, thus emphasizing the importance the presentation of a set of results across all possible methods, to avoid selective reporting. Further work is needed to understand the patterns of disproportionality analyses results vibration and the ADR characteristics influencing the correlation with ADR risk.

Introduction

Disproportionality analyses are statistical methods widely used by national drug agencies, industries and researchers for safety signal detection in pharmacovigilance spontaneous reporting systems databases. They aim at quantifying the extent to which a drug-event pair is reported more often than it would be at random. A signal of disproportionate reporting (SDR) is a statistical association that does not imply a causal relationship between the administration of the drug and the occurrence of the adverse event (1,2). Generally, disproportionality analyses cannot be used to assess a drug-relative risk because they don't provide risk quantification (3). Nevertheless, Maciá-Martinez and colleagues found a significant correlation between disproportionality analyses and relative risks estimated in epidemiological studies and meta-analyses (4). Moreover, several studies have recently used a mixed pharmacoepidemiological-pharmacodynamic design to explore the pathophysiology of some adverse drug reactions (ADR), hypothesizing a correlation between the measures of disproportionality and drug-related risks of ADR⁵⁻⁸. However, disproportionality analyses are affected by several bias, which may modify disproportionality effect sizes, performances for SDR detection and eventually the correlation with drug-related risks, if not properly controlled (3). Among them, the time after drug approval (Weber effect), the reporting region (induced by heterogeneity in pharmacovigilance national systems), media attention (notoriety bias) affect drug reporting (9,10). A competition between the studied drug or event with other drugs or events widely reported can also affect disproportionality effect sizes (competition bias or masking effect) (11,12). Lastly, the choice of the control group does affect disproportionality and is an important concern in every epidemiological study. Several strategies to minimize the above-mentioned bias have been developed (e.g. time trend analysis, exclusion of competitors, subgroup analysis) but their impact on the relationship between disproportionality effect sizes and drug-related risks has never been studied (13). The vibration of effect is a concept recently developed by Patel et al. initially applied to observational researches (14). The vibration of effect is an estimation of the variation in effect sizes and p-values due to model selection. Thus this concept could also be applied to describe the result variation of disproportionality analysis due to design specifications.

In this context, several questions remain (1) Does the correlation between disproportionality analyses and drug-related risks exists for a variety of ADR? (2) Could the disproportionality analyses methodological choices modify this correlation?

To further address these questions, we compared and correlated ADR risks obtained from meta-analyses of clinical-trials to disproportionality analyses and assessed the impact of study design and bias minimization methods on the results.

Methods

1. Data sources

1.1. Selection of meta-analyses

We extracted all meta-analyses published over five years (August 2013-August 2018) in seven high impact journals: NEJM, JAMA, Plos Medicine, BMJ, JAMA Internal Medicine, Lancet and Annals of Internal Medicine. Then, we selected the meta-analyses evaluating the safety of a pharmacological drug class, with at least 5 different pharmacological treatments, on specific ADRs.

1.2. Pharmacovigilance database

VigiBase[®] is the World Health organization's (WHO) global safety database of individual case safety reports (ICSRs). At the time of extraction, the database contained over 18 million ICSRs from 131 member countries and 29 associated members of the WHO Programme for International Drug Monitoring. VigiBase[®] provides ICSRs with patient information such as gender, age, medical history, country, drug and concomitant drug taken with chronological information, indication of the drug, adverse effects and their severity and outcome (15).

1.3. Adverse drug reactions

In VigiBase[®], all ADRs are coded according to the Medical Dictionary for Regulatory Activities (MedDRA) (16). In the present study, we identified Standardized Medical Queries and MedDRA terms corresponding to ADRs extracted from meta-analyses. We characterized

each ADR by its frequency, severity and time to onset (Text S1). Furthermore, for each ADR, a potential protopathic and media bias was searched. A protopathic bias occurs when a drug is initiated in response to the first symptoms of an undiagnosed disease that will cause the studied ADR (17). To assess influence of media safety alerts on the reporting rate of an ADR, we retrieved FDA drug safety alerts (10).

2. Analyses

2.1. Meta-analysis

All risk estimates from the selected meta-analysis were extracted. When several drug dosages were available for a given drug, they were meta-analyzed to obtain a summary measure. Moreover, continuous outcomes were back transformed into odds ratio (OR) through standardized mean differences (18).

2.2. Disproportionality analyses

Several disproportionality methods have been developed and are currently applied in pharmacovigilance databases, but no gold standard in terms of performance, accuracy and reliability has been established (19,20). In the present study, disproportionality analyses were performed using the Reporting Odds Ratio (ROR) method, with 95% confidence intervals (CI) (21). Given the poor accuracy of the ROR calculated with less than three individual cases, drug-adverse effect pairs for which the number of cases was lower than 3 were excluded from analysis (13,20,22).

To explore factors that may influence the correlation between RORs and ORs, several disproportionality analyses were performed in Vigibase®. A primary disproportionality analysis was performed for each drug or drug class considered as suspect at the publication date of the corresponding meta-analysis, to minimize the notoriety bias and the modification of reporting rates over time. Then, the following secondary analyses were performed: (1) adding the concomitant reports to the studied drug; (2) standardizing the time on the market of different drugs five years after approval date, to assess the influence of time on the market on the reporting rate (9,23); (3) adjusting on the reporting continent (North America: Canada and USA) (24); (4) restricting SDR detection within a therapeutic area to limit indication and

confusion bias (25,3,26); (5) excluding competitors to take into account potential drug-event competition bias. Competitors were identified using the competition index at the PT level with a cut off at 5% (11,27,28).

2.3. Correlation analysis

To take into account the variability of the point estimates of both values we performed an orthogonal regression analysis with the assumption of equal variance. We used natural logarithms of both values to reduce heteroscedasticity (29). Moreover, t-tests of the regression slope were performed, a p-value<0.05 was considered significant. In addition to the secondary disproportionality analyses above described, we performed two sensitivity analyses: (1) to take in account the variability associated with the point estimate, we correlated the lower boundary of the 95% CI of both estimates; (2) we performed the regression with a robust regression method to minimize the influence of outliers, through an MM-type regression estimator (30).

We adapted the measures of vibration of effect developed by Patel at al. to the disproportionality analyses. (14) For each ADR, we thus estimated the “relative ROR” (RROR) which is the ratio between the higher and the smaller ROR estimated according to the primary and secondary analyses previously described. (14)

We also calculated the relative bias of ROR compared to OR. We thus calculated the prediction errors between the two estimates divided by the ROR value; using this formulae $RB = (|pred - obs|) / pred$; pred=predicted value (ROR); obs=observed values (OR) (31). Lastly, we calculated the agreement between OR and ROR through Bland-Altman method (32). All analyses were performed using R statistical software (Version 3.3.0)

Results

1. Meta-analyses selection

Among the 425 meta-analyses published between 2013 and 2018 in the 7 selected high impact journals, 43 assessed the safety of several pharmacological interventions. However, only five meta-analyses assessed the risk of specific ADRs and were selected (33–37). The reasons for exclusion were the presence of only pooled results (n=8), no safety analysis or no

specific ADR (e.g. proportion of severe ADR or drop out for ADR) (n=21), not enough drug groups (n=8) and only combined treatment results (n=1) (Table S1) Drug classes were anti-psychotics, anti-depressants, blood pressure-lowering agents, oral anticoagulants and anti-diabetics.

2. Description of adverse drug reactions

These 5 meta-analyses evaluated 13 ADRs: extrapyramidal syndrome, prolactin increase, QTc prolongation, sedation, weight gain with antipsychotics; cough hyperkalemia, oedema, presyncope with blood pressure-lowering agents; suicide with antidepressant; gastrointestinal and intracranial bleeding with oral anticoagulants and hypoglycemia with anti-diabetics. The ADRs are described in Table 1.

Table 1. Description of 13 selected adverse drug reactions

Drug class	Adverse drug reaction	Frequency	Seriousness	Time to onset	Type	Protopathic bias	Media bias
Antipsychotics	Weight gain	Very Common	NS	D	A	Yes	No
	Extrapyramidal syndrome	Very common	S	D/I	A	No	No
	Prolactin increase	Common	NS	D	A	No	No
	QTc prolongation	Uncommon	S	I	A	No	Yes
	Sedation	Very common	NS	I	A	Yes	No
Blood pressure-lowering agents	Hyperkalemia	Common	S	D/I	A	No	No
	Presyncope	Common	S	I	A	No	No
	Cough	Common	NS	D/I	A	No	No
	Oedema	Common	NS	I	A	Yes	No

Antidepressant	Suicide	Uncommon	S	D	B	Yes	Yes
Oral anticoagulants	Intracranial bleeding	Uncommon	S	I	A	No	No
	Gastrointestinal bleeding	Common	S	I	A	No	No
Anti-diabetics	Hypoglycemia	Common	NS	I	A	No	No

D: Delayed; I: Immediate; NS: Non serious - S: Serious. A: type A adverse drug reactions (i.e. pharmacological and dose-related adverse effects); B: type B adverse drug reactions (idiosyncratic, bizarre or novel responses that cannot be predicted from the known pharmacology of a drug)

We found three FDA drug safety alerts in relation to the studied drug-ADR pairs. In October 2004, the FDA issued a black-box warning on antidepressants indicating that they were associated with an increased risk of suicidal thinking, feeling, and behavior in young people (38). Six months after this safety alert, the number of spontaneous reports involving an antidepressant in the occurrence of a suicidal ideation raised significantly ($p < 0.05$). In December 2001, the FDA released a warning on droperidol because of reported cases of QT-interval prolongation. Likewise, the number of cases reported in Vigibase[®] significantly increased ($p < 0.05$). Contrariwise, safety alert in 2007 on haloperidol and QTc prolongation was not followed by an increase in spontaneous reporting database (39).

3. Disproportionality analyses

The criteria (MedDRA terms, cases selection, drug class, drug competitors) used for ROR calculations in Vigibase[®] are described in Table 2.

Table 2. Criteria used for disproportionality analyses

Meta-analysis	Adverse reaction	drug	MedDRA Term(s) (Hierarchy level)	Cases selection Patient age Date of inclusion	Therapeutic area (ATC classification)	Drug competitor(s)
Leucht, 2013 (33)	Weight gain		Weight increased (PT)	18 to \geq 75 years old	Antipsychotics (N05A)	Levonorgestrel
	Extrapyramidal syndrome		Extrapyramidal syndrome (SMQ narrow)	27/06/2013		Metoclopramide
	Prolactin increase		Hyperprolactinaemia (PT) Blood prolactin increased (PT)			Fluoxetine
	QTc prolongation		Electrocardiogram prolonged (PT)	QT		None
	Sedation		Sedation (PT)			None
Palmer, 2015 (34)	Hyperkalemia		Hyperkalaemia (PT)	18 to \geq 75 years old	Cardiovascular system (classe C)	Potassium
	Presyncope		Presyncope (PT)	23/05/2015		None
	Cough		Cough (PT)			Influenza vaccine
	Oedema		Oedema peripheral (PT) Localised oedema (PT) Oedema (PT)			None
Cipriani, 2016 (35)	Suicide		Suicidal ideation (PT)	0 to 20 years old 08/06/2016	Antidepressants (N06A)	Isotretinoin Atomoxetine Montelukast
López-López, 2017 (36)	Gastrointestinal bleeding		Gastrointestinal haemorrhage (PT)	18 to \geq 75 years old 28/11/2017	Antithrombotic agents (B01A)	Warfarine
	Intracranial bleeding		Haemorrhage intracranial (PT)			Warfarine Alteplase Heparin
Palmer, 2016 (37)	Hypoglycemia		Hypoglycaemia (PT)	18 to \geq 75 years old 19/07/2016	Drugs used in diabetes (A10)	None

ATC: Anatomical Therapeutic Chemical – PT: Preferred terms – SMQ: Standardized Medical Queries

The vibration of disproportionality analyses results ranged from a relative ROR of 2.7 (risperidone and prolactin increase) to 710.8 (quetiapine and QTc prolongation) (Figure 1). In the later, the ROR for QTc prolongation with quetiapine was of 1.20 when restricting the analysis by therapeutic area and was of 854.80 when conducting the analysis 5 years after FDA labelling. The prediction error of ROR ranged from 0.1% to 2742%. Only 8.3% of the ROR displayed a prediction error inferior to 50% of the OR value.

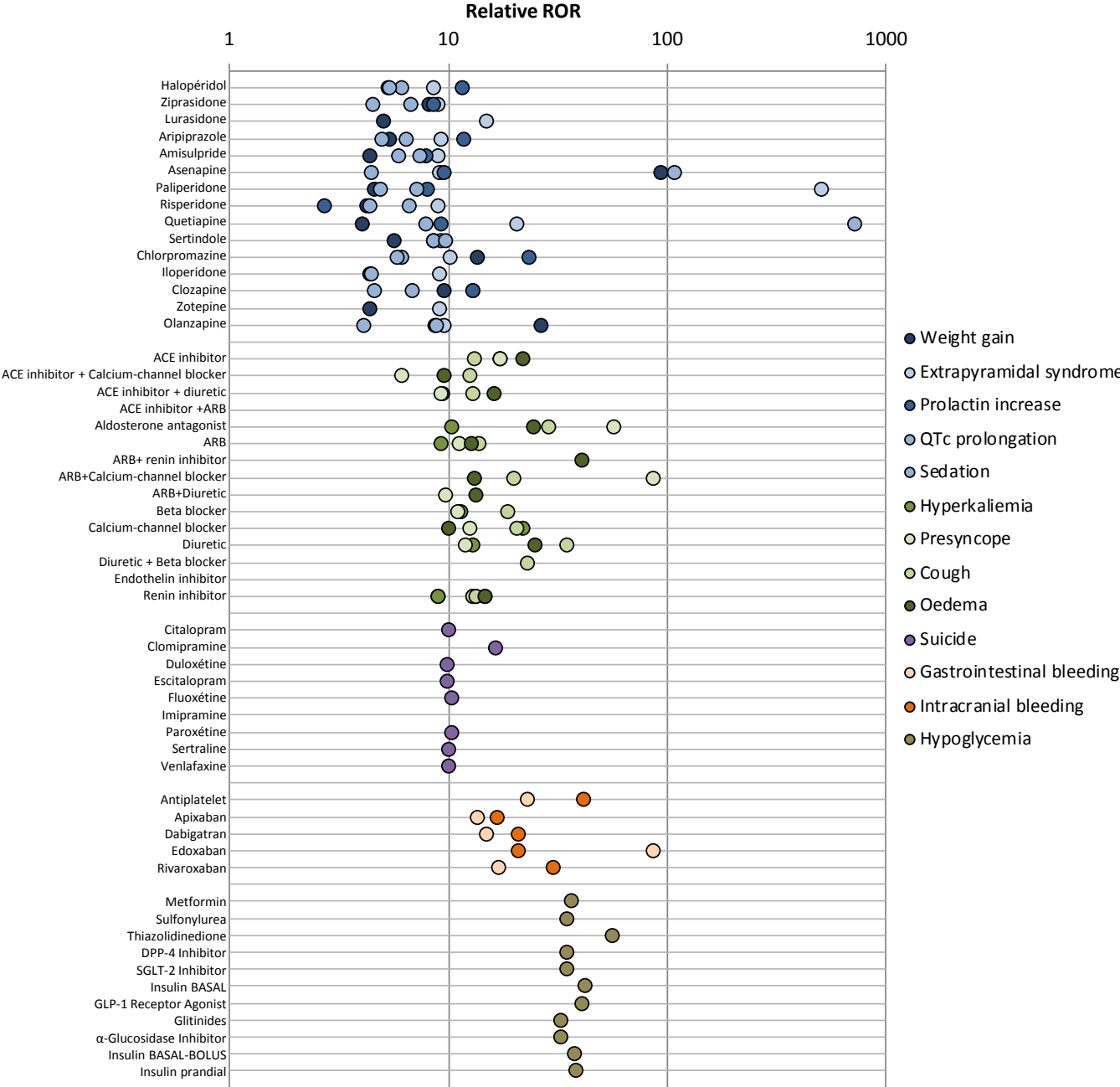


Figure1. Relative Reporting Odds Ratio (ROR) calculated as a ratio between the higher and the smaller ROR estimated according to the primary and secondary disproportionality methods.

4. Correlation analysis

In the primary analysis, we found a significant correlation between ORs from meta-analyses and the corresponding RORs for 7 of the 13 ADRs studied: extrapyramidal syndrome and prolactin increase with antipsychotics, hyperkalemia and cough with blood pressure-lowering agents, gastrointestinal bleeding with oral anticoagulants and hypoglycemia with anti-diabetics (Figure 2). Interestingly, some correlations remain significant in all secondary and sensitivity analyses despite the vibration of effect. Intercept, slope and goodness of the fit for each correlation are presented in supplementary material (Table S2) and examples of the best and the worst correlations are presented in Figure 3.

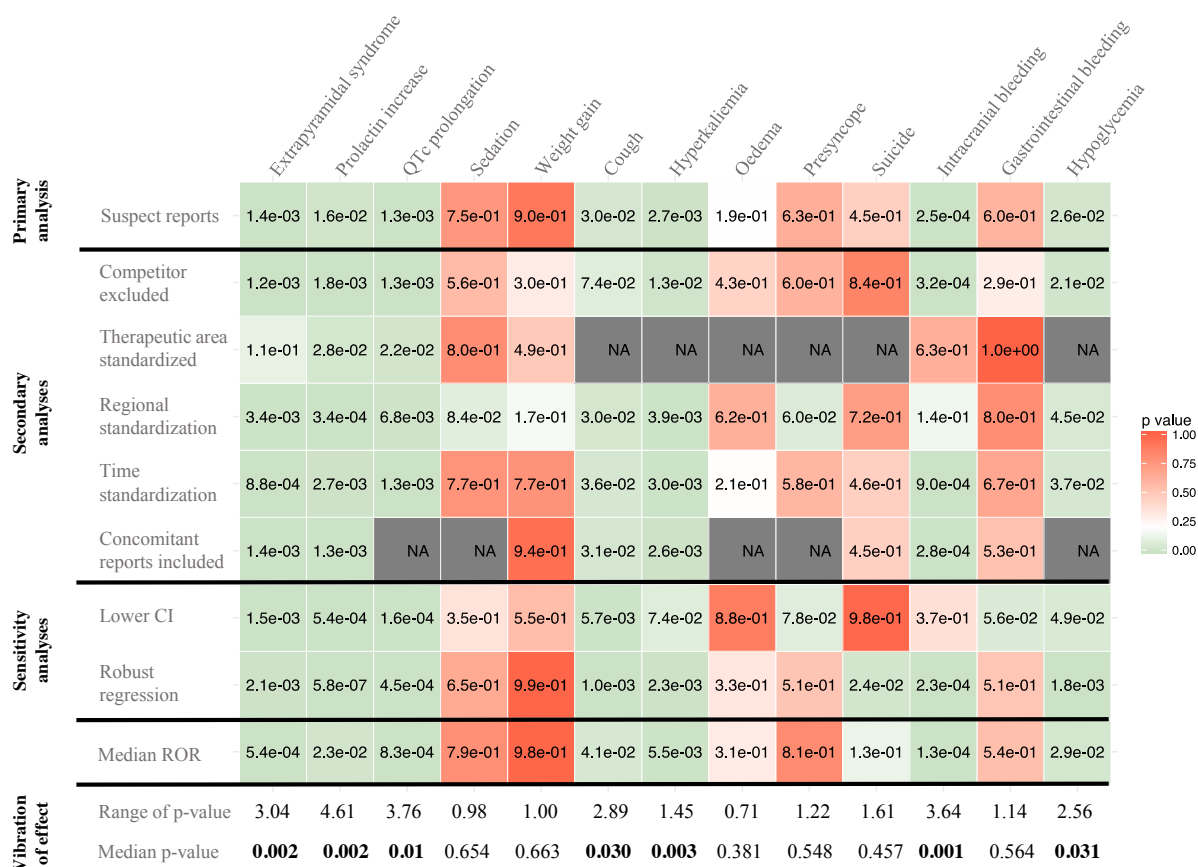


Figure 2. Heat map representing the p-value of the t-test of the slope for each adverse drug reactions in primary, secondary and sensitivity analyses. Relative and median p-value are also presented.

None of the methods for ROR calculation (time, region or population standardization, drug considered concomitant) systematically improved the correlation results. Excluding competitors slightly improved the correlation in 7 on the 8 affected ADRs. The wider variation in p-value was induced by time and regional standardization. Using the lower boundary of the confidence intervals and using a robust correlation method provided better results than the primary analysis for 8 and 11 ADR respectively.

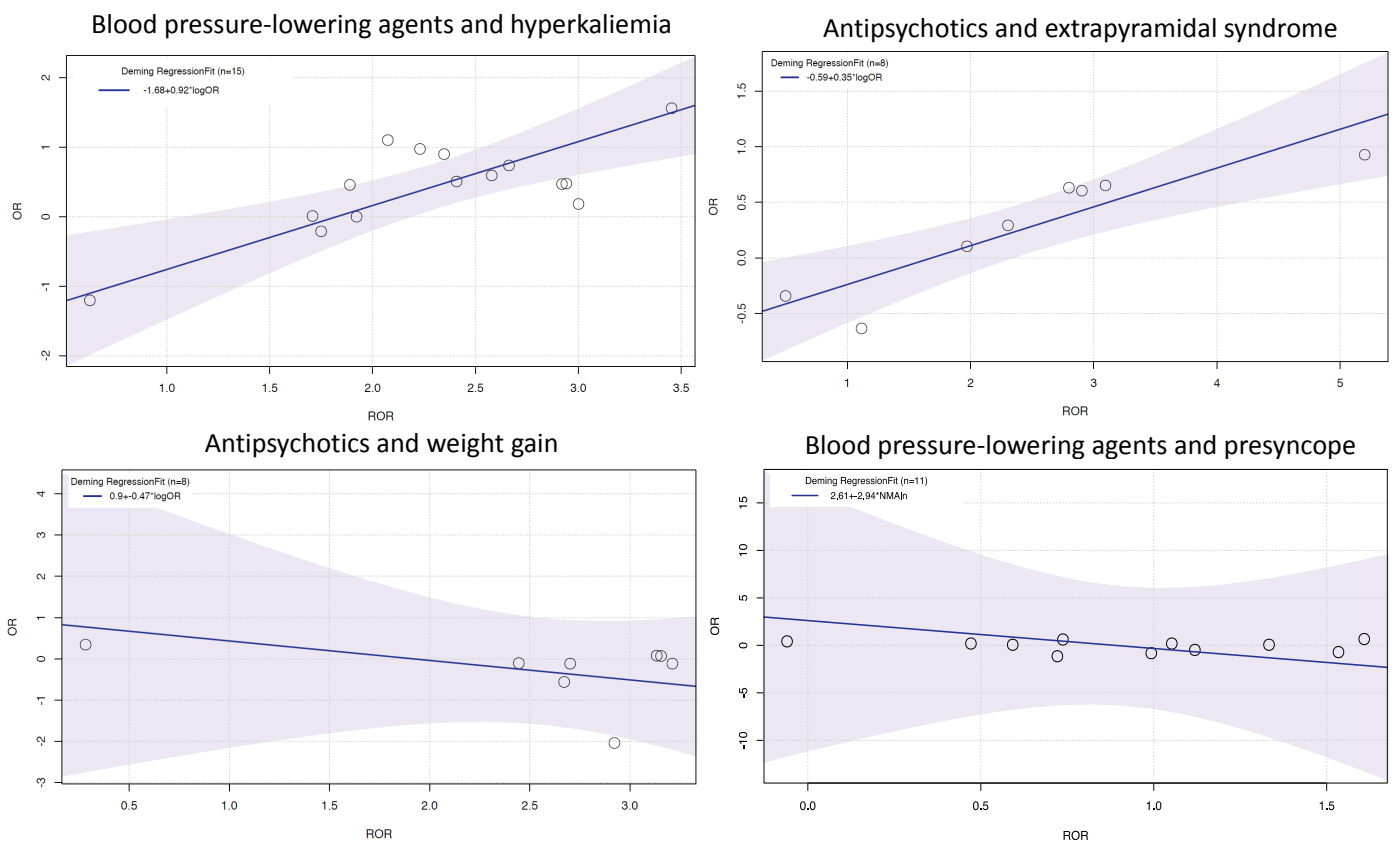


Figure 3. Examples of the best and the worst correlations between measures of association from meta-analyses (expressed as OR) and from Vigibase® (expressed as ROR) in the primary analysis. Each dot represents a drug.

Whereas correlation was found between OR and ROR agreement was poor. According to Bland and Altman method, mean differences between OR and ROR ranged from 37.6 for time standardization to -0.1 in restricting the analysis to a therapeutic area (Table S3)

Discussion

To our knowledge, this is the first study exploring the impact of disproportionality analyses study design for ROR calculation on the correlation with drug-related ADR risk from clinical trials.

We found a significant correlation for 7 out of the 13 ADRs studied in the primary analysis. Within ADR characteristics, the presence of a protopathic bias seems to be associated with weaker correlations. This is striking in the first meta-analysis in which sedation and weight gain, frequently induced by multifactorial etiologies and drugs, are far less correlated than prolactin increase, extrapyramidal syndromes and QTc prolongation. The result of the 5 secondary analyses provided heterogeneous results, and none of the methods systematically improved the results.

Among the two sensitivity analyses performed, the correlation between the lower boundary of the confidence intervals of RORs and ORs resulted in the wider variation in p-values. Overall, in addition to the use of a correlation method robust to outliers, these methods could be used in order to test the robustness of the results.

In this study, despite significant correlation between ROR and OR, agreement was poor and in about half of cases the relative bias was extremely important. This finding is similar to the study conducted by Maciá-Martinez and colleagues (4) and should be related to the heterogeneity of the control group or to the preferential reporting of already reported ADRs. Thus, a ROR value cannot be used as a surrogate for an OR. However, surprisingly, the agreement between OR and ROR calculated in restricting the background to a homogeneous therapeutic area was good, and the robustness of this results remains to be further validated.

Importantly, this study highlighted the wide vibration of effect associated with disproportionality analyses. Considering the growing use of disproportionality analyses and the lack of standardized methods, this could lead to a major distortion of pharmacovigilance SDR. The patterns of vibration in disproportionality analyses according to ADR characteristics and to disproportionality measures is unknown. Moreover, the relationship between vibration of effect and the performances of SDR should be further explored.

This study suffers from several limitations. First, only one pharmacovigilance database was studied, we did not tested for other databases, such as national databases which may be more homogenous. On another hand, the WHO pharmacovigilance database has the advantage of being more representative and is larger. Furthermore, it should be interesting to

compare these results with some Bayesian estimates of disproportionality such as the Multi-item Gamma Poisson Shrinker or the Bayesian Confidence Propagation Neural network which probably significantly differ from frequentist methods. We selected only meta-analyses to extract the most accurate risk estimates. However, safety data from clinical trials are generally insufficient to assess drug-related risk. Indeed, the lack of power, the exclusion of patients with multiple comorbidities and short-term follow-up do not allow accurate identification of rare adverse effects (type B) and/or adverse effects with a long latency (type C). All ADRs in this study were type A (i.e. pharmacologically-expected and dose-related adverse effects), except suicide with antidepressants. However, pharmacovigilance databases are known to be mainly helpful in detecting type B adverse effects (i.e. effects that are often allergic or idiosyncratic reactions, characteristically occurring in only a minority of patients and usually unrelated to dosage and that are serious, unexpected and unpredictable), as well as unusual type A ADRs (40). Furthermore, drug dosage was not taken into account in ROR calculation. Lastly, in this study we mixed individual and collection of PTs to reflect the data used in the meta-analyses. The impact of the PT selection at a medical concept level or at individual level on the vibration of effect and on the disproportionality analyses performances remains to be further explored. It would be of interest to conduct this study with large epidemiological studies exploring other types and scarce ADRs which could be better correlated with pharmacovigilance data.

This study provides further evidence that effect sizes obtained from meta-analyses and from disproportionality analyses to assess drug related ADR risks sometimes correlate. However, there is large vibration of effect of disproportionality analyses, thus emphasizing the importance the presentation of a set of results across all possible methods, to avoid selective reporting. Further work is needed to understand the patterns of disproportionality analyses results vibration and the ADR characteristics influencing the correlation with ADR risk.

Author contributions

CK designed the research; CP extracted the data; CK analysed the data; all authors interpreted the data and wrote the manuscript.

Data Availability Statement

All data used for this study are available on demand to the corresponding author

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2. Conclusion et perspectives

L'étude que nous avons réalisée démontre la très grande vibration des résultats des analyses de disproportionnalité en fonction des designs utilisés. Ces choix méthodologiques impactent les résultats ainsi que les corrélations avec les risques d'effets indésirables estimés par des méta-analyses. Cette étude soulève donc de nombreuses questions :

- Sachant les limites des méta-analyses d'effet indésirables, quelle est la corrélation entre des études de pharmaco-épidémiologie et des analyses de disproportionnalité, notamment en étudiant des effets indésirables plus rares ?
- Quelle corrélation existe avec les autres métriques de disproportionnalité, notamment bayésiennes ?
- Quel est l'impact du type d'effet indésirable sur ces corrélations ?
- Des niveaux de vibration des résultats importants reflètent-ils de moins bonnes valeurs pronostiques positives et négatives des analyses de disproportionnalité ?

Nous tenterons d'apporter des réponses à ces questions à l'avenir par d'autres études. Nous envisageons, par exemple, de réaliser une étude sur les performances des différents designs, et sur la vibration de l'effet, en termes de sensibilité et spécificité à partir de tables tests comprenant des contrôles positive et négatifs comme celle développée par l'OMOP. (129)

Par ailleurs, cette étude a également mis en évidence la possibilité de publication sélective des résultats et de p-hacking dans le domaine de la pharmacovigilance. L'adaptation des méthodes de méta-recherche, le développement de méthodes standardisées et de transparence des résultats est un domaine à développer en pharmacovigilance.

PARTIE 6. DISCUSSION GENERALE

Ces différentes études nous ont donc permis d'identifier de nombreuses classes pharmacologiques potentiellement à l'origine d'une induction ou d'une aggravation d'un phénomène de Raynaud, de troubles trophiques ou d'une HTAP. Au sein de ces étiologies, peu d'entre elles peuvent être considérées comme certaines et des études confirmatoires et mécanistiques restent à mener. Par ailleurs, l'impact en santé publique de ces effets indésirables reste encore à étudier.

Les différentes étiologies à ce jour identifiées de façon certaine, probable ou possible sont résumées dans le tableau ci-dessous (Tableau 5).

Tableau 5. Résumé des étiologies iatrogènes des pathologies microvasculaires actuellement identifiées comme (A) certaine (EI démontré et risque quantifié) ; (B) probable (EI démontré) ; (C) possible (signal à confirmer).

Substances	Phénomène de Raynaud	HTAP	Troubles trophiques
Béta-bloquants	A		
Dérivés de l'ergot	B		
Sympathomimétiques	B		
Inhibiteurs recapture sérotonine	C		
Amphétaminiques	B	A	
Inhibiteurs de tyrosine kinase	C	A	A
Chimiothérapies alkylantes	A	B	
Antiangiogéniques (bevacizumab++)			A
Anti-métabolites (methotrexate, hydroxycarbamide)			A
Leflunomide		B	C
Ciclosporine	B		
Inhibiteurs mTOR			A
Interférons	B	B	B
Inhibiteurs cox 2			C
Antiviraux directs anti-hépatite C		B	
Bisphosphonates			C

Ce tableau met en évidence la possibilité d'atteintes microvasculaires généralisées par certains médicaments, ou au contraire d'atteintes spécifiques. Il paraît donc important d'étudier l'impact de chaque substance identifiée dans une pathologie sur les autres. Le parallèle fait dans ce travail entre ces 3 pathologies permet en effet de soulever des pistes sur de

potentielles étiologies iatrogènes encore non identifiées. Par exemple, quel est l'impact du leflunomide dans le phénomène de Raynaud, des bisphosphonates dans l'HTAP et le phénomène de Raynaud ou des amphétaminiques sur les troubles trophiques. Enfin, l'étude de cette hétérogénéité pourrait permettre de mieux comprendre les similarités et spécificités physiopathologiques de ces maladies.

Avenir de la détection et de l'évaluation de signaux en pharmacovigilance

L'avènement de méthodes statistiques puissantes associées à la possibilité d'exploiter de nouvelles sources de données permet d'entrevoir l'avenir de la détection de signaux de pharmacovigilance dans quelques années.

La combinaison de larges bases de données de pharmacologie permet de transposer les concepts de la biologie des systèmes à la pharmacologie et donc d'appréhender l'effet une molécule sur le système entier et non sur une cible spécifique. (130) De nombreuses bases de données comprenant des informations pharmacologiques sont, en effet, maintenant disponibles dans la littérature (Tableau 6). Ces approches ont déjà démontré leur capacité prédire la toxicité de nouvelles molécules mais aussi à générer des signaux de pharmacovigilance. (69,105)

Tableau 6. Bases de données en « open source » comprenant des informations moléculaires et pharmacologique Extrait de (Basile et al., Trends Pharmacol Sci. 2019) (108).

Name	Description
DrugBank	Bioinformatics and cheminformatics resource about drug data and drug target
SIDER	Database on marketed medicines and their recorded adverse drug reactions
ChEMBL	Manually curated database of bioactive molecules with drug-like properties
ChEBI	Freely available dictionary of molecular entities focused on 'small' chemical compounds
PubChem	Open chemistry database at the National Institutes of Health
Reactome	Curated and peer-reviewed pathway database
KEGG	Database resource for understanding high-level functions and utilities of the biological system, the organism and the ecosystem, from molecular-level information

Pour détecter des signaux de pharmacovigilance on pourra également s'appuyer, au-delà de la notification spontanée, sur des données provenant de l'analyse textuelle de forums et de réseaux sociaux mais aussi de tous les appareils connectés, montres, smartphones, lentilles digitales...



Figure 10. Nouvelles sources de données exploitables en pharmacovigilance. Extrait de (Spooner et al. 11th Stakeholder forum on the Pharmacovigilance legislation; 2017) (131)

L'analyse textuelle via des méthodes de « natural language processing » permettra d'extraire des informations de données non structurées comme les cas publiés dans la littérature, les dossiers médicaux ou les observations de pharmacovigilance. (132–134) Ces méthodes promettent également un remplissage automatisé des observations et des cas de pharmacovigilance. (135)

Enfin, les travaux actuels sur l'utilisation de modèles de données communs, l'amélioration des méthodes de pharmaco-épidémiologie, la création de plateformes permettant la fusion et

l'interrogation simultanées de plusieurs bases de données permettra une détection automatisée de signaux directement sur ces bases de données et leur évaluation rapide avec une très grande puissance. (136–139)

De nombreux travaux seront à réaliser pour définir la place et l'intérêt de chacune de ces méthodes par rapport aux autres, et notamment à la notification spontanée.

CONCLUSION

Pour conclure, ce travail de thèse nous a permis d'explorer le rôle de nombreux médicaments dans les pathologies micro-vasculaires, champs qui restait encore peu étudié dans la littérature. Ces travaux nous ont permis d'identifier plusieurs classes pharmacologiques dont le rôle était encore non décrit dans ces pathologies. De nombreux travaux restent à mener dans ce domaine afin de démontrer le lien causal entre certains médicaments et l'induction ou l'aggravation de pathologies microvasculaires, de quantifier le risque et l'impact de ces effets indésirables et d'en identifier le mécanisme. L'étude des mécanismes pharmacologiques à l'origine de ces effets indésirables permet également d'émettre de nouvelles hypothèses physiopathologiques à l'origine de ces maladies.

Les traitements utilisés dans ces différentes pathologies microvasculaires sont à l'heure actuelle encore peu efficaces et spécifiques. Des travaux de recherche important doivent encore être menés afin d'identifier de nouvelles pistes thérapeutiques et de personnaliser la prise en charge des patients.

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AUTRES TRAVAUX EN RELATION AVEC LA THESE

- 1. Targeting the Prostacyclin Pathway: Beyond Pulmonary Arterial Hypertension.**
- 2. Geographic Variations in Controlled Trials.**
- 3. The pathophysiological continuum of endothelial dysfunction across the spectrum of cardiometabolic health: a systematic review and network meta-analysis.**
- 4. French translation and linguistic validation of the Raynaud's Condition Score.**

Review

Targeting the Prostacyclin Pathway: Beyond Pulmonary Arterial Hypertension

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Pioneering work demonstrated that an unstable substance isolated from rabbit and pig aortas could relax arterial smooth muscle and inhibit platelet aggregation. Since then, prostacyclin (prostaglandin I₂, PGI₂) and its analogs have raised much pharmacological interest. In this review we detail how the PGI₂ signaling pathway is much more complex than was initially anticipated, involving peroxisome proliferator-activated receptors (PPARs), prostaglandin transporters (PGTs), and PGI₂-thromboxane A₂ (TXA₂) receptor (IP TP) heterodimerization. We discuss the distinct affinities of PGI₂ analogs for prostanoid receptors. In addition, we introduce the new direct and indirect pharmacological approaches to targeting the PGI₂ pathway within the systemic circulation, including non-prostanoid agonists of the prostacyclin receptor (IP) and PGT inhibitors, as well as transcutaneous pathways using iontophoresis and nanostructured lipid carriers.

Prostacyclin Pathway in Vascular Disease

The pioneering work of Moncada et al. in 1976 demonstrated that an unstable substance isolated from rabbit and pig aortas could relax arterial smooth muscle and inhibit platelet aggregation [1]. Since then, **prostacyclin** (PGI₂, see [Glossary](#)) and its analogs have mostly been used to treat pulmonary arterial hypertension (PAH). PGI₂ analogs induce a rapid decrease in pulmonary vascular resistance and increase cardiac output. The development of more stable and more selective drugs that target the PGI₂ receptor (IP receptor) in PAH has led to the recent development of non-**prostanoid** IP agonists [2].

The PGI₂ signaling pathway is now realized to be much more complex than was anticipated, and still remains incompletely elucidated. New findings, particularly concerns the **peroxisome proliferator-activated receptors** (PPARs), the importance of **prostaglandin** (PG) transporters (PGTs), and the interplay between the IP receptor and the thromboxane A₂ (TXA₂) receptor (TP receptor) through heterodimerization, and have also provided evidence of dysfunctional IP receptor variants. In addition to its vasodilator properties, PGI₂ can exert long-term effects such as promoting angiogenesis. The potent pharmacological properties of drugs that target the prostacyclin pathway have suggested new targets for the treatment of vascular diseases other than PAH [3–9].

This review discusses these pharmacological approaches to targeting the PGI₂ pathway within the systemic circulation as well as local targets [10–13].

Trends

PGI₂ is mainly synthesized by endothelial and vascular smooth muscle cells. It exerts a variety of pharmacological effects including platelet aggregation inhibition, vasodilation, inhibition of cell adhesion, and gastroprotection.

PGI₂ and some of its analogs are PPAR α and PPAR β/δ ligands.

PGI₂ may be a substrate for PGT and multidrug-resistance protein 4 (MRP4). Thus, inhibition of MRP4 and PGT could lead to higher PGI₂ concentrations.

Focusing development on greater specificity towards the IP receptor has led to the recent marketing of selexipag, a non-prostanoid agonist drug which metabolite MRE-269 has an increased IP receptor affinity.

Microsomal prostaglandin E synthase 1 inhibition may indirectly enhance PGI₂ synthesis.

IP receptor variants could cause an increased risk of cardiovascular events and lead to therapeutic failure with prostacyclin analogs.

PGI₂ analogs can be delivered to the skin through iontophoresis or nanostructured lipid carriers.

Genetically modified human mesenchymal stem cells can produce PGI₂.

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PGI₂ – A Regulator of Vascular Homeostasis

In vascular endothelial cells, PGs are major metabolites of arachidonic acid (AA). They are produced by **prostaglandin G/H synthases** (PTGS). They convert AA into prostaglandin H₂ (PGH₂) in a two-step process that combines cyclooxygenase activity and peroxidase activity. PTGS1 (otherwise known as COX-1) is constitutively expressed in most cells, whereas PTGS2 (COX-2) is expressed during oxidative stress or during the release of cytokines. The prostanoid precursor PGH₂ undergoes isomerization through the activity of PGI synthase (CYP8A1) to form PGI₂.

PGI₂ is mainly produced by endothelial and vascular smooth muscle cells [14,15], and exerts a variety of pharmacological effects including platelet inhibition, vasodilation, atheroprotection through the inhibition of cell adhesion, and gastroprotection. In addition, PGI₂ acts in synergy with another prostanoid, prostaglandin E₂ (PGE₂), in the mediation of pain and inflammation [15,16]. The two main signaling pathways underlying these effects operate via G protein-coupled receptors (GPCRs) and PPAR.

Prostanoids and GPCR-Mediated Effects

In the extracellular compartment, prostanoids bind to prostanoid receptors that belong to the GPCR family. GPCR is composed of seven transmembrane domains plus six intra- and extracellular loops. PGI₂ binds to several members of the GPCR family located on vascular smooth muscle cells. IP receptor activation causes vascular smooth muscle relaxation through G_s-protein-adenylyl cyclase signaling and increased cAMP concentrations [17,18] (Figure 1, Key Figure). Although the human IP receptor preferentially activates the G_{sα} subunit of the heterotrimeric G protein, it can also activate the G_{qα} subunit, leading to activation of phospholipase C [19]. The structure–function relationship between the sequences of the intracellular loops determines the specificities of G proteins for the IP receptors. Indeed, the second and third intracellular loops of the IP receptor are key elements in G_{sα} coupling, and some regions of specific intracellular loops can switch G protein specificity between the IP and TP receptors [20]. In addition, four distinct anchoring sites on the IP receptor transmembrane domain appear to be crucial for binding PGI₂ [21].

Activation of other prostanoid receptors, such as EP2, EP4 (PGE₂ receptors), and DP1 (prostaglandin D₂ [PGD₂] receptors) also leads to vasodilation through similar mechanisms. By contrast, the PGE₂ type 3 receptor (EP3) inhibits adenylyl cyclase via a G_i protein-dependent pathway [17,18,22]. Activation of the PGE₂ type 1 receptor (EP1) also induces vasoconstriction through G_q protein and activation of the PLC-β-IP₃-Ca²⁺ pathway. Of note, PGI₂ analogs

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Table 1. Affinity of Available Prostacyclin Analogs and Non-Prostanoid IP Agonists for Prostanoid Receptors.^a

Drug	Receptor					
	IP	EP1	EP2	EP3	EP4	DP1
Beraprost	+++	0	0	++	0	0
Prostacyclin/epoprostenol	++++	++	ND ^b	++++	0	ND
Iloprost	++++	++++	++++	++	++	+
Treprostinil	+++	++	++++	+	+	++++
Selexipag ^c	++	0	0	0	0	0
MRE-269 ^c	++++	0	0	0	0	+

^aKey: +++++, very high affinity; +++, high affinity; ++, low affinity; +, very low affinity; 0, no affinity.

^bND, no data available.

^cSelexipag is an active drug and is metabolized into MRE-269, a more active major metabolite.

activate the various types of PG receptors differently, thus explaining the differences in the pharmacological effects of these compounds (Table 1).

Another GPCR-mediated pathway that contributes to vasodilation involves the PGE₂ type 3 receptor subtypes, EP3C and EP4, that are found on local sensory nerves. Their stimulation by PGE₂ not only provokes sensory neuron sensitization but also increases the local release of calcitonin gene-related protein (CGRP) [23], a neuropeptide with potent vasodilatory activity. Thus, PGI₂ analogs that target EP3C and EP4 receptors may cause vasodilation via this pathway.

In addition to signaling through the monomeric IP receptor, the IP receptor can form active homodimers or heterodimers with the TXA₂ receptor [20]. In the latter case, the formation of an IP–TP heterodimer contributes to an IP-mediated shift of TP function to an IP-like function. This effect can be suppressed by elevated plasma cholesterol [24].

PPAR-Mediated Effects

PPAR receptors belong to the nuclear hormone receptor superfamily. There are three isoforms: PPAR α , PPAR β/δ , and PPAR γ . By heterodimerization with the retinoid X receptor (RXR) they control the expression of multiple genes. PPAR α mostly controls lipid metabolism but also plays a role in the suppression of stress-induced **apoptosis** of vascular smooth muscle cells [14]. PGI₂ and some of its analogs (such as iloprost and carbacyclin) bind directly to PPAR α and PPAR β/δ [14].

PPAR β/δ activation leads to vasodilation. This effect has been described in human umbilical vein endothelial cells (HUVECs) and has been, in part, explained by activation of **endothelial NO synthase** (eNOS), which may be related to the PI₃K–Akt–NOS pathway [25]. Moreover, PPAR β/δ controls endothelial cell apoptosis through various mechanisms, including the 14-3-3 ϵ pathway [14]. Drugs that target IP may also induce vasodilation through indirect synthesis of PGI₂, which activates PPAR β/δ and its heterodimerization with RXR. However, it remains unclear which receptor is intermediate [26].

PPAR is an important signaling pathway and explains part of the vasodilating effect of prostacyclin as well as its properties for cell cytoprotection. This ability of PGI₂ to bind to PPARs is also shared by some PGI₂ analogs used in therapeutics, such as treprostinil and carbacyclin. Cicaprost binds to PPAR γ through an IP receptor-dependent mechanism [27].

Other Effects of PGI₂ and PGE₂

Effects on angiogenesis and cellular protection have been reported, some of which involve vascular endothelial growth factor (VEGF)/NADPH oxidase 4 (NOX4) signaling. PGI₂ and PGE₂ regulate angiogenesis primarily through the receptors IP and EP4. Hoang et al. stimulated cell migration and tube formation in HUVEC, and these were suppressed by IP and EP4 antagonists [28]. In both PTGS2 knockout mice and IP-receptor knockout mice with experimental focal wounds in colonic mucosa, insufficient VEGF-dependent angiogenesis was observed [29]. *In vitro*, treprostinil enhanced VEGF-A synthesis by mesenchymal stem cells, potentiating endothelial colony-forming cells to develop a vessel-forming ability [30]. These data suggest that PGI₂ signaling is involved in activating VEGF-dependent angiogenesis. NOX4 has been recently identified as a pro-angiogenic factor and increases endothelial cell cytoprotection. NOX4 upregulation by the PGI₂ analog cicaprost *in vivo* and *in vitro* implicates the IP receptor–cAMP/protein kinase A (PKA)/response element-binding (CREB) pathway. Thus, triggering NOX4 expression with cicaprost improves the preservation and protection of endothelial cell function [9].

Glossary

Apoptosis: deletion of individual cells by fragmentation into membrane-bound particles which are phagocytosed by other cells.

Bioavailability: the proportion of the administered dose that is absorbed into the bloodstream.

Endothelium-derived hyperpolarization factors: diffusible factors causing smooth muscle hyperpolarization and thus vasodilation. It should be distinguished from the spread of hyperpolarizing current from the endothelium to the vascular smooth muscle, termed endothelium-dependent hyperpolarization.

Endothelial NO synthase (eNOS): an enzyme in endothelial cells that catalyzes the reaction of L-arginine with 2 O₂ and 1.5 NADPH to form NO, L-citrulline, 1.5 NADP⁺, and 2 H₂O.

Iontophoresis: method for transdermal drug delivery based on the transfer of charged molecules using a low-intensity electric current. It is non-invasive and has several advantages compared to passive transdermal administration, such as faster drug release and better control of the dose delivered. Factors involved in iontophoretic transfer include the concentration and the size of the molecule, the proportion ionized, the intensity of the current, whether it is continuous or discontinuous, and its duration. The nature of the skin surface (thickness, glabrous or not) and its integrity also play key roles.

Nanostructured lipid carriers: lipid particles produced by mixing solid and liquid lipids that become solid but do not crystallize, with dimensions of <100 nm.

Nitric oxide (NO): a gaseous mediator of cell-to-cell communication and a potent vasodilator formed from L-arginine in bone, brain, endothelium, granulocytes, pancreatic β cells, and peripheral nerves by constitutive NOS, and in hepatocytes, Kupffer cells, macrophages, and smooth muscle by inducible NOS. NO activates guanylate cyclase, mediates penile erection, and may be the first known retrograde neurotransmitter.

Paracrine: relating to a type of hormone function in which the

Furthermore, in a model of experimental autoimmune encephalomyelitis in mice, newly synthesized endothelial cell-derived PGI₂ (following inflammatory lesions) boosted corticospinal-tract fiber development and axonal refurbishing, whereas iloprost promoted axonal remodeling and motor recovery. These results could suggest a beneficial effect of PGI₂ on restoration of neuronal function after injury to the central nervous system [31].

The PGI₂ Pathway as a Target for Treating Endothelial Dysfunction

Together with **nitric oxide** (NO) and **endothelium-derived hyperpolarization factors**, the PGI₂ pathway is one of the three pathways responsible for endothelium-dependent vasodilation [1,32]. These mediators decrease intracellular calcium levels leading to vascular smooth muscle cell relaxation. PGI₂ inhibits platelet aggregation, whereas TXA₂ plays an opposing but crucial role in the vasculature. The PGI₂ pathway has been shown to be dysregulated in PAH [33], diabetes [34,35], atherosclerosis [36], and Raynaud's phenomenon [37]. In addition to its vasodilator properties, paradoxical vasoconstriction caused by PGI₂ acting through a TP receptor-dependent mechanism has been described *in vivo* in some vascular beds [38]. In pathological conditions, such as obesity and diabetes, Baretella et al. reported that PGI₂-related vasoconstriction was enhanced through an endothelin-1/thromboxane-dependent pathway, to the detriment of its vasodilatory effects [38]. Given that such an effect appears to be mostly absent when PGI₂ or its analogs are administered exogenously, from a therapeutic perspective the consequences of this observation are probably minimal.

Administration of PGI₂ has long been thought of as a therapeutic strategy to restore the vasodilation/vasoconstriction balance. Prostacyclin analogs have been the gold standard for PAH since the 1990s. This disease is characterized by excessive remodeling and tightening of the pulmonary arteries, triggering pulmonary vascular resistance that can ultimately lead to right ventricular failure, a drop in cardiac index, and death. Infusion of prostacyclin analogs decreases pulmonary vascular resistance and right atrial pressure, increases the cardiac index, and leads to longer survival [39]. Prostacyclin in PAH also preserves endothelial cell function through the PPAR pathway [22]. However, because synthetic PGI₂ (also known as epoprostenol) is unstable, continuous intravenous administration is required. Thus, subsequent adverse effects, together with invasive and cumbersome delivery systems, limit its therapeutic use. Drug development has therefore focused on improving the pharmacokinetic and pharmacodynamic profiles of PGI₂ analogs.

Selexipag, a non-prostanoid agonist of the IP receptor, was recently authorized for use to treat PAH [2] (Table 1). Other than PAH, iloprost is the only prostacyclin analog approved for vascular disease. Indeed, iloprost is indicated for severe peripheral ischemia and severe Raynaud's phenomenon [40]. The recent advances in understanding the cellular mechanisms of PGI₂ (described above), together with the innovative formulations and new routes of administration, have opened up new therapeutic perspectives for other vascular diseases.

Towards New Strategies for Targeting the IP Receptor

Since the marketing of epoprostenol, several analogs have been developed. Table 2 shows the different prostacyclin analogs authorized for PAH. The different approaches initially consisted of improving the stability of prostacyclin analogs, leading to the development of subcutaneous treprostinil, which has a prolonged half-life, and epoprostenol thermostable salt. Thereafter, inhaled (iloprost, treprostinil) and oral (selexipag, beraprost) forms were developed to avoid the need for continuous and cumbersome delivery systems. These drugs also differ in terms of specificity towards IP and EP receptors (Table 1), which explains their different pharmacodynamic profiles. EP and DP receptors are involved in inflammation and immunity [41], and this effect may counterbalance the beneficial effect obtained in vascular tone [42].

effects of the hormone are restricted to the local environment.

Peroxisome proliferator-activated receptors (PPARs): a family of transcription factors that recognize response elements in the promoters of their target genes. Three main isoforms exist (PPAR α , PPAR β/δ , PPAR γ) and may be activated by a wide variety of endogenous or exogenous ligands.

Prostacyclin (PGI₂): also known as prostaglandin I₂, the molecule has a short half-life (4 minutes) and is produced by the endothelium. It acts as a physiological antagonist of TXA₂. Epoprostenol is a synthetic PGI₂ molecule used as a drug for pulmonary arterial hypertension (PAH).

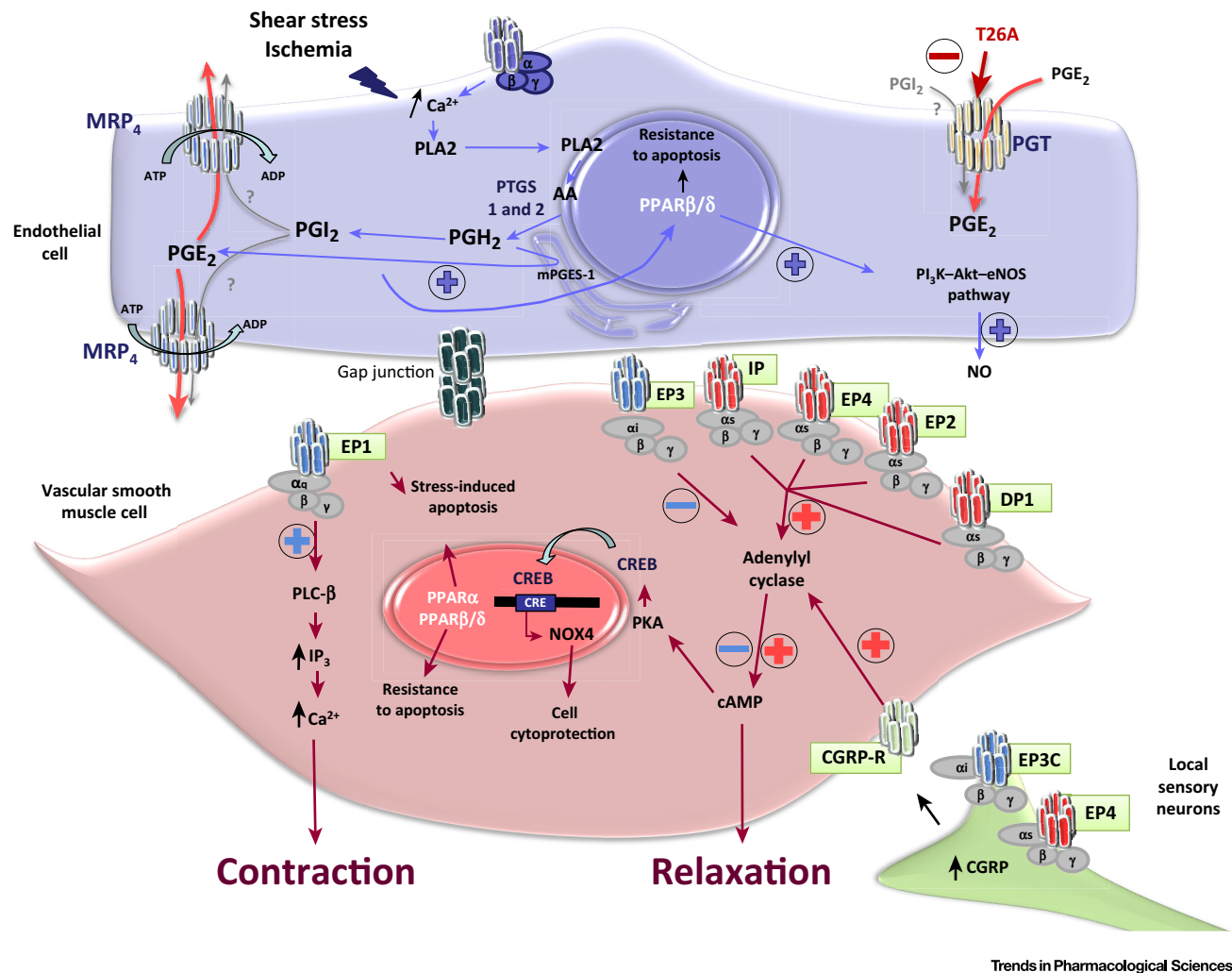
Prostaglandins (PGs): physiologically active and ubiquitously produced lipid compounds derived from fatty acids; contain 20 carbon atoms including a five-carbon ring.

Prostaglandin G/H synthase (PTGS): a key enzyme in PG biosynthesis that converts arachidonic acid into PGH₂ in a two-step process that combines endoperoxide activity and peroxidase activity.

Prostanoids: cyclic lipid mediators that arise from enzymatic cyclo-oxygenation of linear polyunsaturated fatty acids. Active prostanoids derived from arachidonic acid (AA) include PGs and thromboxane A₂ (TXA₂).

Key Figure

Prostacyclin Pathways in the Systemic Circulation



Trends in Pharmacological Sciences

Figure 1. G protein-coupled receptors (GPCRs) are the primary targets of prostacyclin (PGI₂) and its analogs. By activating adenylyl cyclase, agonism of IP, EP2, EP4, and DP1 leads to vasodilation, in contrast to EP1 agonism which causes vasoconstriction through a variation in cAMP levels. In addition, both PPARα and β/δ can be activated by PGI₂ analogs or IP agonists. PPARβ/δ activation induces vasodilation through the PI3K-Akt-eNOS pathway, and confers resistance to apoptosis. PPARα prevents stress-induced apoptosis. It remains unclear whether PGI₂ analogs or agonists can directly activate PPARα and β/δ, or indirectly affect PGI₂ production. The prostaglandin transporter (PGT) is involved in PGI₂ clearance. Multidrug-resistance protein 4 (MRP4), also expressed on vascular smooth muscle cells, has a role in prostaglandin E₂ (PGE₂) cell efflux, although its part in PGI₂ export remains hypothetical. EP3C and EP4 activation of local sensory nerves increases CGRP production. In this schema, the signal transduction pathways are simplified for clarity, but the human IP receptor can couple to G_q in addition to G_s, and can form IP-TP heterodimers that contribute to an IP-mediated shift in TP function. Symbols: ↑, increase; + activation; - inhibition.

Development that has focused on greater specificity towards the IP receptor and the formulation of oral pharmaceuticals has led to the recent marketing of selexipag, a non-prostanoid agonist with increased IP specificity [2]. Its advantage is that it avoids infusion-delivery systems, which adversely impact on quality of life. Selexipag is metabolized into its more active metabolite, MRE-269, which has a high affinity for the IP-receptor and a weak affinity for the DP receptor. However, whether drug development should focus on IP receptor selectivity is

Table 2. Pharmacokinetics, Side Effects, and Disadvantages of Prostacyclin Analogs and IP Agonists in the Treatment of Vascular Diseases.

Molecule	Route of administration	Therapeutic indication	Disadvantages in vascular disease	Pharmacokinetics	Side effects
Beraprost	Oral	Peripheral vascular disorders, PAH (Japan, South Korea)		<i>F</i> : NA	Common: hypotension, flushing, headache, nausea and vomiting
				$t_{1/2}$: 1 h	Serious: hemorrhage
Epoprostenol sodium	Intravenous ^b	PAH	Systemic side effects	$t_{1/2}$: 3–5 minutes	Common: arrhythmia, hypotension, flushing, headache, dizziness, diarrhea, nausea and vomiting,
		Renal dialysis	Cumbersome delivery systems: impact on quality of life	Metabolism: inactive plasma metabolite	arthralgia, jaw pain, musculoskeletal pain
				Excretion: renal	Serious: hemorrhage, hypersplenism, splenomegaly
Iloprost	Intravenous	Severe chronic limb ischemia	Systemic side effects	$t_{1/2}$: 20–30 minutes	Common: hypotension, flushing, headache, nausea, trismus
		Severe Raynaud's phenomena with evolving trophic disorders	Cumbersome delivery systems: impact on quality of life	Metabolism: hepatic	
				Inactive metabolite	
				Excretion: renal/biliary	
Iloprost	Inhalation	PAH	Route of administration not exploitable for vascular diseases	<i>F</i> : 10–20%	Common: cough
				$t_{1/2}$: 20–30 minutes	Serious: bronchospasm
				Metabolism: inactive hepatic metabolite	
				Excretion: renal/biliary	
Selexipag	Oral	PAH	Modification of absorption with food	<i>F</i> : 49%	Common: headache, diarrhea, nausea, jaw pain
			Systemic side effects	$t_{1/2}$: 0.8–2.5 h	
				Metabolite $t_{1/2}$: 6.2–13.5 h	
				Metabolism: hepatic active metabolite	
				Excretion: biliary	
Treprostinil	Subcutaneous and intravenous	PAH	Systemic side effects	$t_{1/2}$: 4 h	Common: flushing, headache, dizziness, diarrhea, nausea, injection site pain, rash
			Painful subcutaneous injection	Metabolism: hepatic inactive metabolite	Serious: hemorrhage, hemoptysis
			Cumbersome delivery system or subcutaneous injection: impact on quality of life	Excretion: renal/biliary	
Treprostinil	Inhalation	PAH	Route of administration not exploitable for vascular diseases	<i>F</i> : 64–72%	Common: flushing, headache, nausea, cough, throat irritation
				$t_{1/2}$: 4 h	
				Metabolism: inactive hepatic metabolite	
				Excretion: renal/biliary	

Table 2. (continued)

Molecule	Route of administration	Therapeutic indication	Disadvantages in vascular disease	Pharmacokinetics	Side effects
Treprostinil	Oral	PAH	Modification to absorption with food	<i>F</i> : 17%	Common: headache, diarrhea, nausea
				<i>t</i> _{1/2} : 4 h	
				Metabolism: inactive hepatic metabolite	
				Excretion: renal/biliary	

^aAbbreviations: *F*, bioavailability; NA, not available; PAH, pulmonary arterial hypertension; *t*_{1/2}, half-life.

^bEpoprostenol sodium is available with mannitol/glycine excipients or thermostable arginine/sucrose excipients.

debated, particularly because there is recent evidence that the PGI₂-IP interaction itself has proinflammatory properties – that J. Stitham has aptly termed the ‘prostacyclin inflammatory paradox’ [43].

New compounds are currently being investigated to enhance pro-angiogenic activity, such as ONO-1301, a novel PGI₂ receptor agonist that also has inhibitory activity on thromboxane synthase. In a murine sponge model, injected ONO-1301 stimulated angiogenesis by enhancing hepatocyte growth factor (HGF) and VEGF synthesis, and increased cAMP levels through IP receptor agonism, as well as having a long-acting effect [44]. ONO-1301 has been tested and injected subcutaneously into rats with ischemia/reperfusion injury. Cardiac function was improved as was angiogenesis, which was detected by HGF synthesis [8]. In a murine, obese, type 2 diabetic model with nephropathy, subcutaneously injected ONO-1301 reduced the urinary albumin/creatinine ratio, glomerular hypertrophy, and cellular infiltration, suggesting its potential usefulness in renal disease [45].

In addition to new compounds that target PG receptors, other innovative approaches include the identification of new cellular targets involved in the metabolism of PGI₂, which enhance its **bioavailability** within endothelial cells. An emerging alternative strategy in vascular diseases (except for PAH) is the local administration of PGI₂ analogs to avoid the side effects of systemic therapies.

New Cellular Targets

Prostaglandin Transporter

PGT, also known as the solute-carrier organic anion 2A1 transporter (SLCO2A1/OATP2A1), is an antiporter that plays a major role in PG influx in exchange for lactate [46]. It is expressed by many human tissues [47] and, under shear-stress conditions, PGT is upregulated in HUVEC and human vascular endothelial cells [48,49]. A study on its affinity for the various PGs found in HeLa cell monolayers showed high affinities for prostaglandin E₁ (PGE₁) and PGE₂ > thromboxane B₂ (TXB₂) > 6-keto-prostaglandin F_{1α} (6-keto-PGF_{1α}) (an inactive PGI₂ metabolite). By contrast, TXA₂ was not a substrate for PGT. There are no data for PGI₂ transport by PGT (because of its *in vitro* instability) or for the PGI₂ analogs. Limited data suggest that iloprost is not significantly transported by PGT [47,50,51].

In normotensive anesthetized rats, intravenous T26A, a PGT inhibitor, increased extracellular PGE₂ concentrations [52]. Oral T26A increased PGE₂ urinary excretion and PGE₂ plasma concentration in hypertensive rats and mice. It also heightened urinary sodium excretion, inhibited serotonin-induced vasoconstriction, and potentiated vasodilation induced by exogenous PGE₂ [3]. Syeda et al. demonstrated that PGT expression is increased in human dermal

microvascular endothelial cells (HDMCs) when exposed to hyperglycemic conditions or *in vivo* by diabetes and during the initiation of wound healing. This decreases PGE₂ levels and angiogenesis, thus impairing wound healing [53]. Interestingly, intravenous and topical T26A accelerated wound healing in non-diabetic and streptozotocin-induced diabetic rats [4].

Multidrug-Resistance Protein 4 (MRP4)

Once synthesized, PGI₂ and other PGs exit the cell through several mechanisms that are not yet fully understood. In addition to passive diffusion through the lipid bilayer, attention has been drawn to efflux through MRP4 [54]. MRP4 is an ATP-binding cassette (ABC) transporter that is localized both on endothelial and vascular smooth muscle cell membranes [55,56]. MRP4 is non-specific, being involved in the transport of several other endogenous mediators such as lipid mediators, glutathione, and amphiphilic anions [54,57], as well as exogenous compounds (e.g., ganciclovir, 6-mercaptopurine) [56]. PGE₁, PGE₂, prostaglandin F_{1α} (PGF_{1α}), prostaglandin F_{2α} (PGF_{2α}), and TXB₂ are transported by MRP4, whereas PGF_{1α}, PGF_{2α}, and TXB₂ are MRP4 inhibitors [54,55]. Given that PGI₂ has a short half-life, MRP4 involvement in PGI₂ efflux remains likely but currently remains hypothetical.

Although PAH is outside the scope of this review, it is interesting to note the MRP4 is highly expressed in the pulmonary arteries of patients with idiopathic PAH. *In vitro*, MRP4 inhibition has been associated with decreased cell proliferation and migration, and *in vivo* with reversion of hypoxia-induced pulmonary hypertension in mice [58]. In a murine model of type 2 diabetes and obesity, MRP4 was overexpressed in kidney and liver [59]. MRP4 inhibition was associated with higher cAMP levels in platelets and coronary artery vascular smooth muscle cells. Given that raised cAMP level is a negative signal for platelet aggregation and enhances vasodilation, MRP4 inhibition may be a potential therapeutic strategy for cardiovascular disease [60].

Microsomal Prostaglandin-E Synthase-1 (mPGES-1)

mPGES-1 is the major enzyme involved in PGE₂ synthesis. Two mechanisms are affected when this enzyme is inhibited or deleted: first, PGE₂ production decreases and, second, PGH₂ accumulates and becomes available in higher concentrations to be transformed to PGI₂ through PGI synthase. In a recent report substrate diversion to PGI₂ and subsequent IP activation was shown to limit thrombogenesis, while reduced PGE₂ levels led to restricted atherogenesis [5]. Therefore, mPGES-1 inhibition may provide a new strategy to counter the thrombotic complications of nonsteroidal anti-inflammatory drugs and contribute towards improving cardiovascular efficacy.

Dysfunctional IP Receptor Variants

The IP and TP receptors are concomitantly present in smooth muscle cells. IP and TP can form homo- and heterodimers, and heterodimerization of IP with TP shifts the latter to signal via the IP–G_s pathway, with subsequent cAMP generation, whereas IP counters the activity of TXA₂ [7]. A rare genetic variant, IP^{R212C}, leads to impaired IP signaling when it dimerizes with wild-type IP or TPα [7]. Alteration in a helical interaction motif the fifth transmembrane domain of TPα prevents TPα homodimerization and protein G_q-induced signaling. More interestingly, this alteration does not modify IPTPα heterodimerization or protein G_s-induced signaling. Targeting this region of the TPα receptor enhances indirect IP–TPα heterodimerization through the suppression of TPα homodimerization [6].

Genetic variants of the IP receptor are known and these can alter prostacyclin binding and subsequent G-protein activation. The IP^{R212H} variant (located in the third intracellular loop) exhibited abnormal activation at both pH 7.4 and under stress conditions (pH 6.8), whereas a significant decrease in binding affinity was observed only at pH 6.8 [61]. Likewise, IP^{R77} and IP^{R279} exhibited deficiencies in binding, activation, or expression [62].

To date, 18 rare non-synonymous mutations have been identified by Stitham et al. Of these, eight were associated with a greater risk of coronary artery obstruction in patients who had undergone a coronary angiograph. These eight mutations exhibited abnormal binding, activation, and protein stability/folding of the IP receptor [63].

Thus, these rare dysfunctional IP receptor variants could increase the risk of a cardiovascular event and decrease the efficacy of prostacyclin analogs, factors that need to be studied further.

Local Therapy and New Formulations

PGs have long been used as localized treatments in obstetric gynecology for their contractile effects on the uterine lining [64] or as topical treatments for glaucoma [65]. More recently, other methods of local delivery have been proposed such as **iontophoresis**. This consists of applying a drug-impregnated patch or gel to the skin, and drug delivery into the dermis by electro-repulsion or electro-osmosis is promoted by the application of a low-intensity electric current. The PGI₂ analog, treprostinil, is a good candidate for route of administration because it has a low molecular weight and is negatively charged at neutral pH. Cathodal iontophoresis of treprostinil and iloprost have led to increased cutaneous blood flow in rats, with good local tolerance [66]. Similarly, treprostinil iontophoresis has been reported to increase skin blood flow in the forearm, finger pad, and leg without local side effects in healthy subjects, and in patients with diabetes or scleroderma [10]. Treprostinil was detected locally in the derma at up to 8 h after iontophoresis, and its systemic diffusion was limited [11]. Likewise, treprostinil iontophoresis was safely used to locally modulate the PGI₂ pathway in patients with PAH [33]. Prostacyclin holds promise in the treatment of microvascular skin ulcerations (Box 1), and Phase II trials of prostacyclin iontophoresis are currently planned in this indication.

A highly innovative cell therapy to deliver PGI₂ locally has been recently described [13]. This uses genetically modified human mesenchymal stem cells (hMSCs) to introduce a triple catalytic enzyme that produces PGI₂ (PGI₂-hMSCs). When injected into a mouse hindlimb ischemia model, PGI₂-hMSCs improved perfusion and muscle function compared to control hMSCs or iloprost alone [13]. In a **paracrine** manner, PGI₂-hMSC delivery upregulated long non-coding RNA H19,

Box 1. Prostacyclin as a Local Treatment for Microvascular Skin Ulcers

Skin ulcers are hard-to-heal damaged cutaneous areas that may be associated with microvascular dysfunction in patients with diabetes or systemic sclerosis. They require thorough treatment to avoid serious complications, such as infections and diminished quality-of-life with disabilities. Systemic sclerosis (SSc) is a rare disease characterized by vasculopathy and fibrosis. Microvascular dysfunction is an early feature of the pathophysiology of SSc [69] and is associated with decreased endothelium-dependent vasodilation [70] and abnormal neurovascular microcirculatory responses [71]. Digital ulcers (DUs) represent the major complication of SSc vasculopathy [72]. Iloprost, a prostacyclin (PGI₂) analog, is the only recommended prostacyclin analog for the treatment of active SSc-related DUs [73].

There is growing interest in the treatment of SSc-related ulcers with locally delivered PGI₂ analogs via vascular and non-vascular effects. Indeed, PGI₂ has been shown to play a key role in tissue repair through VEGF-dependent enhancement of angiogenesis [29]. Although there has been little interest in the non-vascular effects of PGI₂, these could also play a key role in wound healing. First, PGI₂ has been shown to promote cell migration in a wound model of cultured human fibroblasts [74]. Moreover, iloprost reduced skin tightness in patients with SSc by blocking the induction of connective tissue growth factor (CTGF) and increased collagen synthesis in fibroblasts exposed to TGF-β [75]. This finding is particularly interesting because CTGF is a biomarker of the extent of skin disease in patients with diffuse cutaneous SSc [76]. Iloprost has been recently and consistently shown to reduce collagen deposition and procollagen expression in the right ventricle, and was associated with a reduction in CTGF mRNA and protein levels. This antifibrotic effect was mediated through reduced rhTGF-β1-induced fibroblast activation and migration, and increased gene expression and activity of MMP-9 [77]. Such properties may also be beneficial in other types of microvascular wounds such as diabetes-related ulcers. Increasing the local concentration of vasodilatory prostaglandins (PGE₂ and PGI₂) by inhibiting PGT, which is involved in prostaglandin clearance, resulting in improved re-epithelialization and accelerated wound healing in non-diabetic and diabetic rats [4,38].

which is involved in cell proliferation in progenitor cells under hypoxic conditions. It remains to be determined whether long-term effects can be achieved using PGI₂-hMSCs.

Another exciting perspective for local delivery includes **nanostuctured lipid carriers** that have been developed during the past decade and exploit the concept of using the skin as a site for particle delivery, particularly in the context of skin damage. To stimulate encapsulation of cationic lipids, stearylamine or 1,2-di-(9Z-octadecenoyl)-3-trimethylammonium-propane is incorporated into drugs that contain liposomes. Enhanced vasorelaxation of murine pulmonary arteries was observed at half the usual non-encapsulated concentrations of iloprost [12]. Other groups are currently working on nano-encapsulation of PGI₂ analogs to target the systemic circulation.

Poly(lactide-co-glycolide) microspheres that encapsulate ONO-1301 have been developed to obtain slow-release properties. The stability of microspheres containing antioxidants were compared; 10% butylated hydroxytoluene (BHT) gave consistently better stability and a higher area under the curve after subcutaneous injections in rats compared to microspheres without BHT. This formulation also showed better efficacy in an angiogenesis murine sponge model [67]. ONO-1301 has also been developed and tested *per os* in rats [68].

Concluding Remarks

This review has described the latest advances in targeting the PGI₂ pathway in vascular disease beyond PAH. This pathway involves many complex factors, for example, the multiplicity of activated prostaglandin receptors, the difficulties of intravenous delivery, and the systemic side effects. These were, in fact, the starting points to identify new targets and new methods of drug delivery, and now include the development of pharmacogenetic studies and precision medicines (see Outstanding Questions). These advances are encouraging and demonstrate that targeting the PGI₂ pathway is a promising approach to the treatment of vascular disease.

The next step will be to prove that these new insights are both safe and efficient *in vivo*, while also taking into account the wide complexity of the PGI₂ pathway. The 'old' PGI₂ pathway may not yet have fully revealed its potential therapeutics.

Conflict of interest statement

M.R. and J.-L.C. have received research grants from Pfizer, GlaxoSmithKline, and Bioprojet for other studies. M.R., J.-L.C. and S.B., through Grenoble Alps University, hold a patent of a topical formulation of treprostinil for wound healing.

Acknowledgments

We thank Alison Foote (Grenoble Alps University Hospital) and Susanna Lyle for correcting the English.

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Outstanding Questions

Is binding of PGI₂ to PPAR α or PPAR β/δ a major pathway for the vascular effects of PGI₂? Should we attempt to specifically target these pathways?

Inhibition of PGT or MRP4 has been identified as a new target to modify PGE₂ and PGI₂ concentrations. What beneficial effects can we expect in human disease? Could this approach be specific?

Can mPGES-1 inhibition switch the thromboxane/PGI₂ risk/benefit ratio to favor the PGI₂ pathway?

How can we favor heterodimerization of IP receptors with TP receptors?

Can IP receptor variants be sequenced to avoid the therapeutic failure of PGI₂ analogs?

Can iontophoresis of PGI₂ analogs or nanostuctured lipid carriers for skin delivery of PGI₂ analogs be used to treat microvascular diseases of the skin?

What is the potential of genetically modified human mesenchymal stem cells that produce PGI₂?

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Geographic Variations in Controlled Trials

TO THE EDITOR: In their exploration of multinational clinical trial analysis, Yusuf and Wittes (Dec. 8 issue)¹ appropriately emphasize the necessity of distinguishing true heterogeneity across countries from chance variation. We wish to point out additional limitations of testing for country-level heterogeneity.

A positive heterogeneity test could be driven by especially low efficacy (i.e., harm) in some countries rather than by high efficacy in others. Trialists should consider supplementing such tests with shrinkage estimation analysis, a statistical tool that refines subgroup estimates with the use of data beyond the subgroup. This technique provides more accurate estimates of efficacy in subgroups (with confidence intervals) by pulling subgroup findings toward the overall mean in proportion to the uncertainty underlying the results in that subgroup.^{2,3}

Even when the estimate for one country indicates unusually high efficacy and chance is not the cause, the benefits may not materialize if the trial intervention is implemented nationally, because study sites are only a small nonrandom sample within the country. The study sites may not represent the care system, study population, enrollment practices, and other elements in the country more broadly. Trialists should proceed with caution.

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No potential conflict of interest relevant to this letter was reported.

1. Yusuf S, Wittes J. Interpreting geographic variations in results of randomized, controlled trials. *N Engl J Med* 2016;375:2263-71.
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DOI: 10.1056/NEJMc1700529

TO THE EDITOR: Yusuf and Wittes report examples of regional differences in the results of trials and provide interpretation regarding whether such differences are likely to be due to chance. A recent and striking example of such regional differences concerns the cardiovascular safety of glucagon-like peptide 1 (GLP-1) analogues and inhibitors of sodium–glucose cotransporter 2 (SGLT2). Overall, trials evaluating these drugs have shown a benefit with regard to cardiovascular outcomes, yet a subgroup meta-analysis from four trials¹⁻⁴ that enrolled 25,725 patients reveals significant differences in cardiovascular outcomes according to region (Fig. 1). The global effect size is driven by Latin America, Africa, and Asia, whereas the effects in Europe and North America are extremely small or nonexistent. How should we interpret these variations?

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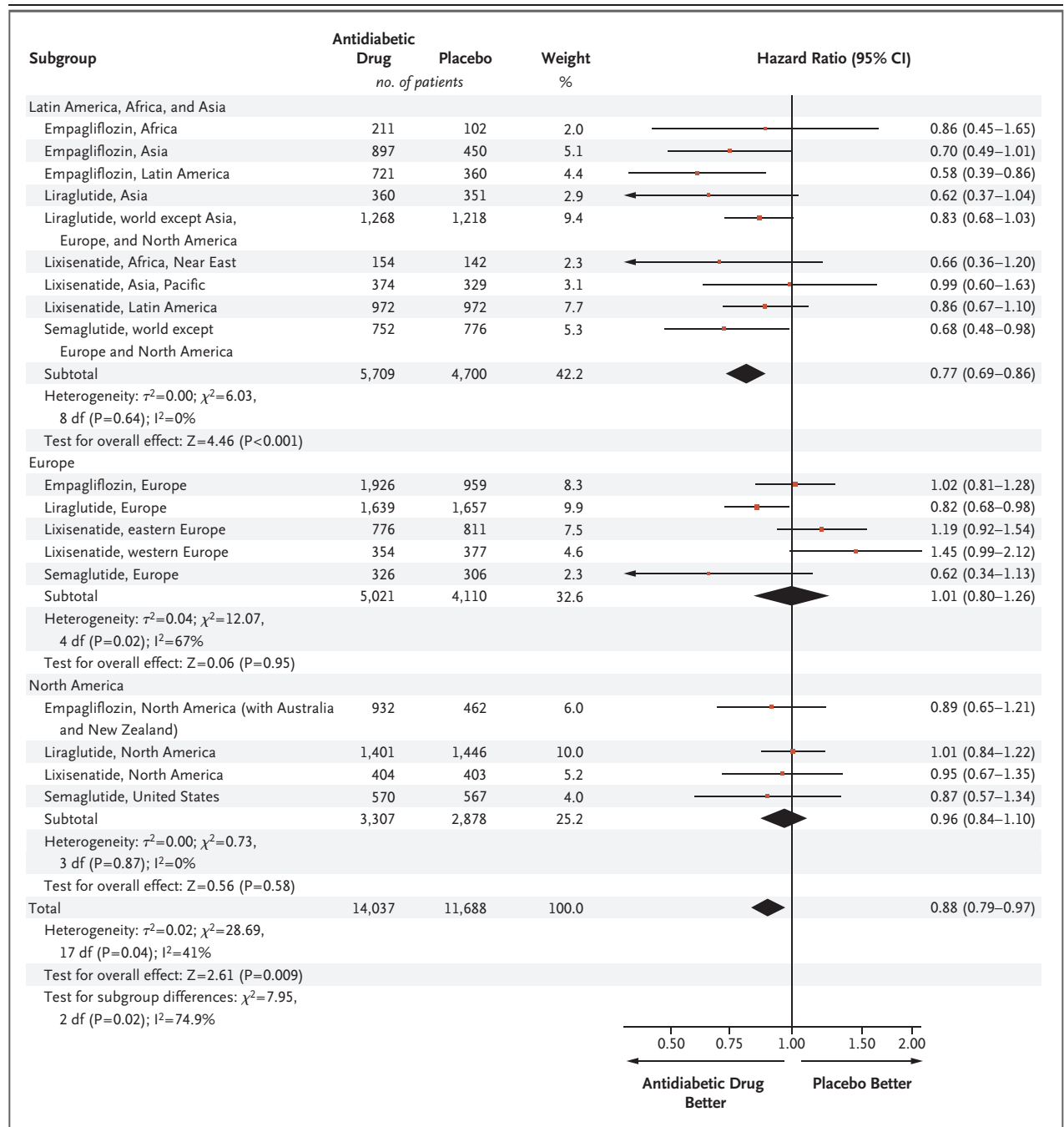
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Figure 1 (facing page). Forest Plot of an Inverse Variance Random Effect Meta-Analysis of Trials That Assessed GLP-1 Analogues or SGLT-2 Inhibitors in Patients with Type 2 Diabetes.

The trials included in the meta-analysis evaluated glucagon-like peptide 1 (GLP-1) analogues and inhibitors of sodium–glucose cotransporter 2 (SGLT2) in patients with type 2 diabetes who were receiving treatment with a standard-of-care regimen. The primary outcome was cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke. Subgroups were based on geographic region and included North America; Europe; and Latin America, Africa, and Asia; only studies from which regional data were available were included. Empagliflozin data are from Zinman et al.,¹ lixisenatide data are from Pfeffer et al.,² liraglutide data are from Marso et al.,³ and semaglutide data are from Marso et al.⁴ RevMan software, version 5.3 (Cochrane Collaboration), was used for analysis. CI denotes confidence interval.



No potential conflict of interest relevant to this letter was reported.

- Zinman B, Wanner C, Lachin JM, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med* 2015;373:2117-28.
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DOI: 10.1056/NEJMc1700529

THE AUTHORS REPLY: We agree with the recommendations from Schwartz and Friedman about the usefulness of shrinkage estimates to assess the effects of treatments in subgroups. The broader issue of the expected effect from implementing the results of an intervention proven to be effective in specific countries within a trial will depend on a large number of factors that go beyond interpretation of subgroup results within trials, including some that Schwartz and Friedman have raised.

Roustit et al. provide an example of an apparent benefit with GLP-1 analogues and SGLT2 inhibitors being confined to patients enrolled from Africa, Asia, and Latin America but not from Europe or North America. In our view, this is probably due to chance. First, Asians, Latin Americans, and Africans are highly heterogeneous in their genetics, lifestyles, and risks of diabetes, and so there is no biologic rationale for putting them into a single group. The decision to group them for this analysis was probably data-derived. Second, we know of no rationale for combining GLP-1 analogues or SGLT2 inhibitors, since their mechanisms of action are quite different from one another. Third, some trials of dipeptidyl peptidase 4 inhibitors have not reported such results.¹ Inclusion of the results from these trials may negate the apparent interaction according to region that was presented by Roustit et al.

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Since publication of their article, the authors report no further potential conflict of interest.

1. Green JB, Bethel MA, Armstrong PW, et al. Effect of sitagliptin on cardiovascular outcomes in type 2 diabetes. *N Engl J Med* 2015;373:232-42.

DOI: 10.1056/NEJMc1700529

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The continuums of impairment in vascular reactivity across the spectrum of cardiometabolic health: A systematic review and network meta-analysis

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Funding information

Australian Government Research Training Program Scholarship; National Health and Medical Research Council of Australia Dora Lush Biomedical Research Postgraduate Scholarship, Grant/Award Number: 114350

Summary

This study aimed to assess, for the first time, the change in vascular reactivity across the full spectrum of cardiometabolic health. Systematic searches were conducted in MEDLINE and EMBASE databases from their inception to March 13, 2017, including studies that assessed basal vascular reactivity in two or more of the following health groups (aged ≥ 18 years old): healthy, overweight, obesity, impaired glucose tolerance, metabolic syndrome, or type 2 diabetes with or without complications. Direct and indirect comparisons of vascular reactivity were combined using a network meta-analysis. Comparing data from 193 articles (7226 healthy subjects and 19344 patients), the network meta-analyses revealed a progressive impairment in vascular reactivity (flow-mediated dilation data) from the clinical onset of an overweight status (-0.41% , 95% CI, -0.98 to 0.15) through to the development of vascular complications in those with type 2 diabetes (-4.26% , 95% CI, -4.97 to -3.54). Meta-regressions revealed that for every 1 mmol/l increase in fasting blood glucose concentration, flow-mediated dilation decreased by 0.52%. Acknowledging that the time course of disease may vary between patients, this study demonstrates multiple continuums of vascular dysfunction where the severity of impairment in vascular reactivity progressively increases throughout the pathogenesis of obesity and/or insulin resistance, providing information that is important to enhancing the timing and effectiveness of strategies that aim to improve cardiovascular outcomes.

KEYWORDS

endothelial dysfunction, insulin resistance, obesity, vascular function

1 | INTRODUCTION

It is well established that endothelial dysfunction is an early predictor of cardiovascular events in at-risk patients.¹⁻³ Impairments in vascular reactivity, stemming from such endothelial dysfunction, as well as

possible disruptions to endothelium-independent activity and maladaptation to the vascular smooth muscle, increase susceptibility to endothelial injury and, thus, promote atherosclerotic change. Furthermore, impaired vascular reactivity may also contribute to the development of obesity and insulin resistance, in what may, indeed, be considered a

ABBREVIATIONS: CVD, cardiovascular disease; FMD, flow-mediated dilation; GRADE, Grading of Recommendations Assessment, Development and Evaluation; NMD, nitrate-mediated dilation; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-analyses; SAQOR, systematic assessment of quality for observational research; SMD, standardized mean difference

vicious cycle where each compounds the other.⁴⁻⁶ It is therefore important from a clinical perspective that the interaction between cardiometabolic health and vascular reactivity is fully understood to enhance the timing and, subsequently, the effectiveness of treatment strategies that aim to improve vascular health and cardiovascular disease (CVD) outcomes.

Currently, several cross-sectional studies provide evidence that, in comparison with healthy controls, vascular reactivity is significantly impaired early in the development of obesity and/or insulin resistance.⁷⁻¹⁶ Although such data may suggest that vascular dysfunction precedes the development of overt disease, many cross-sectional studies often lack the power to accurately estimate the effect size of impairment between groups. Moreover, given that no single study has compared vascular reactivity across the full spectrum of cardiometabolic health, whether there is a continuum in the impairment of macrovascular and microvascular reactivity between early stages and diabetic complications is yet to be properly addressed.

Therefore, considering the large number of vascular studies that have been conducted, the primary objective of this present research is to combine direct and indirect comparisons of vascular reactivity in a network meta-analysis to test this hypothesis and, subsequently, further understand the development of vascular dysfunction.

2 | MATERIAL AND METHODS

The protocol for this systematic review and network meta-analysis was registered on PROSPERO (registration number: CRD42017053411) and is available in full on the National Institute for Health Research International Prospective Register of Systematic Reviews (<https://www.crd.york.ac.uk/PROSPERO>); and it was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement.¹⁷

2.1 | Population and outcomes

Seven health groups that represent key stages in the pathogenesis of type 2 diabetes were included in this study: healthy, overweight, obesity, impaired glucose tolerance, metabolic syndrome, type 2 diabetes, and type 2 diabetes with complications. Complications of interest in those with type 2 diabetes were microvascular (diabetic neuropathy, retinopathy, or nephropathy), macrovascular (peripheral artery disease or coronary artery disease), or both (diabetic foot ulceration). Considering that definitions for these conditions have evolved over time and between countries and, thus, vary between research, a homogenous classification for each health group was applied to all studies included in this network meta-analysis by comparing the average value of the key clinical characteristics (e.g. body mass index, fasting blood glucose concentration) from each health group against the World Health Organization guidelines for classification of overweight and obesity¹⁸; the joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention, harmonizing the criteria for defining metabolic syndrome¹⁹; and the American Diabetes Association criteria for impaired glucose tolerance and diabetes.²⁰ Those considered overweight or with obesity had normal

blood glucose profiles and those with impaired glucose tolerance or type 2 diabetes were overweight or with obesity.

The objective of this study was to assess differences in endothelium-dependent and endothelium-independent vascular reactivity between each of the seven health groups of interest. Outcomes of interest included commonly used tests of vascular reactivity in the microcirculation and macrocirculation. Microvascular endothelium-dependent reactivity could be evaluated using postocclusive reactive hyperemia, pressure-induced vasodilation, local thermal hyperemia, or the administration of acetylcholine, delivered intravenously or by iontophoresis. Microvascular endothelium-independent reactivity could be assessed with the administration of sodium nitroprusside, also delivered intravenously or by iontophoresis. The microvascular response to each test of reactivity could be measured with strain gauge plethysmography or a laser-based perfusion monitoring technology (e.g. laser Doppler flowmetry or laser Doppler imaging or laser speckle contrast imaging).²¹ Macrovascular endothelium-dependent reactivity and endothelium-independent reactivity were assessed using flow-mediated dilation (FMD) and nitrate-mediated dilation (NMD), respectively,²² each in conjunction with ultrasound of the brachial artery.

2.2 | Data sources and searches

The systematic search was performed in MEDLINE and EMBASE databases from their inception until March 13, 2017, using a combination of subject headings for health status (obesity, prediabetes, metabolic syndrome and type 2 diabetes) and methods of assessing vascular reactivity (FMD, NMD, brachial artery ultrasound, postocclusive reactive hyperemia, iontophoresis, skin microdialysis, intradermal injection, pressure-induced vasodilation, local thermal hyperemia, current-induced vasodilation, nerve-axon reflex, laser Doppler flowmetry, laser Doppler imaging, laser speckle contrast imaging, Doppler wires, strain gauge plethysmography and venous occlusion plethysmography). Searches were limited to "human" studies only, but were not limited by study design. The search strategy is presented in Table S1. A manual search of reference citations in identified reviews and original articles selected for full text retrieval was also performed.^{23,24}

2.3 | Study selection

Two investigators (J.L. and F.T.) independently performed study selection using Covidence, an online, Cochrane approved, software for conducting systematic reviews.²⁵ Discrepancies in inclusion or exclusion were solved through consultation with a third (G.W.) or fourth investigator (M.R.). To be included in this review, each study had to assess vascular reactivity in the basal state in two or more of the health groups of interest. Only data from vascular assessments completed on those aged greater than or equal to 18 years of age were included. The complete inclusion and exclusion criteria are available

2.4 | Data extraction and quality assessment

Characteristics of the population, outcomes, covariates of interest and quality assessments were summarized from each study into a preformatted spreadsheet independently by two investigators (J.L. and M.R.). Discrepancies were solved through consultation with a third investigator (G.W.). If data were unclear or were not available in the published manuscripts, the corresponding or first author was contacted by email to request this information. To minimize heterogeneity, research using methods of assessing microvascular reactivity that were not often used in the literature (i.e. in less than five studies) were excluded from the network meta-analyses. The full list of variables extracted and details about data extraction are available as supplemental methods.

A systematic assessment of quality for observational research (SAQOR), previously applied in meta-analyses of observational studies evaluating vascular reactivity,^{23,24} was performed to assess the quality of the studies included in this research. The SAQOR was scored out of 17; quality is deemed better with a greater score. The Grading of Recommendations Assessment, Development and Evaluation (GRADE) was performed to provide assessment of the quality of evidence for outcomes investigated by this meta-analysis.²⁶ The GRADE for each outcome was classified as high quality, moderate quality, low quality, or very low quality. More details about the GRADE quality assessment are available as supplemental methods.

2.5 | Data synthesis and analysis

All statistical analyses were performed using R statistical software (version 3.2.4), using the Metafor, Meta, and Netmeta packages.²⁷⁻²⁹ Considering that macrovascular endothelium-dependent and endothelium-independent reactivity were each assessed with a single method, FMD and NMD, respectively, macrovascular data was synthesized using the mean difference. In contrast, microvascular reactivity was assessed with various techniques; and thus, the standardized mean difference (SMD) summary statistic was used.³⁰ Direct, pairwise meta-analyses were performed first to assess pooled mean differences or SMD, as well as 95% CI, in macrovascular and microvascular data, respectively, between healthy controls and each other health group. A DerSimonian and Laird random-effects model was used when substantial heterogeneity was detected (I^2 statistic, >50%; or P value of the Q statistic, <0.10) (21). A negative mean difference or negative SMD indicated that vascular reactivity was impaired in that health group when compared with another.

A frequentist network meta-analysis was then performed using the graph theoretical method developed by Rucker et al.^{29,31} A network evidence plot was produced with the nodes indicating the health groups being assessed and the thickness of lines referring to the number of direct comparisons between each health group (e.g. the thicker the line, the more direct comparisons). The hypotheses of homogeneity and consistency were explored by the Q statistic and net heat plots.^{32,33} Additionally, the node-splitting method assessed the consistency between direct and indirect comparisons, with a P value of less than 0.05 deemed inconsistent. A Hasse diagram, using partial order

sets (posets), was used to rank the severity of impairment in vascular reactivity for each health group included in the network meta-analysis.³⁴ Publication bias was evaluated by funnel plot asymmetry and by using Egger's regression test, with a P value of less than 0.05 suggesting publication bias when more than 10 studies were available in each health group.³⁵

Several post hoc meta-regressions were performed on the following potential effect modifiers of macrovascular reactivity using a Bayesian approach: age, body mass index, brachial artery diameter at rest, blood pressure, fasting glucose, HbA1c %, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and triglycerides. The Bayesian network meta-analysis was performed using four chains, 10,000 burn-in and 50,000 iterations using gemtc package (version 0.8-2).³⁶ Convergence was assessed using the Gelman-Rubin-Brooks plot.³⁷

3 | RESULTS

3.1 | Study selection and characteristics

The systematic search resulted in the inclusion of 193 from a total of 4641 potential articles (Figure 1). From the 193 studies included in the analyses,^{7-16,38-220} vascular reactivity was assessed in a total of 26570 patients that were considered healthy ($n = 7226$), overweight ($n = 7605$), or those with obesity ($n = 1758$) or that were diagnosed with metabolic syndrome ($n = 2405$), impaired glucose tolerance ($n = 936$), type 2 diabetes ($n = 5254$) or type 2 diabetes with vascular complications ($n = 1386$). The main characteristics for each study are presented in Table S2. Flow-mediated dilation of the brachial artery was the most frequently used test of vascular reactivity ($n = 120$), while an array of tests was used to assess microvascular reactivity.

3.2 | Quality assessment and potential bias

The quality score and risk of bias for each study are reported in Table S2. The mean quality score was 14.3 ± 2.1 out of a possible 17 points. Quality assessments graded two studies with a high risk of bias, 53 with moderate risk of bias, and 138 with low risk of bias. Overall, the quality of evidence for outcomes demonstrating the impairment of vascular reactivity throughout the pathogenesis of type 2 diabetes and its complications was low to very low (Tables S3 to S6). Evaluation of funnel plot asymmetry and Egger's regression test suggested a possible publication bias for microvascular and macrovascular endothelium-dependent reactivity in those with type 2 diabetes, compared with healthy controls (Figure 2). No major asymmetry was found in data for other health groups.

3.3 | Pairwise meta-analyses results

Results of the pairwise meta-analyses, Table S7, demonstrate that macrovascular endothelium-dependent reactivity was impaired in all disease groups, albeit not significantly for patients considered overweight or with obesity. Endothelium-independent vascular reactivity is not significantly affected in patients considered overweight or with

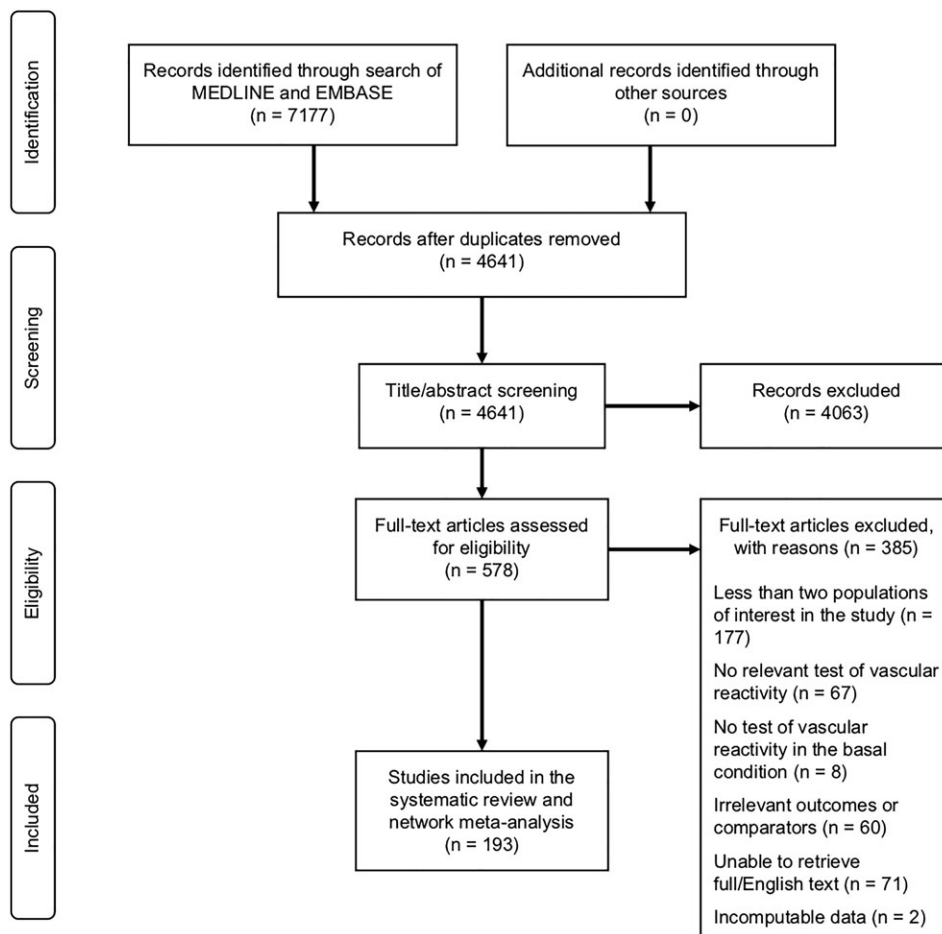


FIGURE 1 Flow diagram of the study selection process

obesity. In the microcirculation, abnormal endothelium-dependent and endothelium-independent vascular reactivity were detected in patients with obesity and in all health groups with cardiometabolic disease, except for patients with impaired glucose tolerance.

3.4 | Network meta-analyses

The networks of available comparisons for endothelium-dependent and endothelium-independent vascular reactivity are represented in Figure 3. These network meta-analyses indicate a progressive impairment of endothelium-dependent reactivity in both the microcirculation and macrocirculation, throughout the pathogenesis of type 2 diabetes and its related complications (Table 1 and Figure 4). A similar pattern was observed for endothelium-independent vascular reactivity in large vessels, while fewer differences were seen in the microcirculation. Forest plots for comparisons of endothelium-dependent and endothelium-independent vascular reactivity between all health groups, using each health group as the reference, are represented in Figure S1, demonstrating similar patterns in the impairment of endothelium-dependent and endothelium-independent vascular reactivity at each comparison.

The consistency between direct and indirect comparisons was assessed by the node-splitting method (Tables S8 to S11). Although several comparisons had significant heterogeneity between direct

and indirect evidence, the difference was primarily driven by the magnitude of the effect size and not by the direction of effect, suggesting consistency in this study's results. Furthermore, only four comparisons differed in direction: type 2 diabetes vs type 2 diabetes with complications, in (a) macrovascular endothelium-dependent reactivity and (b) macrovascular endothelium-independent vascular reactivity; (c) healthy vs obesity, in macrovascular endothelium-independent vascular reactivity; and (d) obesity vs overweight, in microvascular endothelium-independent vascular reactivity. Net heat plots confirmed the overall consistency in the results (Figure S2).

Considering the risk of bias related to the outcome assessment, sensitivity analyses, only including studies in which the outcome assessors were blinded to the health group classification, were conducted (Figure S3). Indeed, a similar trend in the impairment of endothelium-dependent vascular reactivity was observed, but the effect size was smaller. For example, in patients with type 2 diabetes, the mean difference for FMD was about 20% lower when outcome assessment was blinded. Similar results were found for endothelium-independent vascular reactivity.

3.5 | Meta-regressions

Given that methods for assessing macrovascular endothelium-independent reactivity and microvascular reactivity remain largely

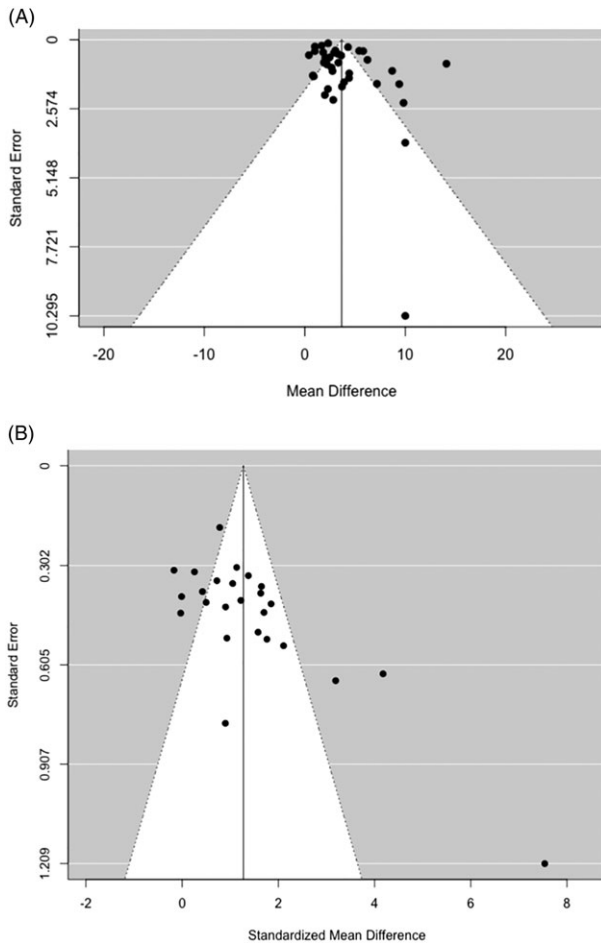


FIGURE 2 Detection of publication bias following evaluation of funnel plot asymmetry in data for (A) macrovascular and (B) microvascular endothelium-dependent reactivity in those with type 2 diabetes

unstandardized and that data, in some cases, were limited, meta-regressions were only performed on FMD data. Among the 11 potential effect modifiers, only fasting blood glucose was significantly, negatively correlated to FMD (Table S12), suggesting that the severity of impairment in macrovascular reactivity worsened as fasting blood glucose concentrations increased (Figure S4). Indeed, for every 1 mmol/l increase of fasting blood glucose concentration, there is a 0.52% decrease in FMD.

3.6 | Microvascular vs macrovascular endothelial dysfunction

To explore whether the pattern of impairment in vascular reactivity is similar between the microcirculation and the macrocirculation, when compared across all cardiometabolic health groups, ranks for macrovascular endothelium-dependent reactivity were plotted against ranks for microvascular endothelium-dependent reactivity (Figure S5A). They were subsequently computed as posets. Overall, the ranks were similar between the macrocirculation and the microcirculation: healthy status preceded impaired glucose tolerance and an overweight status, which preceded both metabolic syndrome and obesity, which preceded type 2 diabetes, which finally preceded type 2

diabetes with vascular complications, as demonstrated in the Hasse diagram (Figure S5B).

4 | DISCUSSION

The objective of this study was to compare vascular reactivity across the spectrum of cardiometabolic health, from healthy populations, through those who are considered overweight or with obesity, impaired glucose tolerance, metabolic syndrome and type 2 diabetes, to those with type 2 diabetes and complications. Combining direct and indirect comparisons from 193 studies, the findings of these network meta-analyses indicate a progressive impairment in microvascular and macrovascular endothelium-dependent reactivity throughout the pathogenesis of type 2 diabetes and its complications. A similar pattern was also observed in endothelium-independent vascular reactivity in the macrocirculation, but not in the microcirculation, which remained relatively unaffected across the health groups. Importantly, this data acknowledges that there is no single perfect time course in the development of obesity and/or insulin resistance (i.e. insulin resistance may be present with or without obesity), indicating that while each stage of abnormal cardiometabolic health is associated with an approximate level of vascular dysfunction, there are numerous possible continuums in the impairment of vascular reactivity. Indeed, the findings of this study indicate roles for both excess adipose tissue and abnormal blood glucose profiles in the impairment of vascular reactivity.

When interpreting these findings, it is important to acknowledge from the outset that this network meta-analysis does not properly address whether impairment in the microcirculation precedes that of the large vessels, a widely accepted hypothesis.²²¹ Indeed, while FMD of the brachial artery is a more standardized procedure than most methods of assessing vascular reactivity, it must be noted that a majority of studies (116 of the 120 included in this network meta-analysis) did not account for changes in shear rate during their data analyses. Subsequently, it is not possible to comprehensively conclude that the impairment in macrovascular endothelium-dependent reactivity is due to intrinsic abnormalities of macrovascular function or if they are partially attributable to downstream abnormalities (e.g. microvascular dysfunction) and/or simply a decrease in the stimulus for conduit artery dilation.²²² Considering this, future vascular studies and the accurate interpretation of their data would be improved by fully adopting previously standardized methodology for evaluating macrovascular reactivity and by work that contributes to establishing a consensus amongst protocols for assessing microvascular reactivity.²²³ In a similar vein, it must also be noted that the progressive decline in FMD is associated with a decrease in NMD, suggesting that impaired vascular reactivity could be caused by one or several factors, including abnormalities in endothelial function, endothelium-independent function, or structural changes within the blood vessel itself.

Regardless of the cause, this study is the first to demonstrate continuums where the severity of impairment in vascular reactivity and, thus, the blood vessel's susceptibility to injury and the potential to develop vascular-related complications progressively increases

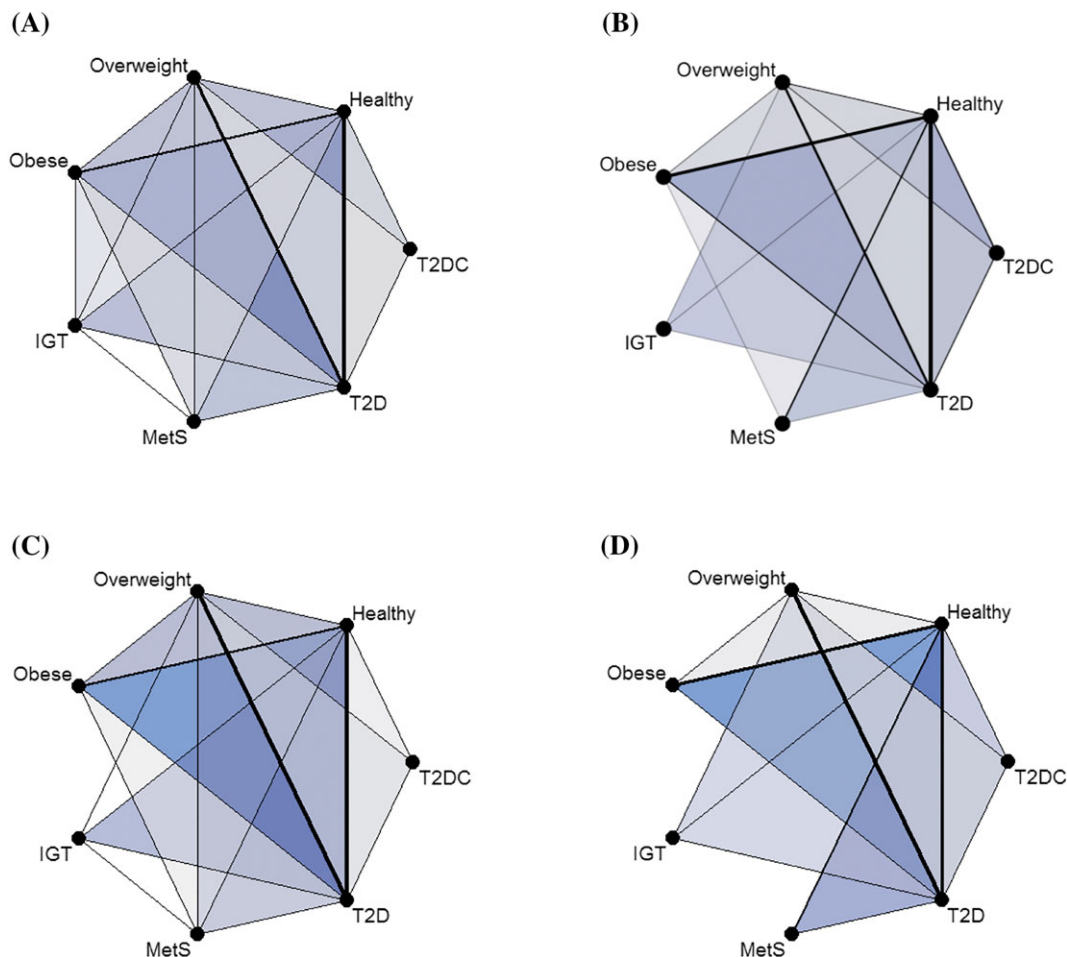


FIGURE 3 The networks of available comparisons between each health group from studies included in the network meta-analysis, for (A) macrovascular and (B) microvascular endothelium-dependent reactivity, as well as (C) macrovascular and (D) microvascular endothelium-independent reactivity. The thickness of lines refers to the number of direct comparisons between each health group, with thicker lines indicating more comparisons. Where there is no line joining two health groups, there was no previous direct comparison of vascular reactivity between those health groups in the literature. IGT, impaired glucose tolerance; MetS, metabolic syndrome; T2D, type 2 diabetes; T2DC, type 2 diabetes with complications [Colour figure can be viewed at wileyonlinelibrary.com]

throughout the pathogenesis of obesity and/or insulin resistance. This data also reinforces that chronic impairments in vascular reactivity present early in the decline of cardiometabolic health, long before the clinical onset of overt diseases. Interestingly, the network meta-analyses indicated that there was potential for the impairment of vascular reactivity in those who are considered overweight or with obesity, but who typically have normal blood glucose profiles, suggesting a role for excess adipose tissue in the impairment of vascular reactivity. Indeed, adipose tissue, including perivascular adipose tissue, produces adipokines such as cytokines (e.g. TNF α and IL-6), chemokines (e.g. IL-8 and MCP-1) and hormones (e.g. leptin and adiponectin), each of which have an influence on vascular function.²²⁴ In those with excess adipose tissue, the production of these adipokines can become dysregulated, reducing the contractility of the vascular smooth muscle by promoting low-grade inflammation and oxidative stress, biological states that inhibit the synthesis of nitric oxide, a potent vasodilating substance.^{224,225} Additionally, this maladaptation can contribute to the pathogenesis of insulin resistance, which itself can mediate an impairment of vascular reactivity. The impact of chronic vascular dysfunction on the pathogenesis of CVD, even in the early stages of abnormal cardiometabolic health (e.g. initial

weight gain), may be significant with data indicating that the risk of coronary heart disease is increased in those who are otherwise metabolically healthy, but are considered obese.²²⁶

Notably, meta-regression analyses of FMD data and potential effect modifiers found one significant, negative correlation; that between FMD and fasting blood glucose concentration, indicating that as fasting blood glucose concentration increases by 1 mmol/l, FMD decreases by 0.52%. In addition to obesity, low-grade inflammation, and insulin resistance (described above), hyperglycemia may also adversely impact vascular function, highlighting that those with excess adipose and impaired blood glucose homeostasis exhibit multiple factors that contribute to the impairment of vascular reactivity. Indeed, elevated generation of reactive oxygen species appears to be a unifying pathway between each of these factors and impaired vascular reactivity.²²⁷ As alluded to, oxidative stress may induce endothelial dysfunction by disrupting the synthesis of nitric oxide, thus, reducing its bioavailability and the capacity of the blood vessel to dilate.²²⁷ Additionally, hyperglycemia-mediated increases in the concentration of the superoxide anion may deactivate available nitric oxide, converting it to the oxidant, peroxynitrite, which induces substrate

TABLE 1 Network meta-analysis results for macrovascular and microvascular reactivity. Results are mean difference (95% CI)

MACROVASCULAR							
Endothelium-dependent reactivity							
Healthy	-0.41 (-0.98, 0.15)	-1.33 (-1.98, -0.67)	-1.75 (-2.80, -0.70)	-1.99 (-2.73, -1.24)	-3.22 (-3.69, -2.75)	-4.26 (-4.97, -3.54)	
-0.98 (-1.79, -0.17)	Overweight	-0.91 (-1.67, -0.16)	-1.34 (-2.33, -0.34)	-1.57 (-2.31, -0.84)	-2.80 (-3.29, -2.32)	-3.84 (-4.57, -3.12)	
-0.34 (-1.39, 0.71)	0.64 (-0.52, 1.81)	Obese	-0.42 (-1.59, 0.74)	-0.66 (-1.57, 0.25)	-1.89 (-2.61, -1.17)	-2.93 (-3.84, -2.02)	
-1.50 (-2.95, -0.06)	-0.52 (-2.00, 0.96)	-1.17 (-2.89, 0.56)	IGT	-0.24 (-1.40, 0.93)	-1.47 (-2.50, -0.44)	-2.51 (-3.68, -1.33)	
-1.47 (-2.48, -0.46)	-0.49 (-1.42, 0.43)	-1.13 (-2.43, 0.16)	0.03 (-1.61, 1.67)	MetS	-1.23 (-1.99, -0.47)	-2.27 (-3.21, -1.33)	
-2.68 (-3.36, -1.99)	-1.69 (-2.35, -1.04)	-2.34 (-3.45, -1.23)	-1.17 (-2.63, 0.29)	-1.20 (-2.18, -0.23)	T2D	-1.04 (-1.69, -0.38)	
-3.14 (-4.20, -2.08)	-2.16 (-3.12, -1.20)	-2.80 (-4.18, -1.43)	-1.64 (-3.31, 0.03)	-1.67 (-2.92, -0.42)	-0.47 (-1.36, 0.43)	T2DC	
Endothelium-independent reactivity							
MICROVASCULAR							
Endothelium-dependent reactivity							
Healthy	-0.20 (-0.48, 0.08)	-0.66 (-0.90, -0.41)	-0.63 (-1.10, -0.16)	-1.01 (-1.39, -0.63)	-1.10 (-1.31, -0.89)	-1.49 (-1.83, -1.14)	
-0.04 (-0.42, 0.34)	Overweight	-0.46 (-0.79, -0.13)	-0.43 (-0.90, 0.04)	-0.81 (-1.27, -0.35)	-0.90 (-1.16, -0.64)	-1.29 (-1.67, -0.90)	
-0.48 (-0.84, -0.13)	-0.45 (-0.90, 0.01)	Obese	0.03 (-0.48, 0.54)	-0.35 (-0.79, 0.09)	-0.44 (-0.72, -0.17)	-0.83 (-1.23, -0.42)	
-0.54 (-1.12, 0.04)	-0.50 (-1.05, 0.04)	-0.06 (-0.71, 0.59)	IGT	-0.38 (-0.98, 0.22)	-0.47 (-0.94, 0.00)	-0.86 (-1.41, -0.30)	
-0.55 (-0.94, -0.16)	-0.51 (-1.04, 0.01)	-0.07 (-0.59, 0.45)	-0.01 (-0.70, 0.68)	MetS	-0.09 (-0.51, 0.32)	-0.48 (-0.98, 0.03)	
-0.50 (-0.80, -0.19)	-0.46 (-0.74, -0.18)	-0.01 (-0.43, 0.41)	0.04 (-0.50, 0.58)	0.05 (-0.42, 0.52)	T2D	-0.38 (-0.73, -0.04)	
-0.87 (-1.32, -0.42)	-0.84 (-1.26, -0.42)	-0.39 (-0.93, 0.15)	-0.33 (-0.97, 0.31)	-0.32 (-0.90, 0.26)	-0.38 (-0.77, 0.01)	T2DC	
Endothelium-independent reactivity							

Values presented in bold font are significantly different. CI, confidence interval; IGT, impaired glucose tolerance; MetS, metabolic syndrome; T2D, type 2 diabetes; T2DC, type 2 diabetes with complications.

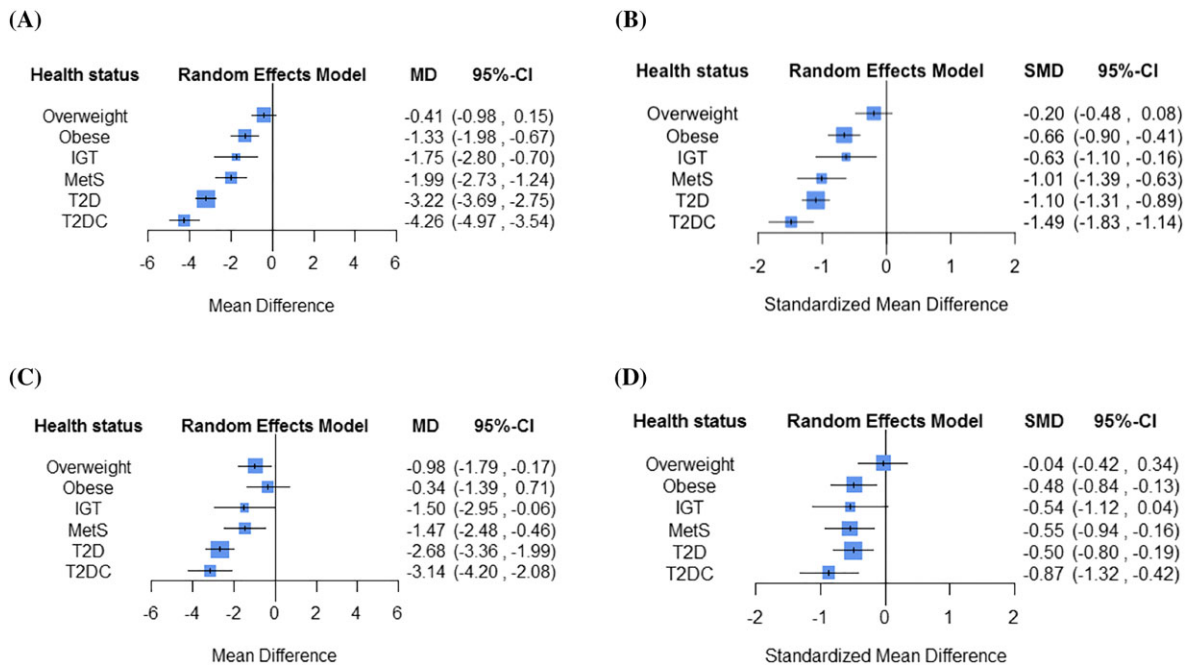


FIGURE 4 Forest plots of the mean difference (MD) in (A) macrovascular endothelium-dependent reactivity and (C) macrovascular endothelium-independent reactivity and the standardized mean difference (SMD) in (B) microvascular endothelium-dependent reactivity and (D) microvascular endothelium-independent reactivity between each health group considered overweight or obese or with cardiometabolic disease, as compared to the healthy group in the network meta-analyses. CI, confidence interval; IGT, impaired glucose tolerance; MetS, metabolic syndrome; T2D, type 2 diabetes; T2DC, type 2 diabetes with complications [Colour figure can be viewed at wileyonlinelibrary.com]

synthase and enzyme activity.²²⁷ Acknowledging that other signalling pathways of vasomotion may be affected by oxidative stress, reduced nitric oxide bioavailability is considered a strong predictor of CVD outcomes.²²⁷ Cardiometabolic diseases are characterized by abnormally frequent hyperglycemic excursions. Such exposure to a hyperglycemic environment that is “chronic” in nature may also induce vascular smooth muscle cell proliferation by disrupting its natural apoptosis.²²⁸

Furthermore, hyperglycemia may enhance the production of advanced glycation end products and collagen cross-linking.²²⁸ Collectively, these mechanisms stiffen the arterial wall, possibly explaining why vascular reactivity may be more severely impacted in those with impaired glucose tolerance, metabolic syndrome, and type 2 diabetes. Ultimately, this increase in myogenic tone elevates the risk of injury to 235 the endothelial wall, an event that is significant in the pathogenesis of

CVD; and potentiates the development of insulin resistance and vascular-related complications (e.g. retinopathy and foot ulceration in those with diabetes).

There are several inherent limitations to this research that must be addressed. Many studies included in this network meta-analysis used control groups that had no specific health classification. Considering this, mean clinical data from all included studies assessing health groups not defined as type 2 diabetes, with or without complications, were checked against current definitions for the different health groups. Although this approach allowed us to obtain more homogeneous health groups while acknowledging the definitions and criteria that have evolved over time, it does not account for the heterogeneity between subjects within each study arm. Furthermore, most studies did not include parameters that define metabolic syndrome and are known to have an influence on vascular reactivity, such as insulin resistance; and therefore, the effect of such factors could not be accounted for in this analysis. It must also be recognised that while there is a large amount of data focusing on endothelium-dependent vascular reactivity, the number of studies that assessed endothelium-independent vascular reactivity was limited, thus, reducing the power to detect differences between health groups and develop conclusions about the changes in endothelium-independent vascular reactivity. Similarly, few studies assessed vascular reactivity in those with impaired glucose tolerance. Although potential bias was detected in several outcomes in this study, the SAQOR revealed a predominantly low risk of bias. Finally, the GRADE indicates that the quality of evidence for outcomes assessed in this study is low to very low, as is the nature of observational data. Although this does not affect the conclusion regarding the trends, the confidence in the effects estimates is more limited. Similarly, sensitivity analyses including only studies in which the outcome assessors were blinded to the health group indicate that the trends remain the same but with lower effects estimates. This highlights evaluation biases, which can be a limitation in techniques such as FMD. Similarly, funnel plot asymmetry and Egger's regression test suggest possible publication bias in patients with type 2 diabetes (endothelium-dependent reactivity data). However, there is no major asymmetry for other health groups and therefore, the impact of publication bias on these findings is difficult to evaluate.

Detailing, for the first time, the change in vascular reactivity across the spectrum of cardiometabolic health, this study provides clinicians and researchers with a unique overview of the numerous time courses in the pathogenesis of vascular dysfunction. Indeed, while this study further detailed the effect of an abnormal blood glucose profile on vascular reactivity, it also addressed discrepant findings in previous studies of vascular function in those considered overweight or with obesity,^{9,11,12,103,112,143,150} demonstrating that obesity and an overweight state is associated with blunted microvascular and macrovascular reactivity. These findings strengthen the hypothesis that excess adipose tissue also has a direct role (i.e. initiating mechanisms) in the impairment of vascular reactivity early in the decline of cardiometabolic health, establishing a greater understanding that is essential to future vascular research. Additionally, acknowledging that endothelial dysfunction is considered a main precursor to the pathogenesis of obesity, insulin resistance and CVD,^{221,229} this research

highlights the need to improve the methods of exploring vascular function. While this present study demonstrates that vascular reactivity may be impaired early, even in those considered overweight, previous research from our laboratory has demonstrated that acute hyperglycemia, induced by excess sugar consumption, transiently blunts endothelium-dependent vascular reactivity in those considered healthy.^{23,230} Considering this, further research is needed to assess if transient impairments in vascular reactivity, mediated by several dietary and lifestyle factors, develop into chronic vascular impairment before, when someone is still considered clinically healthy, or after the clinical onset of an overweight state and/or impaired glucose homeostasis. Furthermore, given that there may be differences in vascular function between ethnicities and gender,²³¹ future research may also assess vascular reactivity, as well as the mechanisms that underlie any impairment (e.g. disruption of the NO pathway), in varying health populations from a range of ethnic backgrounds.

In conclusion, this network meta-analysis demonstrates, for the first time, multiple continuums where vascular reactivity is progressively impaired throughout the pathogenesis of obesity and/or insulin resistance. These results detail the changes in vascular reactivity across the full spectrum of cardiometabolic health, supporting the need for early interventions in at-risk populations to overturn the progressive deterioration of vascular health; indeed, providing important information that may enhance the timing and effectiveness of strategies that aim to improve cardiovascular health outcomes.

ACKNOWLEDGEMENTS

J.L. was responsible for the concept and design of the study. J.L., F.T., G.W., and M.R. acquired the data. J.L., C.K., G.W., and M.R. analysed and interpreted the data and drafted the manuscript. J.L., C.K., F.T., S.S., C.L., J.L.C., G.W., and M.R. provided administrative, technical, or material support and critically revised the manuscript for important intellectual content. All authors approved the final version of the manuscript.

FUNDING INFORMATION

J.L. was supported by the National Health and Medical Research Council of Australia Dora Lush Biomedical Research Postgraduate Scholarship (114350) and an Australian Government Research Training Program Scholarship.

CONFLICTS OF INTEREST

No conflict of interest was declared.

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How to cite this article: Loader J, Khouri C, Taylor F, et al. The continuums of impairment in vascular reactivity across the spectrum of cardiometabolic health: A systematic review and network meta-analysis. *Obesity Reviews.* 2019;1-15. <https://doi.org/10.1111/obr.12831>

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CLINICAL PHARMACOLOGY

French translation and linguistic validation of the Raynaud's condition score

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Received 21 January 2019; accepted 15 March 2019

Available online 2 April 2019

KEYWORDS

Raynaud's
phenomenon;
Systemic sclerosis

Summary The Raynaud's condition score is a 11-point scale severity score used in Raynaud's phenomenon clinical trials since 1998. The Raynaud's condition score diary has been recommended for use in clinical trials assessing efficacy of interventions on scleroderma related Raynaud's phenomenon. However, this score has never been formally validated in French. We thus performed a translation and a linguistic validation of the Raynaud's condition score through a forward/backward translations process followed by an expert review and cognitive patient interviews. The translations led to a French version of the Raynaud's condition score that was linguistically valid, and conceptually equivalent to the original English version. This "Score de Raynaud" will be usable to perform and harmonize clinical trials in French-speaking patients with secondary Raynaud's phenomenon.

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Abbreviations

PRO	patient-reported outcome
RCS	Raynaud's condition score
WHO	World Health Organization

Introduction

The Raynaud's condition score (RCS) is a 11-point scale severity score used in patients with Raynaud's phenomenon. It has been widely used as a patient-reported outcome (PRO) in clinical trials since 1998 [1]. This Raynaud's phenomenon severity score was validated and standardized by Merkel et al. in 2002 [2]. The RCS diary has been recommended for use in clinical trials assessing efficacy of interventions on scleroderma related RP and is usually used as a primary endpoint [3,4]. However, this score has never been formally validated in French and, to our knowledge, in other languages. The aim of the current study was to translate and perform a linguistic validation of the RCS in French.

Linguistic validation of a PRO is a process ensuring that concepts are equivalent and easily understood by people speaking other languages than that of the original version.

Method

Ethical approval was applied from the CPP Île-de-France 10 (RCB: 2018-A01473-52) prior beginning the study.

The translation process was performed through a forward/backward validation process followed by an expert review and cognitive patient interviews, in accordance with the translation guidelines of the World Health Organization (WHO) [5,6].

The following four steps were taken and are synthesized in Fig. 1.

Forward translation

The original RCS was translated by two French-native speaking translators. They performed this translation independently after receiving information about the goal of the RCS and on the way it has been used in research. The two versions were further conciliated and a synthetic version was produced (version A).

Backward translation

The harmonized version A was back-translated by a professional English-native speaking translator (version B). The original RCS version and the back-translated B version were compared, conciliated and a synthetic version was produced (version C).

Clinicians experts validation

The conciliated version C was sent to 4 clinical experts in the field in France (YA, SB, EH, PS). They were asked to review and comment the version C for expression and concepts. The

results of the clinical expert feedback were synthesized and incorporated into the version C to produce the version D.

Patient cognitive interviews

Finally, the version D was presented in a face-to-face meeting to 5 scleroderma patients to ask for comprehension and commentaries. All remarks and commentaries were synthesized and used to produce the version E. A further 5 cognitive face to face interviews with patients were led. Findings were used to make the final French version of the RCS.

Results

The translation process led to a French version of the RCS that was linguistically validated and conceptually equivalent to the original English version. In the first step, we found that the two independent forward translations had different views on the language, which required discussion in order to reach consensus. Main discrepancies between the two translated versions were the use of "*Syndrome de Raynaud*" or "*Phénomène de Raynaud*" terms, and the translation of difficulty by "*difficulté*" or "*gêne*" in French. The "painful sores" translation was also absent from one version. Moreover, adding or not the concept of quantification to translate the English concept of "rating" was also thoroughly discussed during the conciliation process.

The comparison of the back-translated version B to the original English version identified several words for which the meaning differed. "Difficulty" was back translated to "discomfort" and the emphasis "ALONE" (in capital letters) put on Raynaud's phenomenon in the original version was lost in the back translation.

Then, the version C was sent to 4 clinicians experts in the field. Their comments were integrated to the version D. Mainly, more emphasis was put on the importance to rate daily symptoms by underlining "*au cours de la journée*" ("during the day") in the French version. Moreover the French term "*à lui seul*" ("alone") was also underlined to emphasize this point.

The version D was first presented to 5 scleroderma patients in cognitive interviews. Their characteristics and comments are presented in Table 1. Mainly, the concept of daily rating was insufficiently understood. Furthermore, the isolation of Raynaud's symptoms from other hand related scleroderma symptoms (such as sclerodactyly or musculoskeletal disorders) was not easy for patients. A revised version was thus presented to 5 additional patients, and it was well understood by all of them with no further changes.

The original and the final versions of the RCS are presented in Table 2.

Discussion

In this study, we translated and linguistically validated the RCS into French.

Since its first use in 1998 by Wigley et al. the RCS has been widely used in clinical trials, in combination with the frequency and the duration of RP attacks, to assess the efficacy of pharmacological interventions on RP. In 2002 Merkel et al.

Table 1 Characteristics of included patients. The main triggering factor and Raynaud's phenomenon locations are in bold.

Patient characteristics				Raynaud's phenomenon				Ulcers	Linguistic validation			
Age	Gender	Type of scleroderma	Year of diagnosis	Triggering factor	Frequency		Mean crisis duration	Locations	Sores/finger ulcer	Score de Raynaud	Formulation clear and understandable	Comments
					Winter	Summer						
50	F	Diffuse	2003	Temperature variation	5/day	< 1/week	< 10 min	Hand/foot	Yes	2	Yes	
47	F	Diffuse	2011	Cold /moisture	> 5/day	< 1/week	60 min	Hand/foot	Yes recurring every winter	0	No	"Today" should be emphasized (write it at the beginning)
49	F	Limited cutaneous	2004	Cold /moisture/uncaused	> 5/day	1 to 5/day	20 min (winter)/more fleeting in summer	Hand/foot/nose	No	8	Yes	
57	F	Limited cutaneous	2008	Cold /stress	> 5/day	1 to 5/day	20 to 25 min	Hand/foot	No	5	Yes	
48	F	Diffuse	2013	Cold	> 5/day	< 1/week	10 min	Hand/foot	No	0	Yes	No crisis during the summer
63	M	NA	2013	Cold	> 5/day	1 à 5/day	20 to 30 min	Hand/foot/ears	Yes	1 to 2	Yes	
55	F	Diffuse	2015	Cold	1 to 5/day	< 1/week	20 min	Hand	Yes	0	Yes	
84	F	Limited cutaneous	1999	Cold	1 to 5/day	< 1/week	10 min	Hand/foot	Yes	8	Yes	
60	F	Diffuse	1990	Cold	1 to 5/day	< 1/week	15 min	Hand/foot	Yes	5	No	No crisis but permanent disability
56	F	Limited cutaneous	2002	Cold/moisture	> 5/day	< 1/week	10 min to 1 h	Hand	Yes	5	Yes	

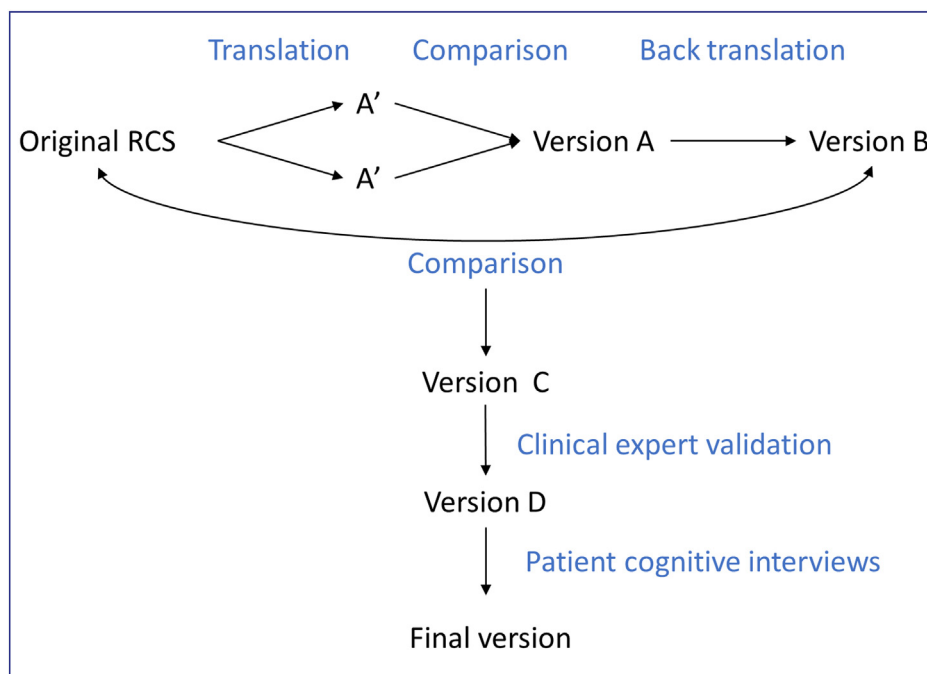


Figure 1. Flow chart of the translation and the linguistic validation process.

Table 2 Original English and French final translated version of the Raynaud's condition score.

Original version	French version
Raynaud's condition score	Score de Raynaud
The Raynaud's condition score is your rating of how much difficulty you had with your Raynaud's TODAY. Consider how many attacks you had and how long they lasted	Nous souhaiterions connaître votre propre évaluation de la gêne ressentie au cours de la <u>journée</u> à cause du phénomène de Raynaud
Consider how much pain, numbness, or other symptoms the Raynaud's caused in your fingers (including painful sores) and how much the Raynaud's ALONE affected the use of your hands today	Prenez en compte le nombre de crises que vous avez eues <u>aujourd'hui</u> ainsi que leur durée ; prenez aussi en compte la douleur, l'engourdissement ou tout autre symptôme (y compris les plaies douloureuses), et à quel point le phénomène de Raynaud <u>à lui seul</u> gêne l'usage de vos mains
CIRCLE below the number that best indicates the difficulty you had today with your Raynaud's condition	Entourez ci-dessous le chiffre qui indique le mieux la gêne que vous avez ressentie aujourd'hui à cause de votre phénomène de Raynaud
No difficulty 0 1 2 3 4 5 6 7 8 9 10 extreme difficulty	Aucune gêne 0 1 2 3 4 5 6 7 8 9 10 Gêne extrême

in a study on 148 scleroderma patients highlighted that the dimension covered by the RCS differed from crisis duration and frequency, thus highlighting the complementarity of the outcomes. Another study conducted in a cohort of patients with primary and secondary RP study further established the minimally important difference for the RCS at 1.4, and patient acceptable symptom state at 3.4, on a 0-10 scale [7]. The cognitive interviews also highlighted the difficulty for the patients to individualize Raynaud's symptoms from other scleroderma hand related symptoms such as sclerodactyly and skin fibrosis. This difficulty is probably more important in late stage scleroderma patients with less paroxysmal RP crises. However, that difficulty is inherent to this global score and is also expected in the original English version [8].

One of the main limitations of the study is the limited number of included patients for cognitive interviews and the absence of primary Raynaud's phenomenon patients. However, the translated version has been promptly well understood by patients and no primary RP patients were included in the original validation study [2].

Conclusion

In this study we translated and validated in French the most used and recommended severity score, the RCS, to assess the efficacy of intervention on scleroderma related RP in clinical trials. This "Score de Raynaud" will be usable

to perform and harmonize clinical trials enrolling French-speaking patients with secondary RP.

Disclosure of interest

The authors declare that they have no competing interest.

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ANNEXES. SUPPLEMENTARY MATERIALS DES ARTICLES

- 1. Peripheral vasoconstriction induced by β -adrenoceptor blockers: a systematic review and a network meta-analysis**
- 2. Proton pump inhibitors and Raynaud's phenomenon: is there a link ?**
- 3. Comparative efficacy and safety of treatments for secondary Raynaud's phenomenon: systematic review and network meta-analysis of randomized trials.**
- 4. Drug repurposing in Raynaud's phenomenon through adverse event profile in the WHO pharmacovigilance database**
- 5. Comparative safety of drugs targeting the nitric oxide pathway in pulmonary hypertension: a mixed approach combining a meta-analysis of clinical trials and a disproportionality analysis from the WHO pharmacovigilance database.**
- 6. Pulmonary Arterial Hypertension associated with Protein Kinase Inhibitors: A pharmacovigilance-pharmacodynamic study.**
- 7. Hierarchical evaluation of electrical stimulation protocols for chronic wound healing: an effect size meta-analysis.**
- 8. SGLT-2 inhibitors and the risk of lower-limb amputation: Is this a class effect?**
- 9. Drug-induced skin ulcer: a combined disproportionality analysis using data from Medline and Vigibase.**
- 10. Impact of pharmacovigilance disproportionality analysis study design on the correlation with drug-related risks.**

Peripheral vasoconstriction induced by beta-blockers: a systematic review and a network meta-analysis.

SUPPLEMENTARY INFORMATION

SUPPLEMENTAL TABLES

Table S1. Quality ratings following GRADE recommendations for comparison of peripheral vasoconstriction induced by beta-blockers. CCB: calcium channel blockers; ACE/ARB: angiotensin converting enzyme inhibitors /angiotensin 2 receptor blockers.

Comparison	Direct evidence	Indirect evidence	Network meta-analysis
	Quality of evidence	Quality of evidence	Quality of evidence
Acebutolol v Alfa blocker		Very low	Very low
Acebutolol v Atenolol		Low	Low
Acebutolol v Betaxolol		Low	Low
Acebutolol v Bevantolol		Very low	Very low
Acebutolol v Bisoprolol		Very low	Very low
Acebutolol v Carvedilol		Very low	Very low
Acebutolol v CCB		Very low	Very low
Acebutolol v ACE/ARB		Very low	Very low
Acebutolol v Labetalol		Very low	Very low
Acebutolol v Metoprolol		Low	Low
Acebutolol v Metoprolol		Low	Low
Acebutolol v Pindolol		Low	Low
Acebutolol v Placebo	Low‡	High	High
Acebutolol v Propranolol	Moderate†	High	High
Acebutolol v Sotalol		Low	Low
Acebutolol v Thiazidique		Low	Low
Acebutolol v Trimetazidine		Very low	Very low
Acebutolol v Xamoterol		Very low	Very low
Alfa blocker v Atenolol	Moderate*	Moderate	Moderate

Alfa blocker v Betaxolol		Very low	Very low
Alfa blocker v Bevantolol		Very low	Very low
Alfa blocker v Bisoprolol		Moderate	Moderate
Alfa blocker v Carvedilol		Very low	Very low
Alfa blocker v CCB		Moderate	Moderate
Alfa blocker v ACE/ARB		Moderate	Moderate
Alfa blocker v Labetalol		Moderate	Moderate
Alfa blocker v Metoprolol		Moderate	Moderate
Alfa blocker v Oxprenolol		Very low	Very low
Alfa blocker v Pindolol		Moderate	Moderate
Alfa blocker v Placebo		Moderate	Moderate
Alfa blocker v Propranolol	Very low**,‡	Low	Low
Alfa blocker v Sotalol		Very low	Very low
Alfa blocker v TD		Low	Low
Alfa blocker v Trimetazidine		Very low	Very low
Alfa blocker v Xamoterol		Very low	Very low
Atenolol v Betaxolol		Low	Low
Atenolol v Bevantolol	Very low**,†	Low	Low
Atenolol v Bisoprolol	High	High	High
Atenolol v Carvedilol		Low	Low
Atenolol v CCB	High	High	High
Atenolol v ACE/ARB	Moderate*	Moderate	Moderate
Atenolol v Labetalol	Moderate†	High	High
Atenolol v Metoprolol	Moderate†	High	High
Atenolol v Oxprenolol		Low	Low
Atenolol v Pindolol	High	High	High
Atenolol v Placebo	Moderate*	Moderate	Moderate
Atenolol v Propranolol		Low	Low
Atenolol v Sotalol		Moderate	Moderate
Atenolol v Thiazidique	Low**	Low	Low
Atenolol v Trimetazidine		Very low	Very low
Atenolol v Xamoterol		Moderate	Moderate
Betaxolol v Bevantolol		Very low	Very low
Betaxolol v Bisoprolol		Very low	Very low
Betaxolol v Carvedilol		Low	Low
Betaxolol v CCB		Very low	Very low
Betaxolol v ACE/ARB		Very low	Very low
Betaxolol v Labetalol		Moderate	Moderate
Betaxolol v Metoprolol	Moderate*	High	High
Betaxolol v Oxprenolol		Low	Low
Betaxolol v Pindolol		Low	Low
Betaxolol v Placebo	Low‡	High	High
Betaxolol v Propranolol		Low	Low
Betaxolol v Sotalol		Low	Low
Betaxolol v Thiazidique		Low	Low
Betaxolol v Trimetazidine		Very low	Very low
Betaxolol v Xamoterol		Moderate	Moderate
Bevantolol v Bisoprolol		Very low	Very low
Bevantolol v Carvedilol		Very low	Very low

Bevantolol v CCB		Very low	Very low
Bevantolol v ACE/ARB		Very low	Very low
Bevantolol v Labetalol		Very low	Very low
Bevantolol v Metoprolol		Very low	Very low
Bevantolol v Oxprenolol		Very low	Very low
Bevantolol v Pindolol		Very low	Very low
Bevantolol v Placebo	Very low**, †	Low	Low
Bevantolol v Propranolol		Very low	Very low
Bevantolol v Sotalol		Very low	Very low
Bevantolol v Thiazidique		Very low	Very low
Bevantolol v Trimetazidine		Very low	Very low
Bevantolol v Xamoterol		Very low	Very low
Bisoprolol v Carvedilol		Very low	Very low
Bisoprolol v CCB		High	High
Bisoprolol v ACE/ARB		Moderate	Moderate
Bisoprolol v Labetalol		Moderate	Moderate
Bisoprolol v Metoprolol		Moderate	Moderate
Bisoprolol v Oxprenolol		Very low	Very low
Bisoprolol v Pindolol		Moderate	Moderate
Bisoprolol v Placebo		Moderate	Moderate
Bisoprolol v Propranolol		Very low	Very low
Bisoprolol v Sotalol		Very low	Very low
Bisoprolol v Thiazidique		Low	Low
Bisoprolol v Trimetazidine		Very low	Very low
Bisoprolol v Xamoterol		Very low	Very low
Carvedilol v CCB		Very low	Very low
Carvedilol v ACE/ARB	Low*, †	Moderate	Moderate
Carvedilol v Labetalol		Low	Low
Carvedilol v Metoprolol	Low*, †	Moderate	Moderate
Carvedilol v Oxprenolol		Very low	Very low
Carvedilol v Pindolol		Low	Low
Carvedilol v Placebo		Low	Low
Carvedilol v Propranolol		Very low	Very low
Carvedilol v Sotalol		Very low	Very low
Carvedilol v Thiazidique		Low	Low
Carvedilol v Trimetazidine		Very low	Very low
Carvedilol v Xamoterol		Low	Low
CCB v ACE/ARB		Moderate	Moderate
CCB v Labetalol		Moderate	Moderate
CCB v Metoprolol		Moderate	Moderate
CCB v Oxprenolol		Very low	Very low
CCB v Pindolol		Moderate	Moderate
CCB v Placebo		Moderate	Moderate
CCB v Propranolol		Very low	Very low
CCB v Sotalol		Very low	Very low
CCB v Thiazidique		Low	Low
CCB v Trimetazidine		Very low	Very low
CCB v Xamoterol		Very low	Very low
ACE/ARB v Labetalol		Moderate	Moderate
ACE/ARB v Metoprolol	Very low**, †	Low	Low

ACE/ARB v Oxprenolol		Very low	Very low
ACE/ARB v Pindolol		Moderate	Moderate
ACE/ARB v Placebo	Very low*,‡	Moderate	Moderate
ACE/ARB v Propranolol		Very low	Very low
ACE/ARB v Sotalol		Very low	Very low
ACE/ARB v Thiazidique	Very low**,†	Low	Low
ACE/ARB v Trimetazidine		Very low	Very low
ACE/ARB v Xamoterol		Very low	Very low
Labetalol v Metoprolol	Moderate†	High	High
Labetalol v Oxprenolol		Very low	Very low
Labetalol v Pindolol	Moderate†	High	High
Labetalol v Placebo		Moderate	Moderate
Labetalol v Propranolol		Very low	Very low
Labetalol v Sotalol		Very low	Very low
Labetalol v Thiazidique		Low	Low
Labetalol v Trimetazidine		Very low	Very low
Labetalol v Xamoterol		Moderate	Moderate
Metoprolol v Oxprenolol		Low	Low
Metoprolol v Pindolol	Low*,†	Moderate	Moderate
Metoprolol v Placebo	High	High	High
Metoprolol v Propranolol		Low	Low
Metoprolol v Sotalol		High	High
Metoprolol v Thiazidique	Low‡	High	High
Metoprolol v Trimetazidine		Very low	Very low
Metoprolol v Xamoterol	High	High	High
Oxprenolol v Pindolol		Low	Low
Oxprenolol v Placebo	Low†,§	High	High
Oxprenolol v Propranolol		Low	Low
Oxprenolol v Sotalol		Low	Low
Oxprenolol v Thiazidique		Low	Low
Oxprenolol v Trimetazidine		Very low	Very low
Oxprenolol v Xamoterol		Very low	Very low
Pindolol v Placebo	High	High	High
Pindolol v Propranolol		Low	Low
Pindolol v Sotalol		High	High
Pindolol v Thiazidique		Low	Low
Pindolol v Trimetazidine		Very low	Very low
Pindolol v Xamoterol		Low	Low
Placebo v Propranolol	Low†,§	High	High
Placebo v Sotalol	High	High	High
Placebo v Thiazidique	Low*,†	Moderate	Moderate
Placebo v Trimetazidine		Low	Low
Placebo v Xamoterol		Moderate	Moderate
Propranolol v Sotalol		Low	Low
Propranolol v Thiazidique	Very low*,‡	Moderate	Moderate
Propranolol v Trimetazidine	Low‡	High	High
Propranolol v Xamoterol		Very low	Very low
Sotalol v Thiazidique		Low	Low
Sotalol v Trimetazidine		Very low	Very low
Sotalol v Xamoterol		Very low	Very low

Thiazidique v Trimetazidine	Very low	Very low
Thiazidique v Xamoterol	Low	Low
Thiazidique v Xamoterol	Very low	Very low
Trimetazidine v Alfa blocker	Very low	Very low

*Risk ok biais. ** Severe risk of biaais. § Inconsistency. † Imprecision. ‡ Severe Imprecision

Table S2. Number of direct comparison included in the network meta-analysis, mean GRADE quality rating summary and percentage of high quality studies for each beta-blocker.

Beta-blocker	Number of direct comparison	Mean quality	Percentage of high quality studies
Acebutolol	18	low	11%
Atenolol	18	moderate	28%
Betaxolol	18	moderate	11%
Bevantolol	18	low	0%
Bisoprolol	4	moderate	50%
Carvedilol	18	low	0%
Labetalol	18	low	17%
Metoprolol	19	moderate	37%
Oxprenolol	17	low	6%
Pindolol	18	moderate	22%
Propranolol	18	moderate	17%
Sotalol	18	low	17%
Xamoterol	18	low	6%

Table S3. Number of direct comparison included in the network meta-analysis, mean GRADE quality rating summary and percentage of high quality studies for each pharmacologic group of beta-blocker. ISA: Intrinsic sympathomimetic activity. VD: Vasodilator activity

Pharmacologic properties	Mean quality	Number of direct comparison	Percentage of high quality studies
Non selective	low	36	17%
β1 selectivity	moderate	95	19%
ISA	moderate	53	13%
VD	low	72	7%

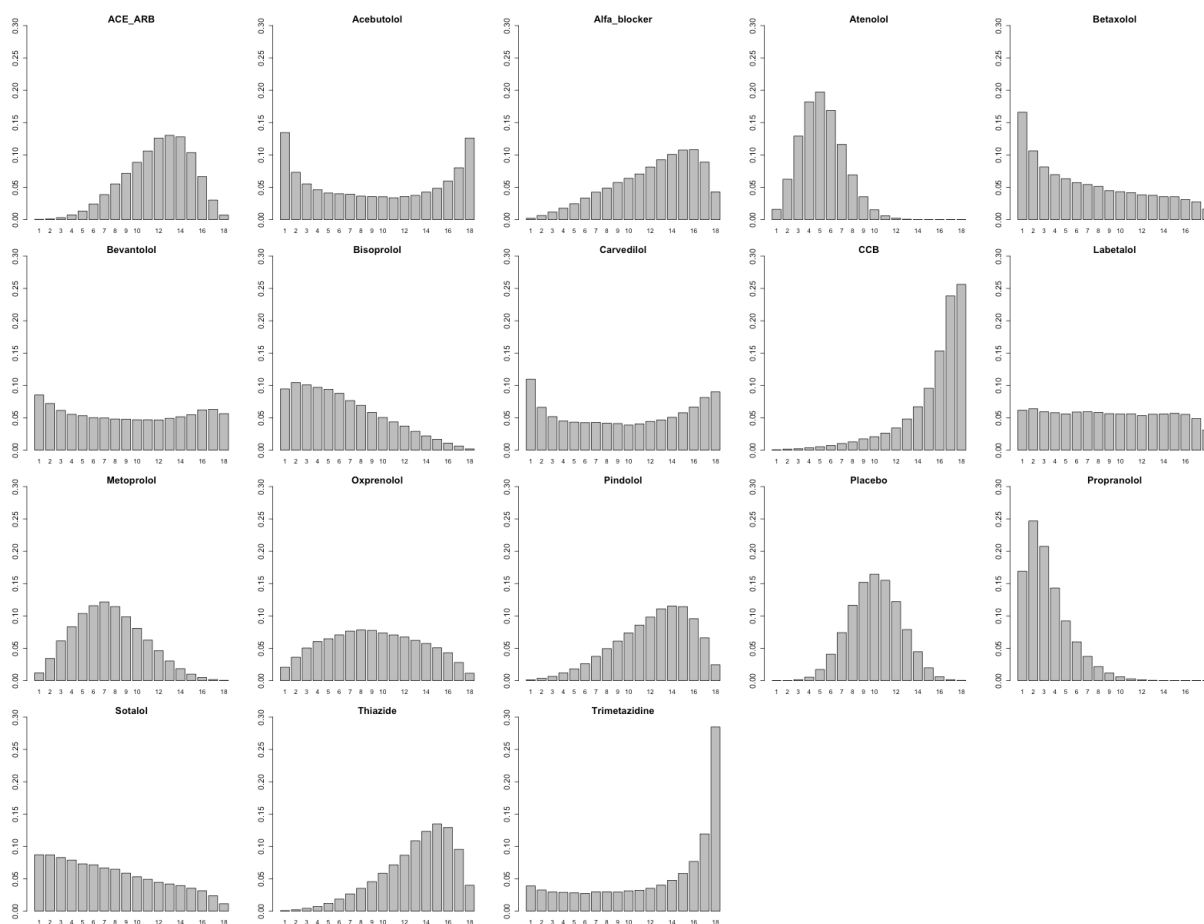
SUPPLEMENTAL FIGURE LEGENDS

Figure S1.The risk of bias summary

	Random sequence generation	Allocation concealment	Blinding participant/personnel	Blinding outcome assessment	Incomplete outcome data	Selective reporting	Other biases
DiBianco 1982	+	+	+	-	-	+	
Dahlöf 2005	+	+	-	+	+	+	
Dahlöf 2002	+	+	+	+	+	+	
Talseth 1991	+	+	+	-	-	+	
NASRC 1988	+	+	+	+	-	+	
Ott 1987	?	?	+	-	-	+	
Fairhurst 1987	?	?	+	?	-	+	
Helgeland 1986	?	?	+	-	-	+	
Rubin 1983	?	?	+	+	-	+	
Julian 1982	+	+	+	-	+	+	
Hansteen 1982	+	+	+	-	+	-	
Persson 1995	?	?	+	-	+	+	
Greenberg 1984	?	?	-	?	+	+	
BHATRG 1982	?	?	+	-	-	+	
Silberstein 2012	+	+	+	+	+	+	
Leren 1980	?	?	+	-	-	-	
Pascal 1987	+	+	-	+	+	+	
Moltzer 2010	?	?	-	-	+	+	
Metra 2000	?	?	+	-	+	+	
Herrick 1989	+	+	+	-	+	+	
Taylor SH 1982	+	+	+	+	+	+	
UKPDS 39 1998	+	+	+	-	+	+	
The DTS Group 1993	+	+	+	+	+	+	
The IPPPSH Group 1985	+	+	+	+	+	+	
Ekbom T 1992	?	?	+	+	+	+	
Garden OJ 1990	?	?	+	-	+	+	
Nielsen 1997	?	?	-	?	+	+	
Beevers 1991	?	?	+	-	+	+	
Khattar 2001	?	?	+	-	+	+	
Mc Neil 1979	?	?	+	-	+	+	
Pasotti 1982	?	?	+	-	+	+	
Iliuta 2009	?	?	+	-	+	+	
Vanderburg 1984	?	?	-	-	+	+	
Detry 1994	?	?	+	+	+	+	
Salonen 1992	?	?	+	-	+	+	
Bühler 1986	?	?	+	-	+	+	
De Muinck 1992	?	?	+	-	+	+	
Pedersen 1976	+	?	+	-	+	+	

+: Low risk of bias; -:high risk of bias; ?: unclear risk of bias.

Figure S2. Rankogram representing on the horizontal axis the possible ranks of the treatment (the more on the left, the higher risk of PV), and on the vertical axis the probability for the treatment to assume each of the possible ranks. CCB: calcium channel blockers; ACE/ARB: angiotensin converting enzyme inhibitors /angiotensin 2 receptor blockers.



Proton pump inhibitors and Raynaud's phenomenon: is there a link?

C. Khouri, B. Revol, JL Cracowski, M. Roustit

Supplementary Material

Supplementary Table 1. Pooled data retrieved from Vigibase® reports of Proton Pump Inhibitors associated with Raynaud's phenomenon compared to H2 antagonists.

	Proton pump inhibitors	H2 antagonists	p-value
Total number of reports	753854	269663	
Number of RP reports	253	48	
Frequency of RP reports (n/1000)	0.34/1000	0.18/1000	>0.01
Gender (M/F) (% of available reports) [§]	56/194 (99%)	14/33 (98%)	0.34
Age (Mean ± sd) (% of available reports) [§]	56.6 ± 15.1 (77%)	59.4 ± 14.4 (67%)	0.31
Secondary RP [n (%)]	44 (17%)	6 (12.5%)	0.40
Scleroderma	9	1	.
Other auto-immune disease [#]	35	4	.
Concomitant drug known to induce RP *	90 (35)	12 (25)	0.16
[number of reports with at least one drug (%)]			

Frequencies of reports and continuous outcomes between drug classes were compared using the χ^2 test and the Student t-test, respectively.

[§] Because all reports were not complete, the percentage of reports providing this variable is reported.

[#] Connective tissue disorder, Systemic lupus erythematosus, Rheumatoid arthritis

* Beta-blockers, ergot alkaloids, Stimulants (amphetamine drugs and methylphenidate), interferons, chemotherapy (cisplatin, bleomycin), ciclosporin.

Supplementary material

Comparative efficacy and safety of treatments for secondary Raynaud's phenomenon: systematic review and network meta-analysis of randomized trials

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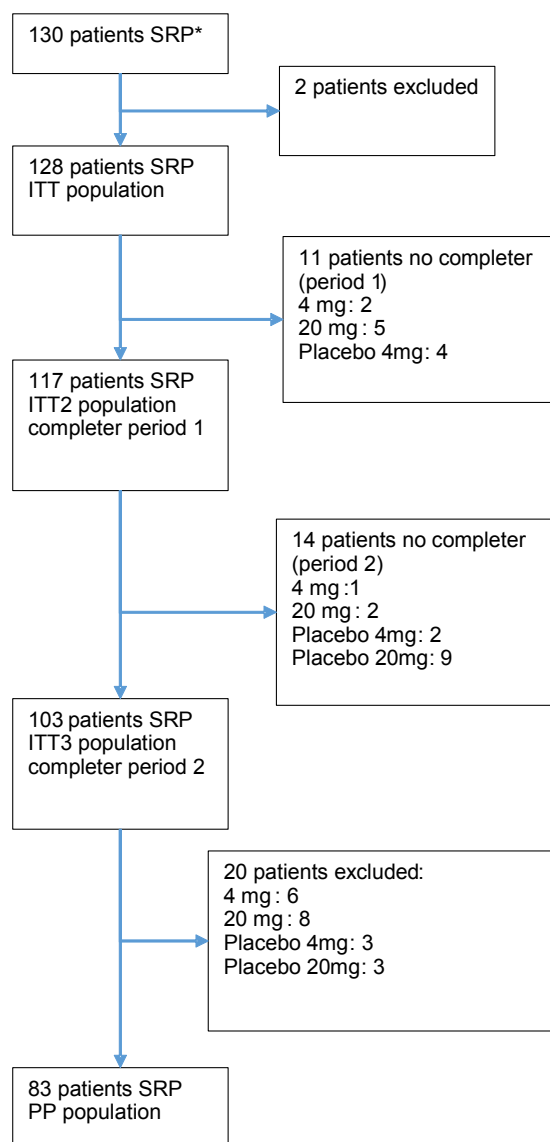
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Appendix Method 1. Summary of literature searches

Database	Keywords	Number of results
Review searches		
Medline	"Raynaud Disease/therapy"[Mesh] AND Review[ptyp]"	301
	"digital[All Fields] AND ("ulcer"[MeSH Terms] OR "ulcer"[All Fields]) AND ("scleroderma, systemic"[MeSH Terms] OR ("scleroderma"[All Fields] AND "systemic"[All Fields]) OR "systemic scleroderma"[All Fields] OR "scleroderma"[All Fields] OR "scleroderma, localized"[MeSH Terms] OR ("scleroderma"[All Fields] AND "localized"[All Fields]) OR "localized scleroderma"[All Fields])) AND Review[ptyp]"	44
Cochrane systematic review database	<i>"Raynaud" in Title, Abstract, Keywords in Cochrane Reviews'</i>	7
Additional RCT searches		
Clicicaltrial.gov	"Raynaud"	77+
	«Systemic Sclerosis »	389
	« Digital Ulcer »	30
From inception to 24.09.2019	"digital[All Fields] AND ("ulcer"[MeSH Terms] OR "ulcer"[All Fields])) AND Clinical Trial[ptyp]"	107
	Raynaud's[tiab] AND Randomized Controlled Trial[ptyp]	205
Embase from inception to 24.09.2019	#1'raynaud phenomenon'/exp OR 'raynaud phenomenon' OR (raynaud AND phenomenon)	16,328
	#2 AND 'randomized controlled trial'/de	226
	#1 'systemic sclerosis'/exp OR 'systemic sclerosis'	34,045
	#2 AND 'randomized controlled trial (topic)'/de	304

AddisInsigt	"Raynaud's phenomenon"	14

Appendix Figure 1: Flow chart of the trial NCT01090492 (PF-00489791 (PDE-5 inhibitor) versus placebo).



* In the initial population 130 patients with secondary RP (SRP) were included, two of whom were excluded at the beginning of the study because there was no collected data. The overall intention to treat (ITT) population was constituted by 128 patients. This is the population we used for safety outcomes. After removing from analysis patients who did not complete period 1 (n=11) and period 2 (n=14), we obtained two additional populations (ITT2 and ITT3, respectively). The latter was used for efficacy outcomes. Finally, from the ITT3 population, 20 patients were excluded to constitute the per protocol (PP) population. The reasons for exclusion were related to treatment compliance (n=11), to the absence of RP attacks during the pre-treatment period (n=6), and unknown for three patients.

Appendix Table 1. Model statistics of random effect models. Mean posterior deviance (Dbar), the effective number of data points (pD) and the Deviance Information Criterion (DIC) are presented.

Outcome	Model	Dbar	pD	Deviance Information Criterion (DIC)	Between study variance (tau ²)	Heterogeneity (I ²)
Frequency	Random	82.68	58.90	141.59	0.035	16.8%
Severity	Random	90.14	62.23	152.37	0	0%
Duration	Random	43.75	32.01	73.83	0	0%
Acceptability	Random	82.5	66.5	148.9	0.077	25.2%
Tolerability	Random	80.4	65.7	146.1	0.23	41.0%

Appendix Method 2. Supplementary statistical methods

All pharmacological treatments were grouped at a therapeutic class level to perform the meta-analysis. When a study reported several arms with different doses of the same drug, those groups were merged.

Pairwise meta-analyses using an inverse variance fixed effects model, or a restricted maximum-likelihood estimator random effects model, were first performed for continuous outcomes to assess pooled mean differences (MD) and their 95% confidence intervals (95% CI). For binary safety outcomes we omitted trials with no events in any arm and we used the incidence rate ratio (IRR) and 95% CI, with exact Mantel-Haenszel method to synthesize the results. Random effects models were used when substantial heterogeneity was detected (I-squared statistic >50% or p-value of the Q statistics <0.10).

Bayesian network meta-analyses were performed using Markov chain Monte-Carlo simulation, with non-informative prior distribution. Data were abstracted and analysed using the MD for continuous outcomes and Incidence Rate Ratios (IRR) with 95% credible intervals (95% CrI). The normal likelihood was used for continuous outcomes and the Poisson distribution with logarithm link function for safety binary outcomes. For the latter, differences in follow-up duration between trials were taken into account by using patient-week follow-up duration to estimate HR. We used generalized linear models with 4 chains and 100,000 iterated simulation, with an initial 10,000 iteration burn-in. Convergence was assessed using the Brooks-Gelman-Rubin statistic, with a cut-off 1.05 (1). Given the diversity in RCTs and patient characteristics included in the meta-analysis we used random-effect models to synthesize the results. The transitivity assumption underlying network meta-analysis was evaluated by constructing summary tables organized by pairwise comparisons to qualitatively assess baseline clinical similarity of trial populations.

We did a statistical evaluation of consistency by comparing statistics for the deviance and DIC in fitted consistency and inconsistency models (2), and by assessing incoherence between direct and indirect comparisons using the node-splitting method (3).

If SDs were not reported and not provided by the authors: 1. When interquartile difference, confidence intervals, SEs, t-statistics or p values were reported, these were back transformed to SDs. 2. If SDs were reported at baseline we used them to impute post treatment SDs. 3. Lastly, the mean value of known SDs from the included studies was calculated and imputed if necessary (4). Missing data for the covariates of interest (i.e. used for meta-regressions) were not imputed.

Appendix Method 3. Methods for assessment of quality-of-evidence of indirect and network effect estimates according to the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) tool.

We GRADEd each drug class versus placebo estimates according to the following criteria.

(1) Study limitations: we estimated the risk of bias as low, moderate, or high for each study. We then derived the judgment for study limitations for each pairwise comparison as an averaged risk of bias, based on the contribution of each direct estimate from the contributions matrix.

(2) Imprecision: We downgraded for imprecision if the information size achieved until date was not considered large enough in the trial sequential analysis.

(3) Inconsistency: we downgraded for inconsistency if substantial inconsistency was found according to the node splitting method ($p < 0.10$). We downgraded for heterogeneity if I^2 was $> 50\%$ in the direct comparison of the pairwise meta-analysis.

(4) Indirectness: we downgraded for indirectness if the result of a meta-regression provided significant results for a variable of interest, and if the point estimate widely varied in subgroup analysis. Furthermore, we downgraded singly-connected nodes for indirectness, because evaluation of transitivity for such nodes is unclear.

(5) Publication bias: we downgraded for publication bias if asymmetry was detected on visual inspection of the funnel plot, or if Egger's regression test was significant ($p < 0.05$). Further, there was no network estimate for which the meta-regression for small-study effects suggested the statistically significant influence of small-study effects.

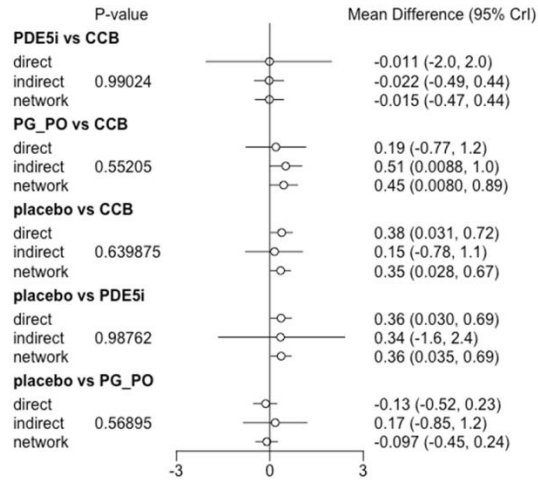
Appendix Table 2. Evaluation of the global inconsistency. Comparison of model statistics between consistency and inconsistency models.

Mean posterior deviance (Dbar), the effective number of data points (pD) and the Deviance Information Criterion (DIC) are presented.

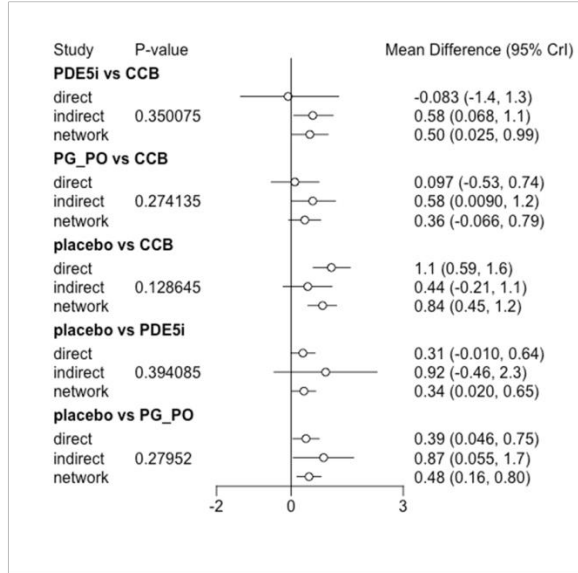
	Dbar	pD	DIC
Frequency			
Consistency	82.68	58.90	141.59
Inconsistency	84.0	84.0	168.0
Serevity			
Consistency	90.14	62.23	152.37
Inconsistency	91.98	91.98	183.96
Duration			
Consistency	43.75	32.01	73.83
Inconsistency	47.86	47.86	95.72
Acceptability: All-cause discontinuation			
Consistency	82.5	66.5	148.9
Inconsistency	87.0	87.0	174.1
Tolerability: Serious adverse events			
Consistency	80.4	65.7	146.1
Inconsistency	81.2	81.2	162.3

Appendix Figure 2. Evaluation of the inconsistency using the node-splitting method.

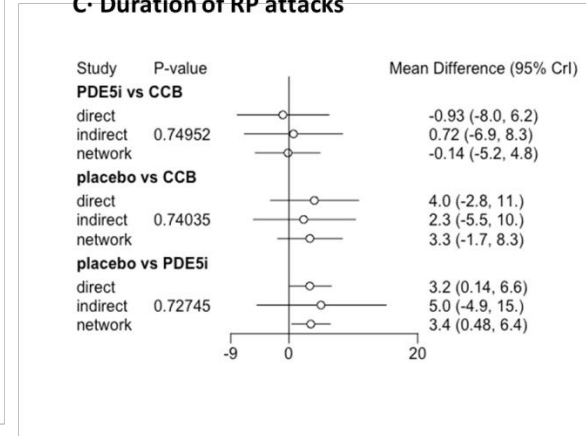
A- Frequency of RP attacks



B- Severity of RP attacks

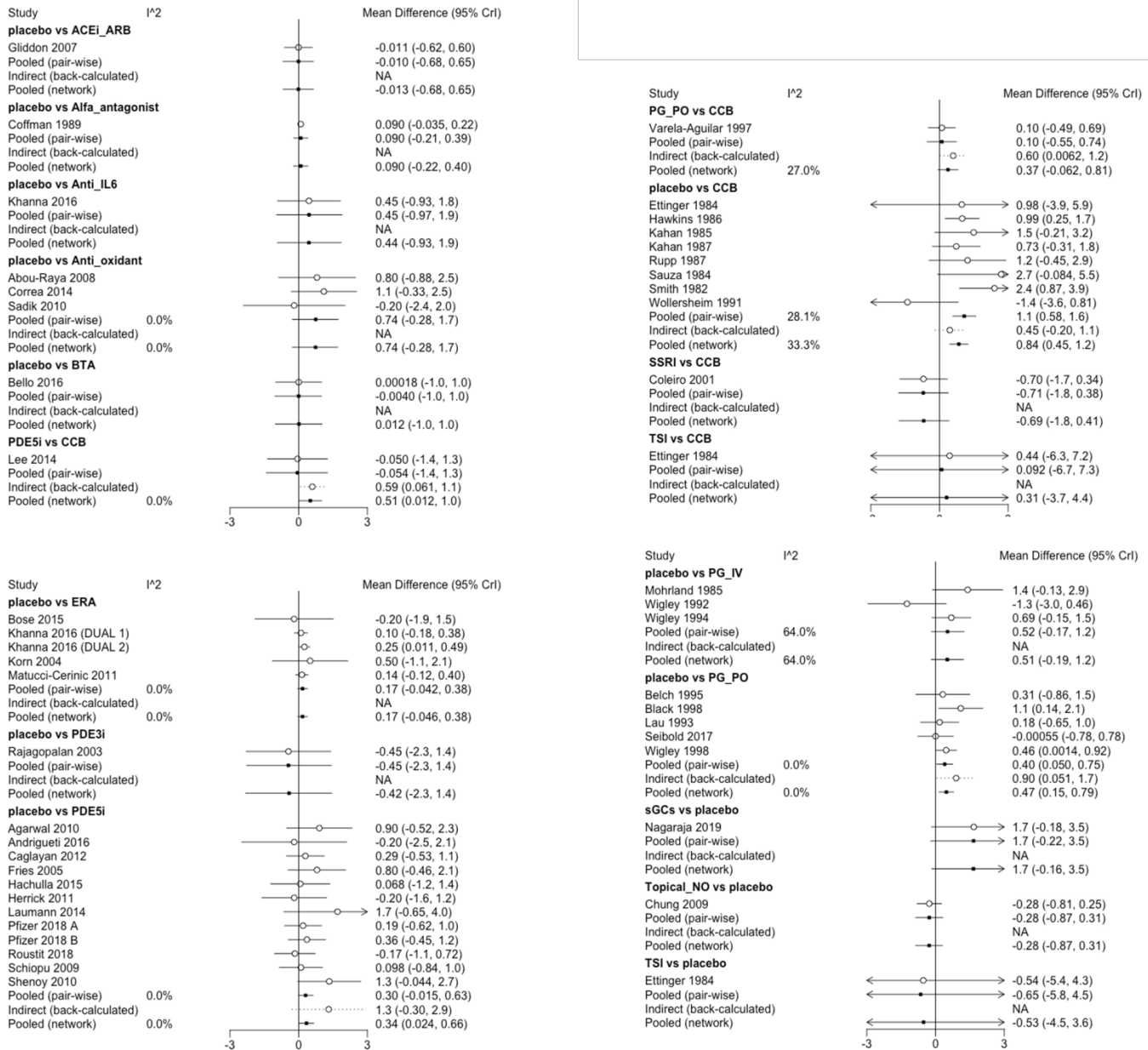


C- Duration of RP attacks

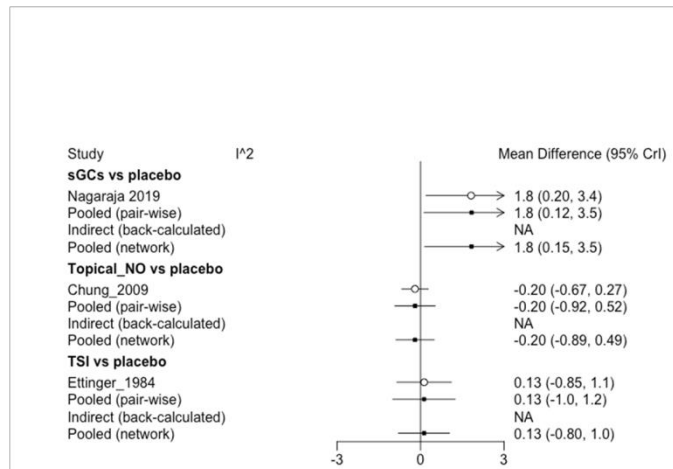
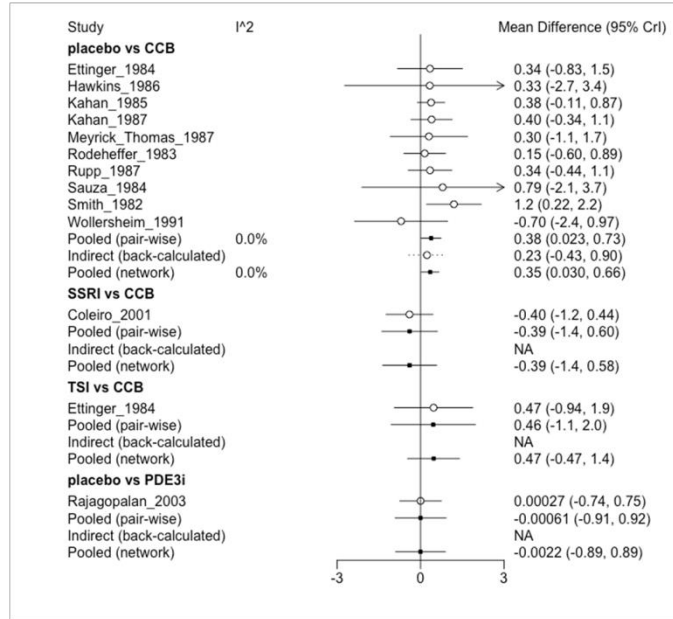
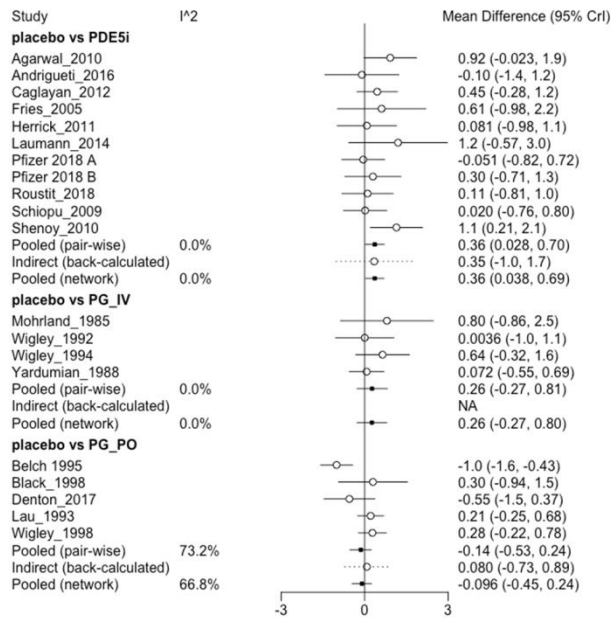
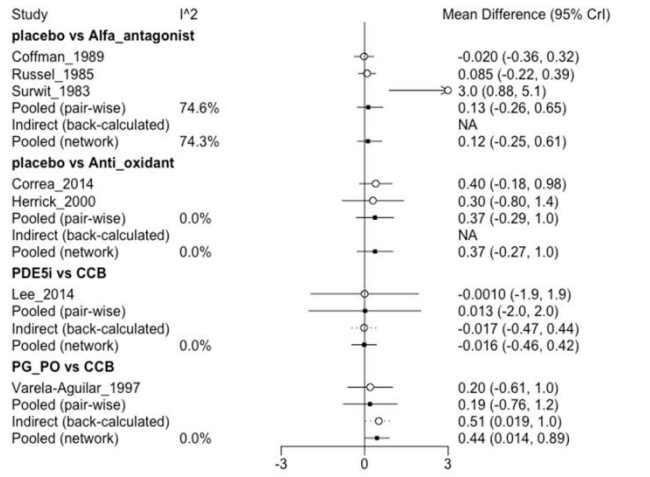


Appendix Figure 3. Evaluation of heterogeneity in each comparison.

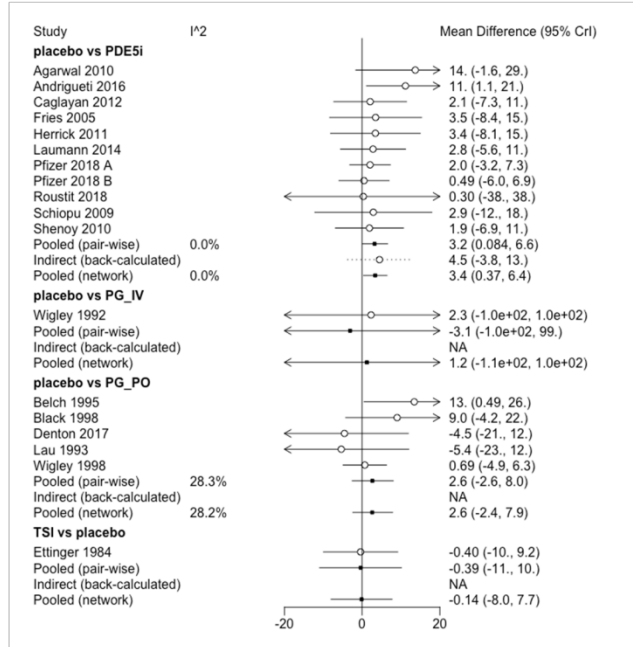
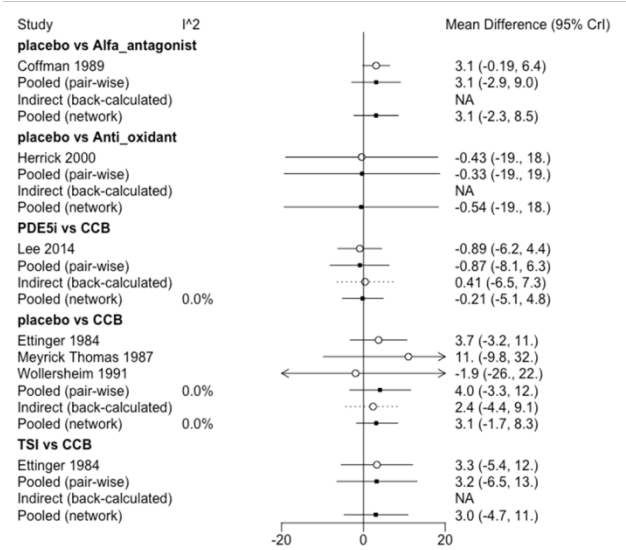
A. Frequency of RP attacks



B. Severity of RP attacks



C· Duration of RP attacks



Appendix Text 1. References of included studies

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Appendix Table 3. Drugs included in each drug class (main analyses)

Drug class	Drugs
α adrenoreceptors antagonists	Ketanserin, prazosin
Anti-oxidants	Atorvastatin, N-acetylcystein, allopurinol+ antioxidant
Anti-interleukin-6	Tocilizumab
Angiotensin-converting enzyme inhibitors / Angiotensin receptor blockers	Quinalapril
Botulinum Toxin A	Botulinum Toxin A
Calcium channel blockers	Nifedipine, sustained-release nifedipine, diltiazem, nicardipine, amlodipine
Endothelin receptor antagonists	Ambrisentan, macitentan, bosentan
Phosphodiesterase type 3 inhibitors	Cilostazol
Phosphodiesterase type 5 inhibitors	Sildenafil, tadalafil, vardenafil, PF-00489791, udenafil
Intravenous prostacyclin analogs	Epoprostenol, iloprost, alprostadil
Oral prostacyclin analogs / non-prostanoid IP-receptor agonists	Iloprost, cicaprost, treprostinil, selexipag
Selective serotonin reuptake inhibitor	Fluoxetine
Soluble guanylate cyclase (sGC) stimulator	Riociguat
Topical nitric oxide donor	MQX-503
Thromboxane synthase inhibitors	Dazoxiben, UK 38,485

Appendix Table 4. Characteristics of included studies.

Study	Design	Treatment arms	Dosage (mg or ng/kg/min)	Country	Patients randomized (n)	Patients included in analysis (n)	Proportion of patients with secondary RP (%)	Follow up (weeks)	Add-on
Abou-Raya 2008	double-blind, parallel	atorvastatin vs placebo	40 mg	Egypt	84	84	100	16	no
Agarwal 2010	double-blind, parallel	tadalafil vs placebo	10 mg	India	53	53	100	8	yes
Andrigueti 2016	double-blind, parallel	sildenafil vs placebo	100 mg	Brazil	41	41	100	8	yes
Belch 1983	double-blind, parallel	epoprostenol vs placebo	7,5 ng/kg/min 5h per day, 3 weeks	UK	14	12	57	3	.
Belch 1995	double-blind, parallel	iloprost PO vs placebo	300 µg/day	UK	63	63	100	1.4	no
Bello 2017	double-blind, parallel	BTA vs placebo	50 units	USA (Maryland)	40	40	100	17	yes
Black 1998	double-blind, parallel	iloprost PO vs iloprost PO vs placebo	100 µg vs 200 µg	Denmark, UK, Netherlands	103	79	100	6	no
Bose 2015	double-blind, parallel	ambrisentan vs placebo	10 mg per day	USA (Ohio)	20	20	100	12	no
Caglayan 2012	double-blind, crossover	varденаfil vs placebo	20 mg	Germany	53	50	89	6	no
Chung 2009	double-blind,	MQX 503 vs placebo	1-4 /day	USA	219	212	68	4	no

	parallel			USA, Canada, Belgium, Denmark, Germany, Italy, Norway, Spain, Sweden, UK					
Coffman 1989	double-blind, parallel	ketanserin vs placebo	120 mg/day		222	222	50	12	no
Coleiro 2001	double-blind, crossover	fluoxetine vs placebo	20 mg vs 40 mg	UK	53	NA	51	6	no
Correa 2014	double-blind, parallel	N-acetylcystein vs placebo	1800 mg/day	Brasil	42	42	100	4	no
Denton 2017	double-blind, parallel	selexipag vs placebo	200-1600 µg/ day	France, Germany, UK	74	64	100	8	yes
Ettinger 1984	double-blind, crossover	nifedipine vs placebo vs dazoxiben	60 mg /day vs 300 mg/day	USA (Maryland)	25	19	73	2	no
Fries 2005	double-blind, crossover	sildenafil vs placebo	100 mg /day	Germany	18	17	89	4	no
Gliddon 2007	double-blind, parallel	quinalapril vs placebo	80 mg/day	UK	210	210	100	144	yes
Hachulla 2016	double-blind, parallel	sildenafil vs placebo	60 mg/day	France	83	83	100	12	yes
Hawkins 1986	double-blind, crossover	nifedipine vs placebo	40 mg/day	UK	71	57	65	6	no
Herrick 2000	double-blind, crossover	Allopurinol+ antioxidant vs placebo	300 mg /day	UK	33	33	100	10	yes

Herrick 2011	double-blind, parallel	sildenafil MR vs placebo	200 mg/day	UK	57	57	100	4	yes
Kahan 1985	double-blind, crossover	diltiazem vs placebo	360 mg/day	France	10	10	63	2	no
Kahan 1987	double-blind, crossover	nicardipine vs placebo	60 mg/day	France	20	20	100	2	no
Khanna 2016	double-blind, parallel	tocilizumab vs placebo	162 mg/sem	Canada, France, USA, Germany, UK	289	280	100	48	no
Khanna (DUAL 1) 2016	double-blind, parallel	macitentan vs placebo	3 mg vs 10 mg/day	USA, Australia, Belarus, Bulgaria, Canada, Chile, Colombia, Croatia, Czech Republic, Denmark, Finland, Germany, Hungary, India, Italy, Poland, Russian Federation, Ukraine	265	255	100	16	yes
Khanna (DUAL 2) 2016	double-blind, parallel	macitentan vs placebo	3 mg vs 10 mg/day	USA, Argentina, Belgium, China,	87	87	100	16	yes

				Colombia, Germany, Greece, Ireland, Israel, Mexico, Netherlands, New Zealand, Poland, Portugal, Puerto Rico, Russian Federation, South Africa, Spain, Turkey, Ukraine, UK					
Korn 2004	double-blind, parallel	bosentan vs placebo	250 mg/day	Europe, North America	122	121	100	16	yes
Lau 1993	double-blind, parallel	cicaprost vs placebo	7.5 vs 15 µg/day	UK	51	49	100	1.4	no
Laumann 2014	double-blind, crossover	tadalafil vs placebo	20 mg/day	USA (Illinois)	10	8	100	4	yes
Lee 2014	double-blind, crossover	udenafil vs amlodipine	100 mg/day vs 10 mg/day	Korea	29	26	100	4	no
Matucci-Cerinic 2011	double-blind, parallel	bosentan vs placebo	250 mg/day	Europe, North America	188	172	100	24	yes
Mc Hugh 1988	double-blind, crossover	iloprost vs placebo	1 to 3 ng/kg/min 3-6h per day, 3 days	UK	25	20	90	6	.

Meyrick Thomas 1987	double-blind, crossover	nifedipine vs placebo	30 mg/day	UK	10	9	100	6	
Mohrland 1985	double-blind, parallel	alprostadil vs placebo	10 mg/kg/min 3 days	USA, UK	55	NA	56	4	no
Nagaraja 2019	double-blind, parallel	Riociguat vs placebo	7.5 mg/day	USA	17	17	17	16	yes
NCT01090492 2018	double-blind, crossover	PF-00489791 vs placebo	4 mg/day vs 20 mg/day	12 countries	17	17	100	4	yes
Nguyen 2010	double-blind, parallel	bosentan vs placebo	250 mg/day	Austria	24	24	100	16	no
Ortonne 1989	double-blind, parallel	ketanserin vs placebo	80 mg/day	France	130	121	100	24	no
Rademaker 1989	double-blind, parallel	nifedipine vs iloprost	30-60 mg/day vs 2 ng/kg/min 8h/day, 3+1 days	UK	23	23	100	16	no
Rajagopalan 2003	double-blind, parallel	cilostazol vs placebo	200 mg/day	USA (Michigan)	21	NA	100	6	no
Rodeheffer 1983	double-blind, crossover	nifedipine vs placebo	30-60 mg/day	USA (Maryland)	15	15	100	2	no
Roustit 2018	double-blind, series of N-of-1 trials	sildenafil "on demand" vs placebo	40 mg/day vs 80 mg/day, on-demand	France	12	12	100	7.74	no
Rupp 1987	double-blind, crossover	nicardipine vs placebo	40 mg/day	USA (Iowa)	15	15	100	4	no
Russel 1985	double-blind,	prazosin vs placebo	1 to 4 mg/day	USA (Texas)	14	9	75	2	no

	crossover								
Rustin 1984	double-blind, crossover		100 mg/day	UK	10	10	76	4	.
Sadik 2010	double-blind, parallel	atorvastatin vs placebo	20 mg/day	UK	36	NA	100	8	yes
Sauza 1984	double-blind, parallel	nifedipine vs placebo	30-60 mg/day (mean=42.2)	Mexico	25	18	94	10	no
Schiopu 2009	double-blind, crossover	tadalafil vs placebo	20 mg/day	USA (Michigan)	45	39	100	4	no
Seibold 2017	double-blind, parallel	treprostinil vs placebo	0.5 to 32 mg/day (mean=7.5 mg/day)	US, Canada, UK	147	147	100	20	yes
Shenoy 2010	double-blind, crossover	tadalafil vs placebo	20 mg/day	India	25	24	100	6	Yes
Smith 1982	double-blind, crossover	nifedipine vs placebo	40 mg/day	Canada (Ontario)	17	17	71	4	no
Surwit 1984	double-blind, crossover	prazosin vs placebo	3 mg/day	UK	20	NA	100	8	no
Varela-Aguilar 1997	double-blind, crossover	nifedipine vs misoprostol	40 mg/day vs 400 µg/day	Spain	20	20	100	1.4	no
Wigley 1992	double-blind, parallel	iloprost vs placebo	1 to 2 ng/kg/min 5 days	USA	35	33	100	10	no
Wigley 1994	double-blind, parallel	iloprost vs placebo	1 to 2 ng/kg/min 5 days	USA	131	122	100	6	no
Wigley 1998	double-blind,	iloprost PO vs placebo	100 µg/day	USA (Maryland)	308	308	100	6	no

Wollersheim 1991	parallel double-blind, crossover	nicardipine vs placebo	90 mg/day	Netherland	9	NA	100	3	no
Yardumian 1988	double-blind, crossover	iloprost vs placebo	1 to 3 ng/kg/min, 5h/day, 3 days	UK	12	9	100	6	no

NA: not available

Appendix Table 5. Available outcomes per study.

10	Main outcome	Frequency	Severity	Duration	Acceptability (drop out)	Tolerability
Abou-Raya 2008	DU		VAS		✓	✓
Agarwal 2010	RP	✓	RCS	✓	✓	✓
Andrigueti 2016	other	✓	RCS	✓	✓	✓
Belch 1983	RP				✓	✓
Belch 1995	RP	✓	Severity score 0-3	✓	✓	✓
Bello 2017	RP		RCS		✓	✓
Black 1998	RP	✓	RCS	✓	✓	✓
Bose 2015	RP		RCS		✓	✓
Caglayan 2012	RP	✓	RCS	✓	✓	✓
Chung 2009	RP	✓	RCS		✓	✓
Coffman 1989	RP	✓	Severity score	✓	✓	

Coleiro 2001	RP	✓	VAS 0-10		✓	✓
Correa 2014	other	✓	Severity score		✓	✓
Denton 2017	RP	✓		✓	✓	✓
Ettinger 1984	RP	✓	Severity score 0-3	✓	✓	✓
Fries 2005	RP	✓	RCS	✓	✓	✓
Gliddon 2007	DU		VAS 0- 10cm*		✓	✓
Hachulla 2015	DU		VAS 0- 100mm		✓	✓
Hawkins 1986	RP	✓	VAS 0-100			✓
Herrick 2000	RP	✓		✓	✓	✓
Herrick 2011	RP	✓	RCS	✓	✓	✓
Kahan 1985	RP	✓	VAS		✓	✓
Kahan 1987	RP	✓	Severity score 0-4		✓	✓
Khanna 2016	other		SHAQ-VAS		✓	✓
Khanna (DUAL 1) 2016	DU		SHAQ-VAS		✓	✓
Khanna (DUAL 2) 2017	DU		SHAQ-VAS		✓	✓
Korn 2004	DU		SHAQ-VAS		✓	✓
Lau 1993	RP	✓	Severity score 0-3	✓	✓	✓

Laumann 2014	RP	✓	RCS	✓	✓	✓
Lee 2014	RP	✓	RCS	✓	✓	✓
Matucci-Cerinic 2011	DU		SHAQ-VAS		✓	✓
Mc Hugh 1988	RP				✓	✓
Meyrick Thomas 1987	RP	✓		✓	✓	✓
Mohrland 1985	RP	✓	VAS		✓	✓
Nagaraja 2019	DU	✓	RCS	✓	✓	✓
Nguyen 2010	RP				✓	✓
Ortonne 1989	other				✓	✓
NCT01090492 2018	RP	✓	RCS	✓	✓	✓
Rademaker 1989	RP				✓	✓
Rajagopalan 2003	RP	✓	Severity score 0-9		✓	✓
Rodeheffer 1983	RP	✓			✓	✓
Roustit 2018	RP	✓	RCS	✓	✓	✓
Rupp 1987	RP	✓	Severity score 1-4		✓	✓
Russel 1985	RP	✓			✓	✓
Rustin 1984	RP	✓		✓	✓	✓
Sadik 2010	other		VAS		✓	✓
Sauza 1984	RP	✓	VAS 0-10			
Schiopu 2009	RP	✓	RCS	✓	✓	✓
Seibold 2017	DU		SHAQ-VAS		✓	✓

Shenoy 2010	RP	✓	RCS	✓	✓	✓
Smith 1982	RP	✓	VAS 0-10cm		✓	✓
Surwit 1984	RP	✓			✓	✓
Varela-Aguilar 1997	RP	✓	Severity score		✓	✓
Wigley 1992	DU	✓	Severity score 1-4	✓	✓	✓
Wigley 1994	RP	✓	Severity score 0-10		✓	✓
Wigley 1998	RP	✓	RCS	✓	✓	✓
Wollersheim 1991	RP	✓	VAS 0-10cm	✓	✓	✓
Yardumian 1988	RP	✓			✓	

*data not used because no data per group available.

VAS: Visual analog scale; RCS: Raynaud's Condition Score; RP: Raynaud's Phenomenon; DU: Digital Ulcer; SHAQ-VAS: VAS for the vascular symptoms (Raynaud's) subcomponent of the Scleroderma Health Assessment Questionnaire.

Appendix Table 6. Result of the pairwise meta-analysis.

Result of efficacy outcomes are expressed as Mean Difference MD (95% CI) and safety outcomes as Incidence Rate Ratio IRR (95% CI).

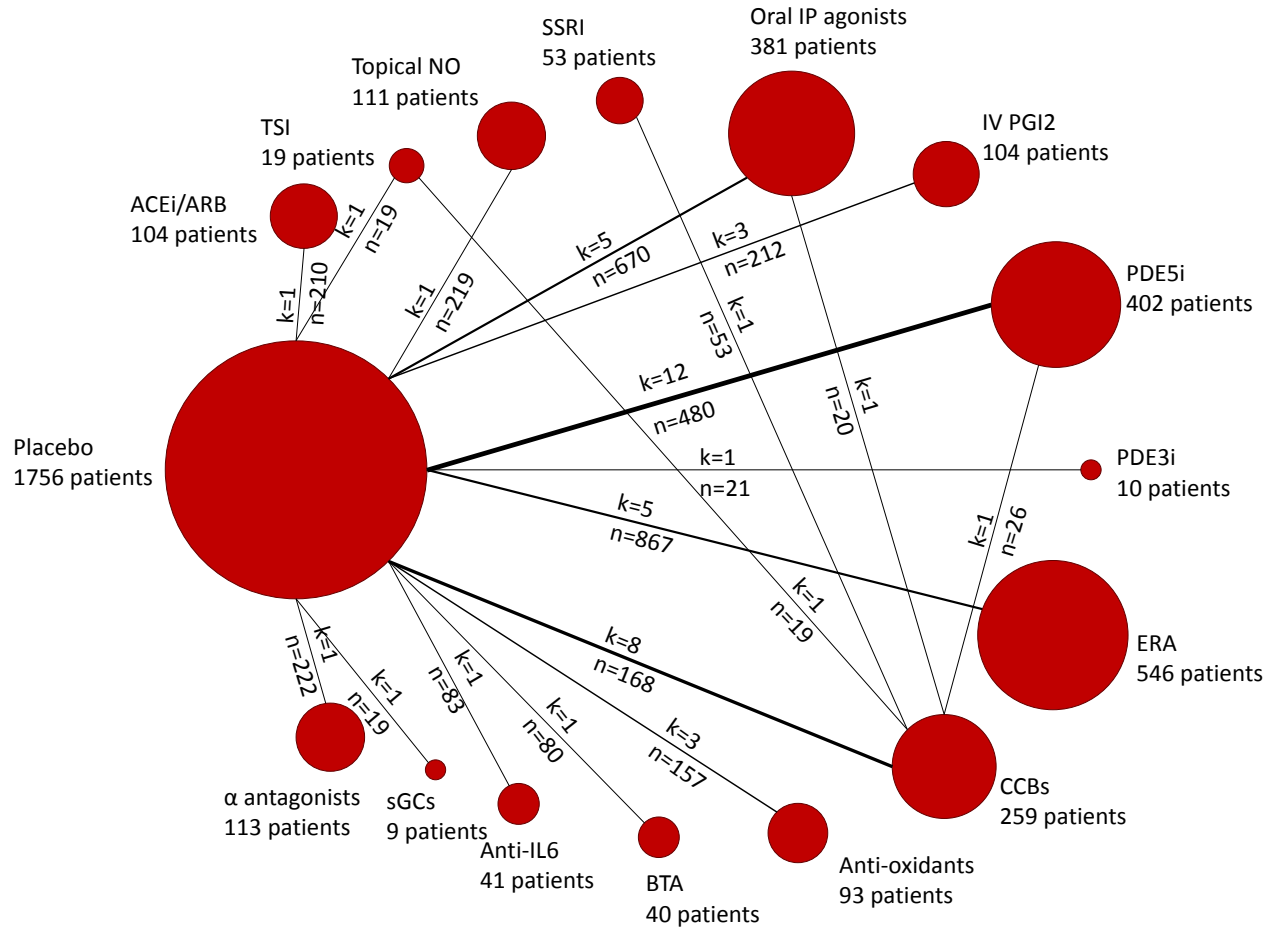
Comparisons	Frequency			Severity			Duration			Acceptability			Tolerability		
	n	MD (95% CI)	I ²	n	MD (95% CI)	I ²	n	MD (95% CI)	I ²	n	IRR (95% CI)	I ²	n	IRR (95% CI)	I ²
PDE5i vs placebo	11	<u>-0.31 (-0.57, -0.04)</u>	0%	12	-0.30 (-0.62, 0.01)	0%	11	<u>-3.05 (-5.79, -0.31)</u>	0%	8	<u>2.64 (1.40, 4.99)</u>	0%	6	<u>3.20 (1.45, 7.09)</u>	15%
CCBs vs placebo	10	<u>-0.38 (-0.66, -0.09)</u>	0%	8	<u>-1.07 (-1.56, -0.59)</u>	28%	3	-4.00 (-10.92, 2.92)	0%	4	1.67 (0.40, 6.97)	0%	5	3.00 (0.97, 9.30)	14%
IV PGI2 analogs vs placebo	4	-0.24 (-0.69, 0.21)	0%	3	-0.36 (-1.74, 1.01)	64%	1	-2.50 (-107.63, 102.62)	.	6	0.91 (0.47, 1.79)	0%	4	<u>2.73 (1.14, 6.50)</u>	0%
α adrenoreceptors antagonists vs placebo	3	-0.71 (-2.27, 0.85)	74%	1	-0.09 (-0.22, 0.04)	.	1	-3.1 (-6.39, 0.19)	.	3	1.46 (0.78, 2.76)	0%	2	4.53 (0.47, 43.23)	0
Oral IP agonists vs placebo	5	0.16 (-0.40, 0.72)	73%	5	<u>-0.40 (-0.72, -0.08)</u>	0%	5	-2.32 (-6.74, 2.11)	29%	6	<u>1.84 (1.23, 2.75)</u>	48%	6	<u>2.97 (1.66, 5.31)</u>	19%
Anti-oxidants vs placebo	2	-0.38 (-0.89, 0.14)	0%	3	-0.75 (-1.72, 0.23)	0%	1	-0.52 (-18.10, 19.15)	.	2	0.67 (0.19, 2.36)	0%	2	0.60 (0.14, 2.51)	0%
Topical NO donor vs placebo	1	-0.20 (-0.67, 0.27)	.	1	-0.28 (-0.81, 0.25)	.				1	0.39 (0.08, 2.01)	.	1	1.95 (0.18, 21.46)	.
PDE3i vs placebo	1	0.00 (-0.75, 0.75)	.	1	0.44 (-1.40, 2.29)	.				1	6.12 (0.32, 118.58)	.	1	6.12 (0.32, 118.58)	.
TSI vs placebo	1	0.13 (-0.88, 1.14)	.	1	-0.54 (-5.36, 4.28)	.	1	-0.40 (-8.82, 8.02)	.	1	1 (0.02, 50.40)	.	1	1.00 (0.02, 50.40)	.
ACEi/ARB vs placebo			.							1	1.64 (0.97, 2.76)	.	1	<u>2.68 (1.19, 6.04)</u>	.
ERA vs placebo				5	<u>-0.17 (-0.32, -0.02)</u>	0%				5	1.22 (0.89, 1.67)	0%	4	1.13 (0.76, 1.69)	0%
BTA vs placebo				1	0.00 (-1.01;1.01)	.						.			
Anti-IL6 vs placebo				1	-0.45 (-1.83, 0.93)	.				1	1.21 (0.54, 2.70)	.	1	0.96 (0.46, 1.98)	.

sGCs vs placebo	1	1.82 (0.20, 3.44)	.	1	1.67 (-0.18, 3.52)	.						1	2.67 (0.28, 25.64)	.
SSRI vs CCB				1	-0.70 (-1.74, 0.34)	.			1	0.44 (0.19,1.02)		1	0.44 (0.19,1.02)	.
CCB vs TSI				1	-0.43 (-5.28, 4.41)	.	1	-3.30 (-10.72, 3.72)	.		6.32 (0.76,52.46)		1	6.32 (0.76,52.46)
PDE5i vs CCB							1	-0.91 (-6.25, 4.43)	.	1	2.00 (0.37, 10.92)		1	4.29 (0.48, 38.34)
CCB vs PG_IV										1	4.36 (0.93, 20.55)		1	7.09 (0.85, 58.90)
CCB vs PG_PO				1	-0.10 (-0.69, 0.49)	.							1	1.67 (0.61, 4.59)

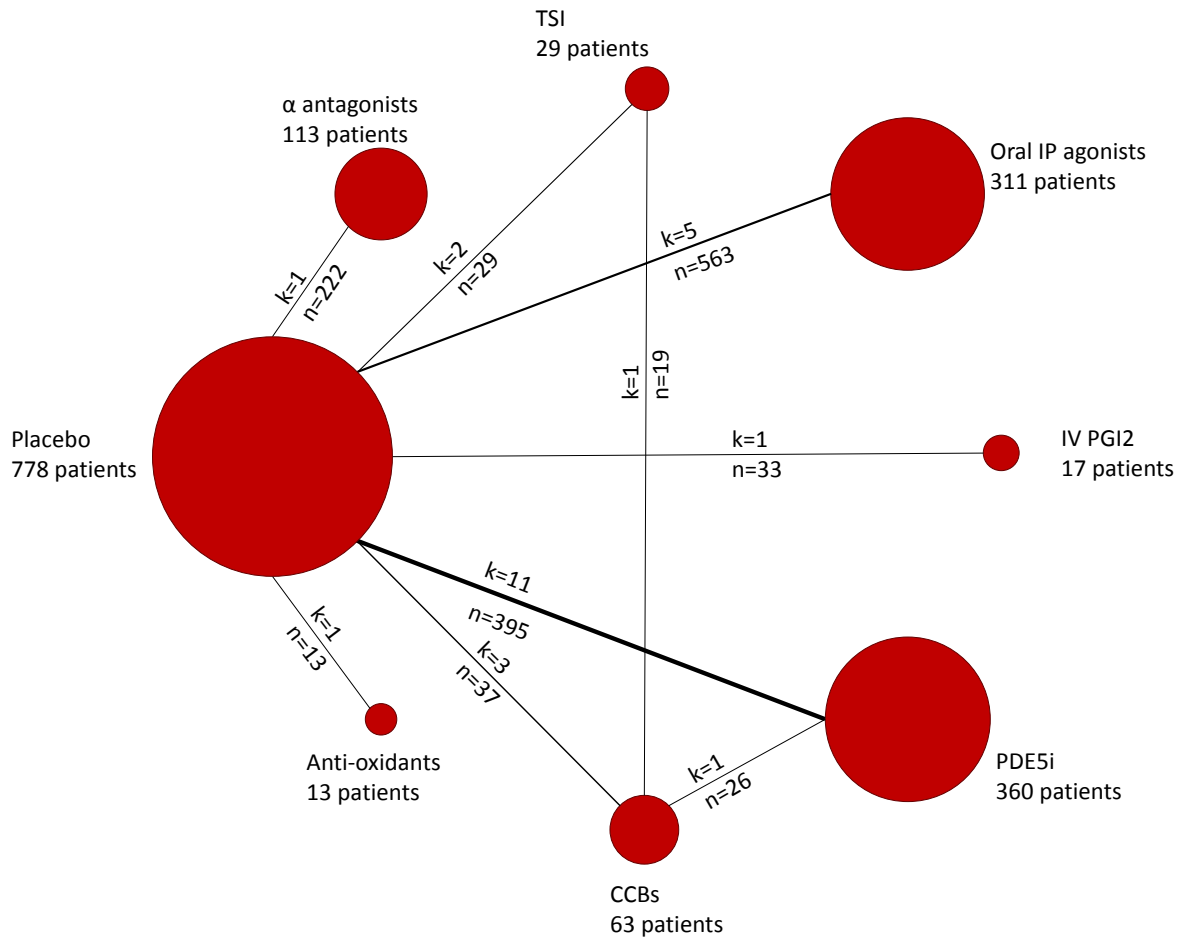
ACEi/ARB : angiotensin-converting enzyme (*ACE*) inhibitor and an angiotensin receptor blocker (ARB); BTA: Botulinum Toxin type A; ERA : endothelin receptor antagonist ; PDE5i: phosphodiesterase 5 inhibitors. CCB: Calcium channel blockers. IV PGI2: intravenous prostacyclin analogues; Oral IP agonists: Oral prostacyclin analogs / non-prostanoid IP-receptor agonists ; PDE3i : phosphodiesterase 3 inhibitors; TSI : Thromboxane Synthetase Inhibitors.

Appendix Figure 4. Graphical representation of the network of included trials for severity, duration and acceptability outcomes. The thickness of lines between nodes is proportional to the number of trials comparing the treatments. The sizes of the nodes are proportional to the number of patients in each treatment group.

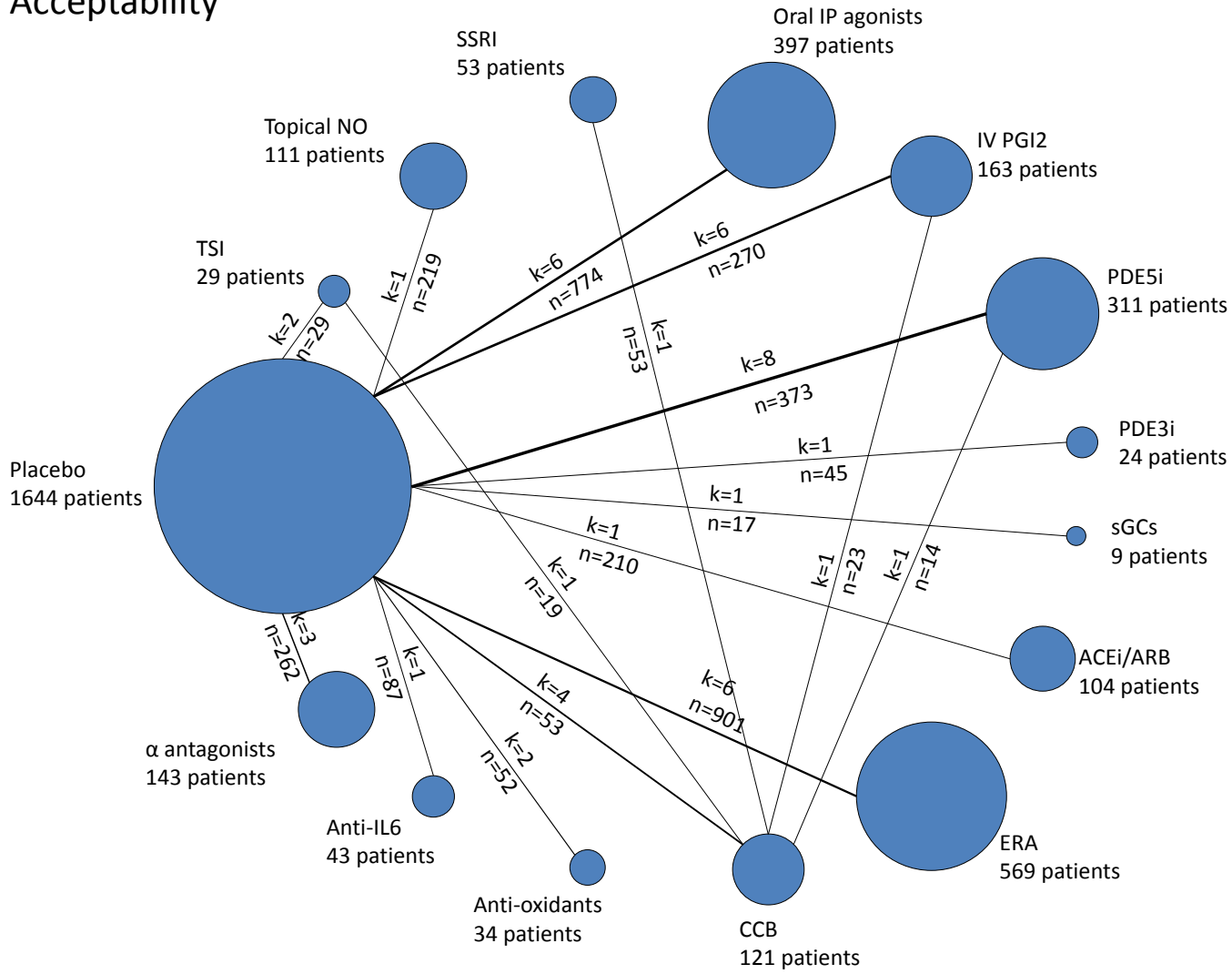
Severity of RP attacks



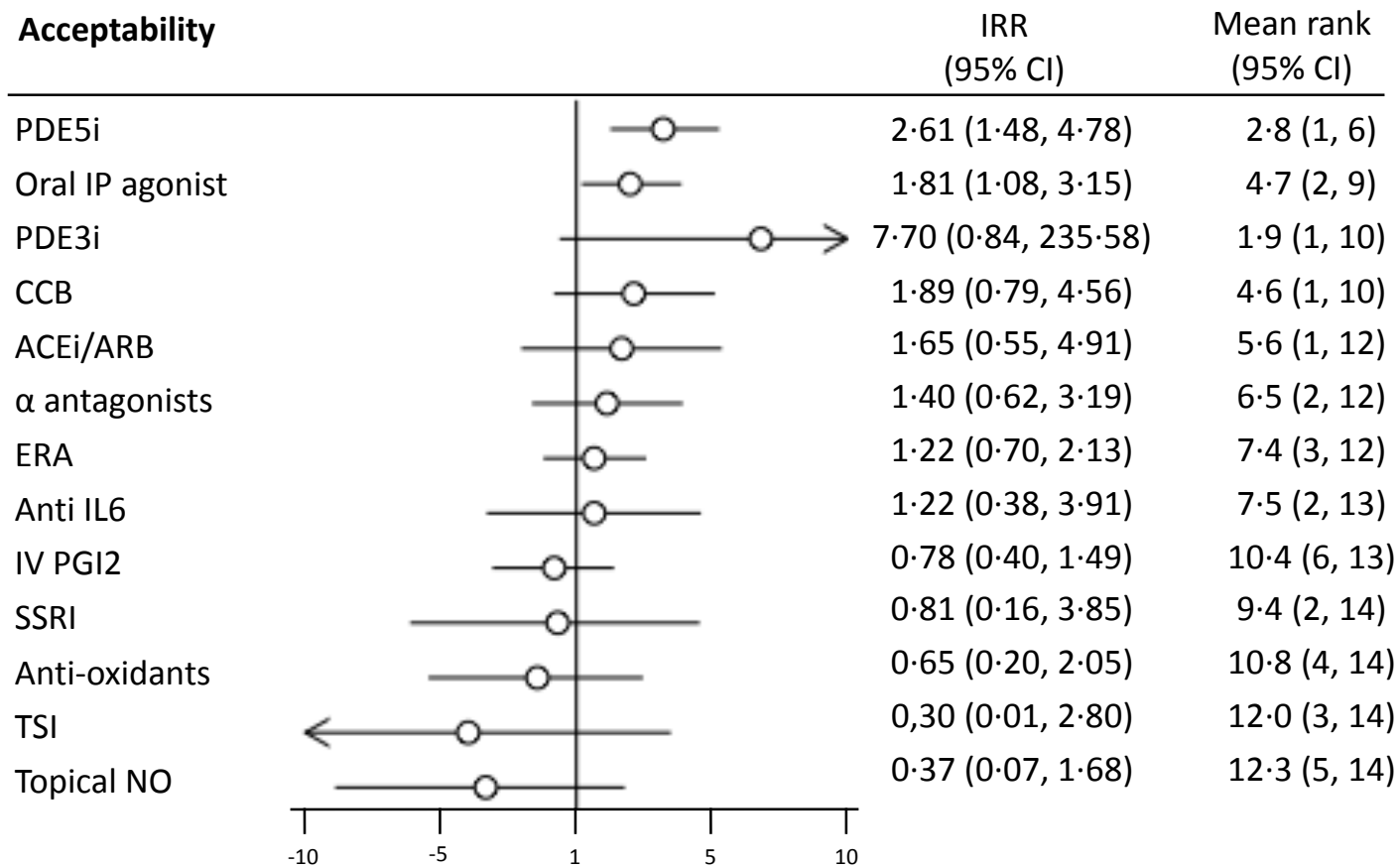
Duration of RP attacks



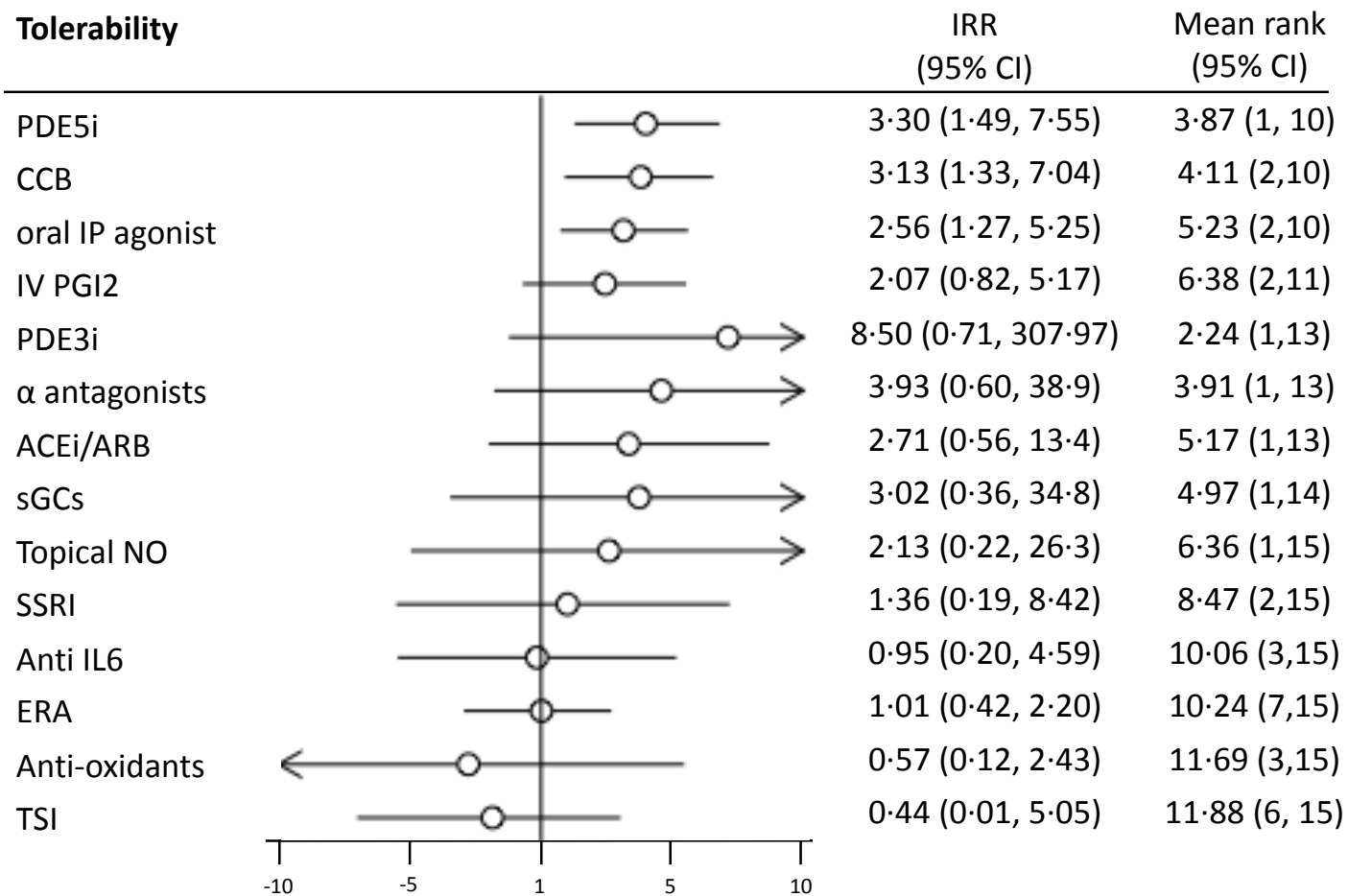
Acceptability



Appendix Figure 5. Forest plots of the network meta-analysis results for safety outcomes. Drug classes are hierarchized according to the lower boundary of the mean rank 95% Credibility Interval (CrI) Data are Incidence Rate Ratios (95% CrI).



Tolerability



Appendix Figure 6. League tables of the network meta-analysis results. Drug classes are hierarchized according to the lower boundary of the mean rank 95% Credibility Interval (CrI) and GRADE evaluations are represented by checked circles, showing very low, low, moderate or high level of evidence. Data are Mean Differences (95% CrI) for efficacy outcomes and Incidence Rate Ratios (95% CrI) for safety outcomes. Comparisons should be read from left to right. The estimate is located at the intersection of the column-defining treatment and the row-defining treatment.

A. Mean difference for daily frequency of RP attacks

PDE5i ☐ ⊗ ⊗ ⊗											
Mean rank 4.1 (1.8)	CCBs ☐ ☐ ⊗ ⊗										
-0.01 (-0.46, 0.44)	Mean rank 4.2 (2, 8)	SSRI ☐ ☐ ☐ ⊗									
0.40 (-0.66, 1.45)	0.40 (-0.56, 1.37)	Mean rank 2.6 (1,10)	Anti oxidants ☐ ☐ ⊗ ⊗								
0.01 (-0.71, 0.72)	0.02 (-0.69, 0.73)	-0.38 (-1.58, 0.81)	Mean rank 4.4 (1,11)	IV PGI2 ☐ ☐ ☐ ⊗							
-0.10 (-0.72, 0.52)	-0.10 (-0.70, 0.52)	-0.5 (-1.64, 0.64)	-0.12 (-0.94, 0.72)	Mean rank 5.3 (1,11)	Topical NO ☐ ☐ ☐ ⊗						
-0.16 (-0.92, 0.59)	-0.16 (-0.91, 0.6)	-0.56 (-1.77, 0.66)	-0.17 (-1.10, 0.76)	-0.06 (-0.93, 0.79)	Mean rank 6.0 (1,11)	α antagonists ☐ ☐ ☐ ⊗					
-0.24 (-0.73, 0.33)	-0.23 (-0.71, 0.34)	-0.63 (-1.70, 0.49)	-0.25 (-0.98, 0.56)	-0.13 (-0.78, 0.56)	-0.07 (-0.83, 0.77)	Mean rank 6.8 (2,11)	PDE3i ☐ ☐ ☐ ⊗				
-0.36 (-1.30, 0.58)	-0.35 (-1.29, 0.58)	-0.75 (-2.10, 0.58)	-0.37 (-1.46, 0.71)	-0.26 (-1.30, 0.77)	-0.20 (-1.32, 0.91)	-0.12 (-1.14, 0.81)	Mean rank 7.6 (1,11)	TSI ☐ ☐ ☐ ⊗			
-0.35 (-1.18, 0.49)	-0.34 (-1.13, 0.47)	-0.75 (-1.99, 0.52)	-0.36 (-1.34, 0.65)	-0.24 (-1.18, 0.68)	-0.19 (-1.21, 0.84)	-0.12 (-1.00, 0.74)	0.01 (-1.16, 1.19)	Mean rank 7.6 (1,11)	Placebo		
-0.36 (-0.69, -0.04)	-0.35 (-0.67, -0.04)	-0.76 (-1.77, 0.25)	-0.37 (-1.01, 0.27)	-0.26 (-0.79, 0.26)	-0.20 (-0.88, 0.48)	-0.12 (-0.60, 0.25)	0.00 (-0.88, 0.88)	-0.01 (-0.79, 0.74)	Mean rank 8.4 (6,11)	Oral IP agonists ☐ ☐ ☐ ⊗	
-0.45 (-0.93, 0.01)	-0.45 (-0.89, -0.02)	-0.85 (-1.91, 0.20)	-0.47 (-1.20, 0.25)	-0.35 (-0.99, 0.26)	-0.29 (-1.06, 0.45)	-0.22 (-0.82, 0.27)	-0.10 (-1.05, 0.84)	-0.11 (-0.96, 0.71)	-0.04 (-1.00, 0.94)	Mean rank 9.2 (5,11)	sGCs ☐ ☐ ☐ ⊗
-2.18 (-3.88, -0.47)	-2.17 (-3.87, -0.45)	-2.58 (-4.53, -0.59)	-2.19 (-3.97, -0.39)	-2.08 (-3.83, -0.31)	-2.02 (-3.81, -0.20)	-1.95 (-3.68, -0.22)	-1.82 (-3.72, 0.08)	-1.82 (-3.68, 0.02)	-1.75 (-3.46, -0.01)	-1.71 (-3.64, 0.22)	Mean rank 11.8 (10,12)

B. Mean difference for severity of RP attacks (scale 0-10)

CCB □ □ ⊗ ⊗																
Mean rank 3.5 (2,6)	SSRI □ □ □ ⊗															
0.70 (-0.38, 1.75)	Mean rank 1.9 (1,7)	Oral IP agonists □ □ ⊗ ⊗														
-0.37 (-0.80, 0.07)	-1.05 (-2.24, 0.09)	Mean rank 6.2 (3,10)	PDE5i □ ⊗ ⊗ ⊗													
-0.5 (-1.00, -0.01)	-1.20 (-2.38, -0.03)	-0.14 (-0.57, 0.31)	Mean rank 7.6 (4,12)	IV PGI2 □ □ □ ⊗												
-0.33 (-1.18, 0.5)	-1.03 (-2.40, 0.31)	0.04 (-0.76, 0.82)	0.17 (-0.62, 0.96)	Mean rank 6.4 (2,13)	ERA □ □ ⊗ ⊗											
-0.67 (-1.13, -0.23)	-1.36 (-2.54, -0.23)	-0.31 (-0.69, 0.07)	-0.17 (-0.56, 0.21)	-0.35 (-1.10, 0.42)	Mean rank 9.6 (6,13)	Anti-oxidants □ □ ⊗ ⊗										
-0.11 (-1.26, 0.97)	-0.82 (-2.37, 0.72)	0.26 (-0.86, 1.31)	0.39 (-0.74, 1.44)	0.23 (-1.10, 1.42)	0.57 (-0.53, 1.58)	Mean rank 5.0 (1,14)	Topical NO □ □ □ ⊗									
-0.57 (-1.28, 0.14)	-1.27 (-2.55, 0.02)	-0.20 (-0.87, 0.47)	-0.07 (-0.74, 0.61)	-0.24 (-1.17, 0.70)	0.11 (-0.52, 0.74)	-0.46 (-1.62, 0.77)	Mean rank 8.4 (3,14)	α antagonists □ □ □ ⊗								
-0.75 (-1.26, -0.26)	-1.45 (-2.63, -0.28)	-0.39 (-0.83, 0.05)	-0.25 (-0.70, 0.18)	-0.43 (-1.20, 0.37)	-0.08 (-0.46, 0.3)	-0.64 (-1.68, 0.47)	-0.18 (-0.85, 0.48)	Mean rank 10.6 (6,14)	Placebo							
-0.84 (-1.25, -0.45)	-1.54 (-2.68, -0.41)	-0.48 (-0.80, -0.16)	-0.34 (-0.66, -0.03)	-0.52 (-1.23, 0.22)	-0.17 (-0.38, 0.05)	-0.74 (-1.73, 0.34)	-0.27 (-0.87, 0.32)	-0.09 (-0.40, 0.23)	Mean rank 12.0 (9,14)	Anti-IL6 □ □ ⊗ ⊗						
-0.39 (-1.90, 1.07)	-1.08 (-3.02, 0.73)	-0.03 (-1.51, 1.43)	0.11 (-1.37, 1.56)	-0.06 (-1.67, 1.50)	0.29 (-1.18, 1.70)	-0.28 (-2.05, 1.49)	0.18 (-1.41, 1.70)	0.37 (-1.13, 1.79)	0.46 (-1.00, 1.85)	Mean rank 7.5 (1,15)	BTA □ □ ⊗ ⊗					
-0.83 (-1.97, 0.25)	-1.54 (-3.1, -0.01)	-0.47 (-1.58, 0.61)	-0.34 (-1.44, 0.74)	-0.51 (-1.79, 0.74)	-0.16 (-1.24, 0.88)	-0.74 (-2.18, 0.71)	-0.27 (-1.49, 0.90)	-0.08 (-1.19, 0.99)	0.01 (-1.05, 1.03)	-0.45 (-2.25, 1.37)	Mean rank 10.6 (3,15)	ACEi/ARB □ □ □ ⊗				
-0.86 (-1.65, -0.09)	-1.56 (-2.88, -0.22)	-0.49 (-1.23, 0.24)	-0.36 (-1.10, 0.38)	-0.52 (-1.51, 0.46)	-0.18 (-0.88, 0.52)	-0.74 (-1.94, 0.46)	-0.29 (-1.18, 0.60)	-0.1 (-0.83, 0.63)	-0.01 (-0.68, 0.65)	-0.47 (-2.02, 1.15)	-0.03 (-1.22, 1.24)	Mean rank 11.2 (4,15)	TSI □ □ □ ⊗			
-0.48 (-4.31, 3.62)	-1.19 (-5.09, 3.01)	-0.1 (-3.94, 3.97)	0.03 (-3.81, 4.11)	-0.14 (-4.01, 3.98)	0.20 (-3.62, 4.27)	-0.31 (-4.35, 3.74)	0.09 (-3.79, 4.21)	0.28 (-3.55, 4.36)	0.37 (-3.45, 4.44)	-0.06 (-4.13, 4.21)	0.40 (-3.64, 4.56)	0.39 (-3.48, 4.52)	Mean rank 8.3 (1,16)	PDE3i □ □ □ ⊗		
-1.27 (-3.14, 0.60)	-1.96 (-4.15, 0.19)	-0.91 (-2.77, 0.94)	-0.77 (-2.64, 1.08)	-0.94 (-2.89, 1.02)	-0.61 (-2.43, 1.24)	-1.17 (-3.24, 0.98)	-0.70 (-2.63, 1.20)	-0.51 (-2.38, 1.33)	-0.43 (-2.26, 1.39)	-0.86 (-3.21, 1.44)	-0.44 (-2.50, 1.74)	-0.42 (-2.36, 1.54)	-0.81 (-5.19, 3.47)	Mean rank 12.1 (1,16)	sGSs □ □ □ ⊗	
-2.48 (-4.39, -0.60)	-3.17 (-5.37, -1.02)	-2.11 (-3.98, -0.25)	-1.98 (-3.86, -0.12)	-2.15 (-4.12, -0.20)	-1.8 (-3.67, 0.05)	-2.37 (-4.5, -0.24)	-1.91 (-3.85, 0.05)	-1.72 (-3.59, 0.14)	-1.63 (-3.48, 0.19)	-2.08 (-4.41, 0.24)	-1.63 (-3.75, 0.44)	-1.63 (-3.58, 0.34)	-2.04 (-6.47, 2.23)	-1.20 (-3.88, 1.37)	Mean rank 15.2 (9,16)	

C. Mean difference for duration of each RP attack

PDE5i □ □ ⊗ ⊗									
Mean rank 3.20 (1,6)	CCBs □ □ □ ⊗			α antagonists □ □ □ ⊗			Oral IP agonists □ □ □ ⊗		
-0.33 (-5.38 , 4.64)	Mean rank 3.53 (1,7)	α antagonists □ □ □ ⊗			Mean rank 3.55 (1,7)	Oral IP agonists □ □ □ ⊗			IV PGI2 □ □ ⊗ ⊗
-0.29 (-6.77 , 5.85)	0.02 (-7.44 , 7.32)	α antagonists □ □ □ ⊗			Mean rank 3.55 (1,7)	Oral IP agonists □ □ □ ⊗			IV PGI2 □ □ ⊗ ⊗
-0.77 (-6.74 , 5.21)	-0.4 (-7.54 , 6.67)	-0.47 (-7.76 , 7.19)	Mean rank 3.92 (1,7)	IV PGI2 □ □ ⊗ ⊗			Anti- oxidants □ □ ⊗ ⊗	TSI □ □ □ ⊗	
2.25 (-100.56 , 102.52)	2.44 (-100.36 , 102.89)	2.59 (-100.21 , 102.74)	2.90 (-99.79 , 103.03)	Mean rank 4.93 (1,8)	Anti- oxidants □ □ ⊗ ⊗			TSI □ □ □ ⊗	
-3.77 (-23.19 , 15.54)	-3.41 (-23.36 , 16.22)	-3.49 (-23.41 , 16.38)	-3.05 (-23.02 , 16.58)	-5.38 (- 109.72 , 99.16)	Mean rank 5.04 (1,8)	TSI □ □ □ ⊗			Placebo
-4.19 (-11.61 , 3.00)	-3.83 (-11.11 , 3.30)	-3.85 (-12.66 , 4.86)	-3.43 (-12.18 , 5.07)	-6.22 (- 106.81 , 96.68)	-0.37 (-20.71 , 20.17)	Mean rank 5.89 (2,8)	Placebo		
-3.42 (-6.63 , -0.29)	-3.06 (-8.07 , 1.91)	-3.09 (-8.58 , 2.41)	-2.62 (-7.84 , 2.38)	-5.63 (- 105.74 , 97.00)	0.38 (-18.65 , 19.45)	0.76 (-6.07 , 7.67)	Mean rank 5.94 (4,8)	Placebo	

D. Acceptability

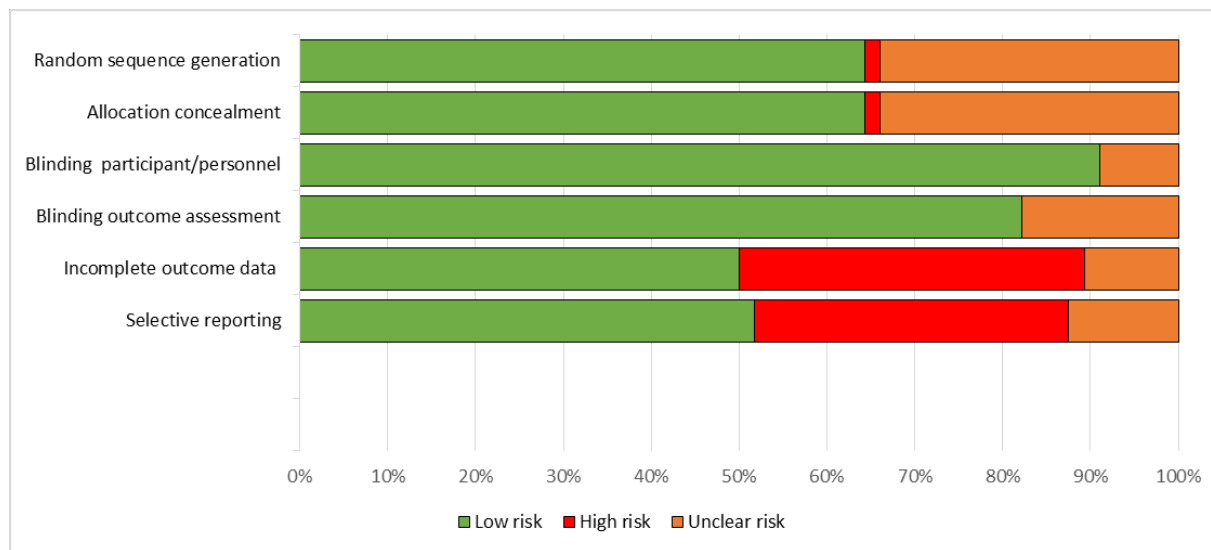
PDE5i

Mean rank 2·8 (1, 6)	Oral IP agonists																
1·45 (0·65 , 3·22)	Mean rank 4·7 (2,9)	PDE3i															
0·34 (0·01 , 3·46)	0·23 (0·01 , 2·36)	Mean rank 1·9 (1,10)	CCBs														
1·39 (0·52 , 3·78)	0·96 (0·35 , 2·72)	4·18 (0·37 , 139·77)	Mean rank 4·6 (1,10)	ACEI/ARB													
1·60 (0·46 , 5·58)	1·11 (0·33 , 3·78)	4·81 (0·39 , 174·16)	1·15 (0·28 , 4·57)	Mean rank 5·6 (1,12)	α antagonists												
1·88 (0·68 , 5·16)	1·30 (0·49 , 3·49)	5·58 (0·51 , 192·48)	1·35 (0·40 , 4·44)	1·17 (0·3 , 4·57)	Mean rank 6·5 (2,12)	ERA											
2·16 (0·97 , 4·85)	1·49 (0·69 , 3·25)	6·42 (0·63 , 210·61)	1·55 (0·55 , 4·35)	1·35 (0·39 , 4·62)	1·15 (0·43 , 3·10)	Mean rank 7·4 (3,12)	Placebo										
<u>2·61 (1·48 , 4·81)</u>	<u>1·82 (1·07 , 3·16)</u>	7·77 (0·83 , 247·15)	1·88 (0·79 , 4·57)	1·65 (0·55 , 4·9)	1·40 (0·62 , 3·19)	1·22 (0·70 , 2·14)	Mean rank 9·1 (6,12)	Anti-IL6									
2·16 (0·58 , 8·08)	1·49 (0·41 , 5·53)	6·49 (0·51 , 235·1)	1·55 (0·36 , 6·69)	1·35 (0·27 , 6·75)	1·15 (0·28 , 4·85)	1·00 (0·28 , 3·71)	0·82 (0·25 , 2·66)	Mean rank 7·5 (2,13)	IV PGI2								
<u>3·35 (1·43 , 8·25)</u>	<u>2·32 (1·02 , 5·58)</u>	9·97 (0·96 , 323·76)	2·41 (0·90 , 6·62)	2·10 (0·60 , 7·69)	1·79 (0·64 , 5·26)	1·55 (0·67 , 3·78)	1·28 (0·67 , 2·51)	1·55 (0·41 , 6·05)	Mean rank 10·4 (6,13)	SSRI							
3·22 (0·63 , 17·64)	2·25 (0·43 , 12·43)	9·97 (0·61 , 407·48)	2·32 (0·63 , 9·03)	2·03 (0·30 , 14·3)	1·73 (0·30 , 10·59)	1·51 (0·29 , 8·33)	1·23 (0·26 , 6·23)	1·51 (0·21 , 11·13)	0·97 (0·18 , 5·10)	Mean rank 9·4 (2,14)	Anti-oxidants						
<u>4·01 (1·11 , 15·33)</u>	2·77 (0·79 , 10·28)	12·18 (0·94 , 441·42)	2·89 (0·67 , 12·68)	2·51 (0·52 , 12·55)	2·14 (0·53 , 9·12)	1·86 (0·52 , 6·96)	1·52 (0·48 , 5·00)	1·86 (0·36 , 9·87)	1·20 (0·32 , 4·62)	1·25 (0·17 , 9·03)	Mean rank 10·8 (4,14)	TSI					
8·5 (0·88 , 284·29)	5·87 (0·61 , 198·34)	<u>29·08 (1·02 , 3041·18)</u>	6·05 (0·70 , 188·67)	5·37 (0·45 , 196·37)	4·57 (0·43 , 157·59)	3·94 (0·40 , 130·32)	3·22 (0·35 , 103·54)	4·01 (0·32 , 149·9)	2·53 (0·25 , 84·77)	2·69 (0·20 , 101·49)	2·16 (0·17 , 77·48)	Mean rank 12·0 (3,14)	Topical NO				
<u>7·17 (1·40 , 41·26)</u>	4·95 (0·98 , 28·22)	<u>22·2 (1·38 , 906·87)</u>	5·16 (0·87 , 32·79)	4·53 (0·69 , 32·14)	3·82 (0·67 , 24·05)	3·32 (0·65 , 19·11)	2·72 (0·59 , 14·15)	3·35 (0·48 , 24·78)	2·14 (0·39 , 12·43)	2·23 (0·23 , 21·33)	1·79 (0·26 , 13·33)	0·83 (0·02 , 13·33)	Mean rank 12·3 (5, 14)				

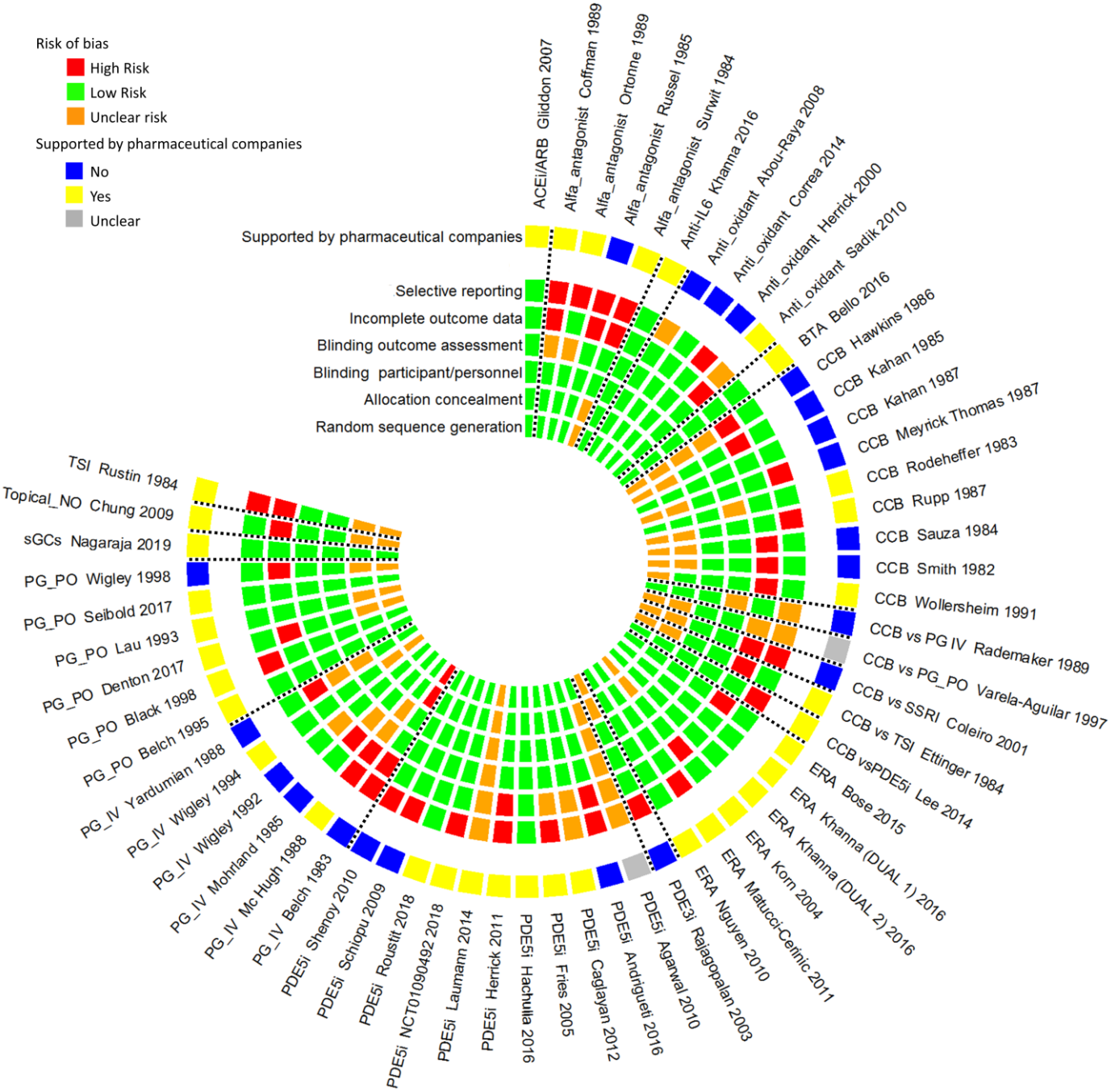
E. Tolerability

PDE5i															
Mean rank 3.9 (1, 10)	CCBs														
1.06 (0.37, 3.29)	Mean rank 4.1 (2,10)	Oral IP agonists													
1.30 (0.44, 3.78)	1.22 (0.44, 3.16)	Mean rank 5.2 (2,10)	IV PGI2												
1.60 (0.48, 5.47)	1.51 (0.47, 4.62)	1.23 (0.40, 3.94)	Mean rank 6.4 (2,11)	PDE3i											
0.39 (0.01, 5.37)	0.36 (0.01, 4.95)	0.30 (0.01, 4.06)	0.24 (0.01, 3.46)	Mean rank 2.2 (1,13)	α antagonists										
0.83 (0.07, 6.69)	0.79 (0.07, 6.17)	0.64 (0.06, 4.90)	0.52 (0.04, 4.31)	2.16 (0.07, 119.1)	Mean rank 3.9 (1, 13)	ACEi/ARB									
1.22 (0.21, 7.24)	1.15 (0.18, 6.55)	0.94 (0.17, 5.42)	0.76 (0.12, 4.71)	3.19 (0.16, 154.47)	1.48 (0.12, 23.57)	Mean rank 5.2 (1,13)	sGCs								
1.08 (0.08, 10.8)	1.01 (0.07, 9.78)	0.84 (0.06, 8.00)	0.68 (0.05, 6.89)	2.83 (0.08, 174.16)	1.3 (0.05, 29.96)	0.88 (0.05, 12.81)	Mean rank 5.0 (1, 14)	Placebo							
3.32 (1.49, 7.61)	3.13 (1.34, 7.03)	2.56 (1.27, 5.31)	2.08 (0.83, 5.16)	8.50 (0.70, 307.97)	3.97 (0.59, 40.04)	2.72 (0.56, 13.46)	3.06 (0.36, 37.71)	Mean rank 10.4 (9,14)	Topical NO						
1.52 (0.11, 16.78)	1.43 (0.10, 15.49)	1.17 (0.09, 12.55)	0.95 (0.07, 10.8)	4.01 (0.12, 262.43)	1.84 (0.08, 44.7)	1.25 (0.06, 19.69)	1.43 (0.05, 39.65)	0.46 (0.04, 4.35)	Mean rank 6.4 (1,15)	SSRI					
2.46 (0.34, 19.69)	2.32 (0.43, 12.94)	1.9 (0.28, 14.3)	1.54 (0.21, 12.3)	6.55 (0.29, 365.04)	3.00 (0.21, 59.15)	2.01 (0.18, 25.03)	2.34 (0.14, 51.94)	0.74 (0.12, 5.16)	1.62 (0.09, 39.25)	Mean rank 8.5 (2,15)	Anti-IL6				
3.49 (0.59, 20.49)	3.29 (0.53, 18.73)	2.69 (0.48, 15.33)	2.18 (0.35, 13.33)	9.12 (0.47, 437.03)	4.18 (0.36, 66.69)	2.86 (0.3, 26.84)	3.25 (0.23, 61.56)	1.05 (0.22, 5.05)	2.29 (0.15, 43.82)	1.42 (0.11, 15.64)	Mean rank 10.1 (3,15)	ERA			
3.32 (1.08, 11.02)	3.13 (0.98, 10.07)	2.56 (0.90, 8.00)	2.08 (0.62, 7.32)	8.67 (0.64, 340.36)	4.01 (0.52, 46.53)	2.72 (0.48, 16.95)	3.10 (0.32, 44.26)	1.00 (0.45, 2.39)	2.2 (0.20, 31.5)	1.35 (0.17, 10.49)	0.95 (0.17, 5.93)	Mean rank 10.2 (7,15)	TSI		
7.69 (0.59, 307.97)	7.1 (0.64, 257.24)	5.93 (0.48, 230.44)	4.81 (0.35, 192.48)	22.2 (0.57, 3010.92)	9.87 (0.40, 607.89)	6.42 (0.34, 314.19)	7.61 (0.26, 566.8)	2.29 (0.2, 85.63)	5.42 (0.18, 379.93)	3.19 (0.16, 156.02)	2.25 (0.12, 108.85)	2.29 (0.17, 91.84)	Mean rank 11.7 (3, 15)	Anti-oxidants	
5.81 (1.11, 33.12)	5.47 (1.00, 30.27)	4.48 (0.90, 24.53)	3.63 (0.65, 21.33)	15.33 (0.84, 713.37)	7.03 (0.63, 109.95)	4.76 (0.56, 42.95)	5.47 (0.40, 102.51)	1.75 (0.41, 8.08)	3.86 (0.26, 72.97)	2.36 (0.21, 25.53)	1.67 (0.20, 14.88)	1.73 (0.32, 9.68)	0.32 (9.68, 0.74)	Mean rank 11.9 (6, 15)	

Appendix Figure 7. Overall Cochrane risk of bias assessment map of included studies.



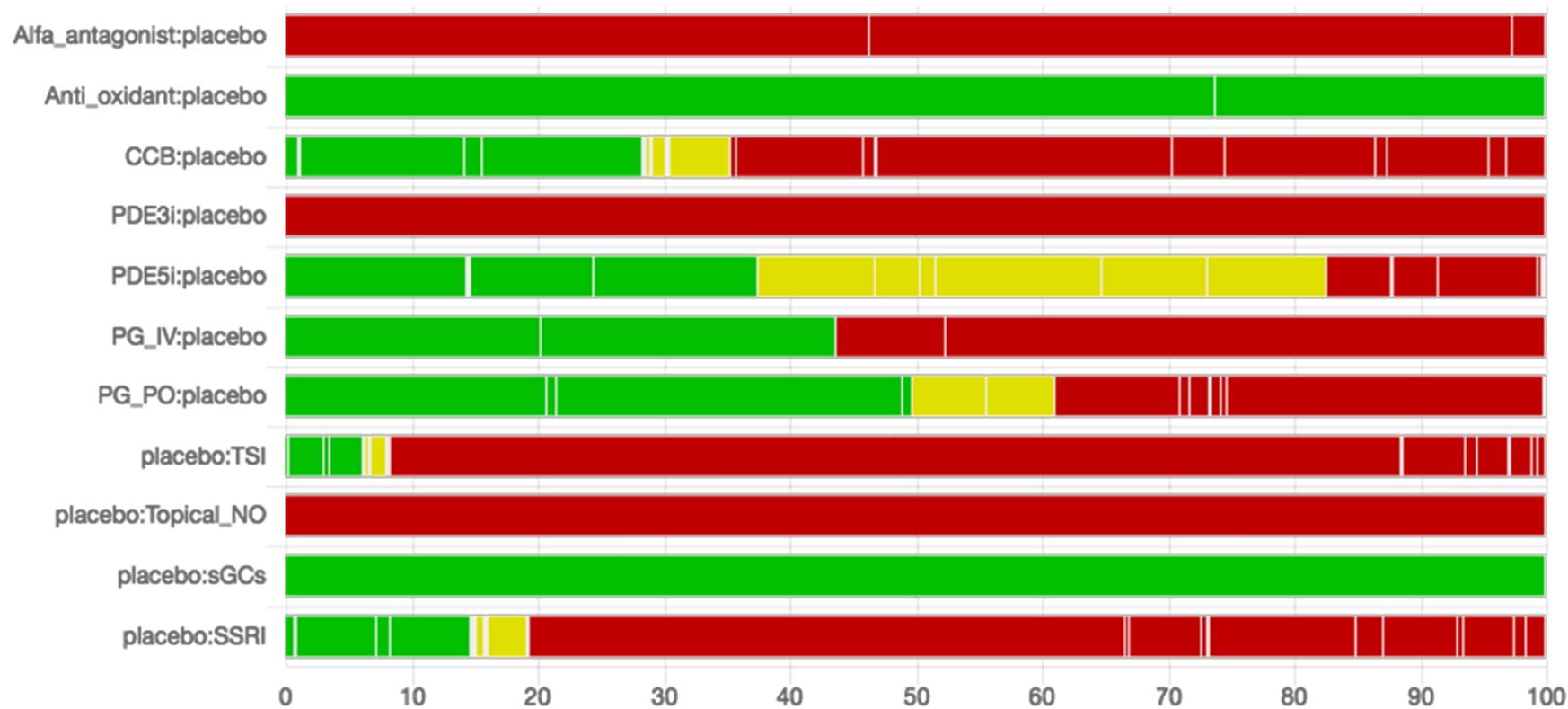
Appendix Figure 8. Circular plot representing the Cochrane domain-specific risk of bias according to each drug class. We considered a study as being “supported by a pharmaceutical company” when it was indicated anywhere in the text that the trial was at least partly funded and/or sponsored by the company which manufactured or marketed the drug being assessed, or if one or more authors were affiliated with the company in question.



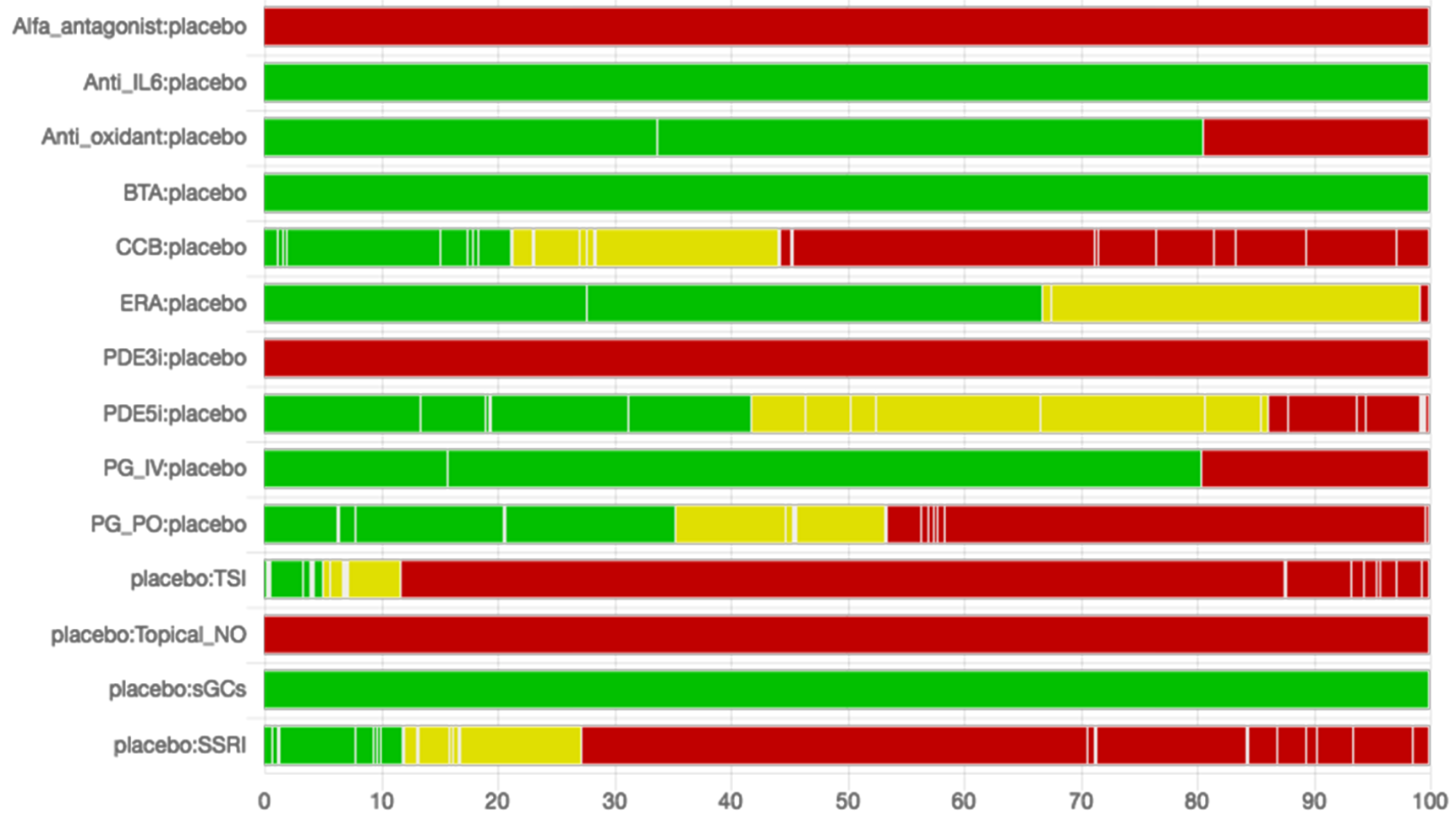
Appendix Figure 9. Study limitations for each drug pairwise estimate versus placebo for efficacy outcomes.

The following figures were generated through the Confidence in Network Meta-Analysis Software (CINeMA) and present the risk of bias for each pairwise estimate versus placebo according to the contribution of each study in the estimate. Low risk of bias is presented in green, unclear risk of bias in yellow and high risk of bias in red.

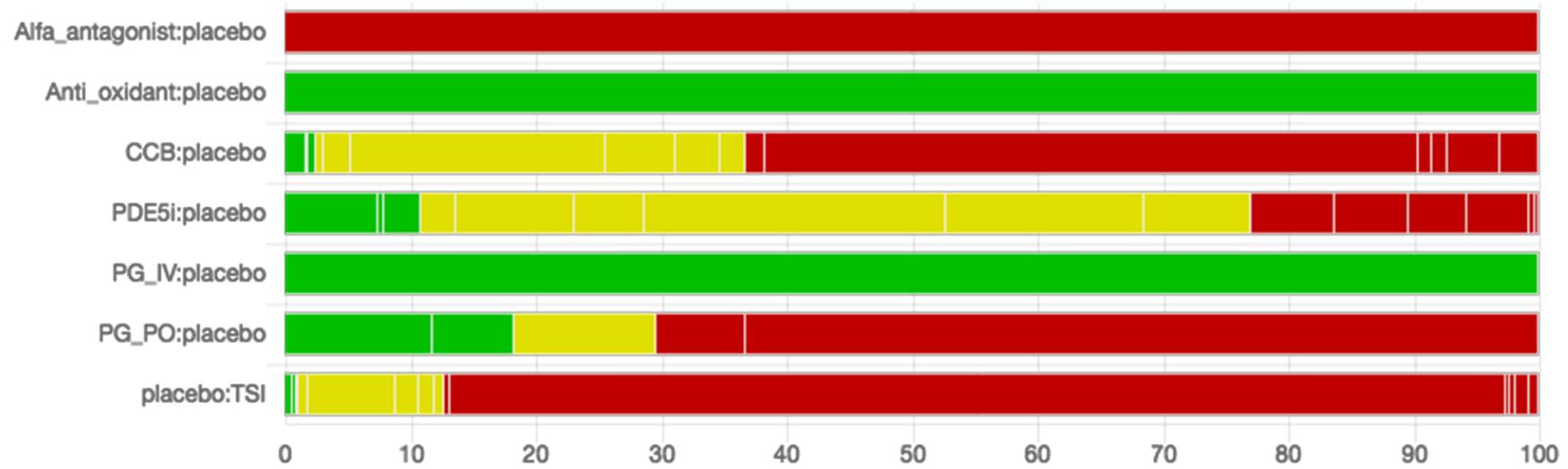
A. Frequency of RP attacks



B. Severity of RP attacks



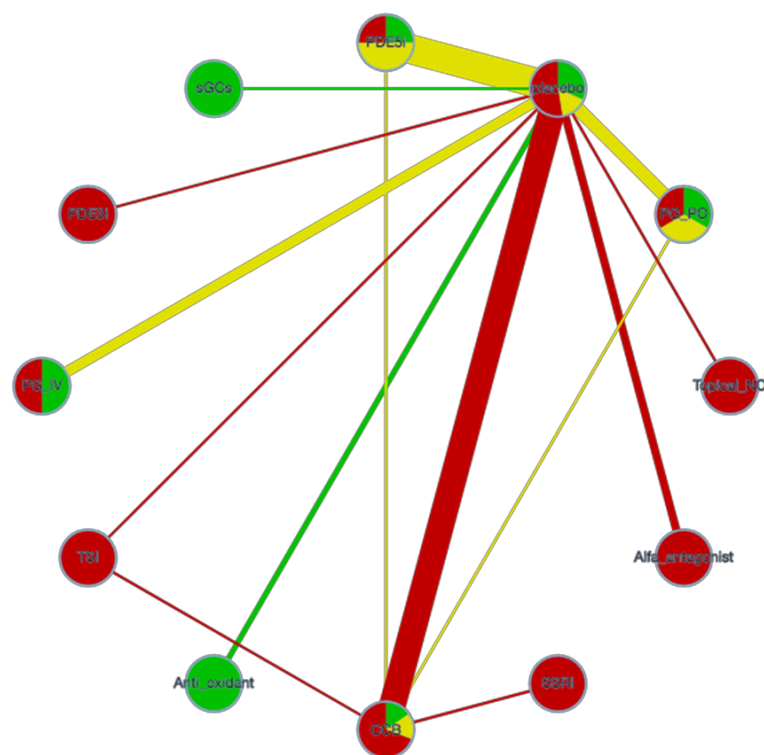
C. Duration of RP attacks



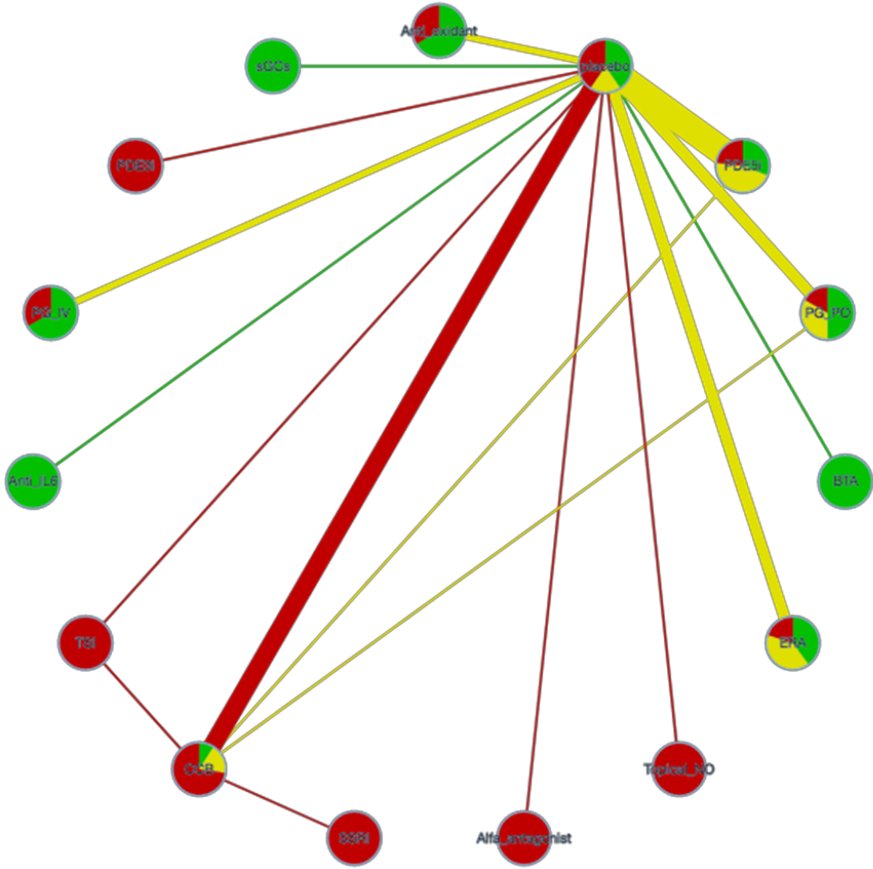
Appendix Figure 10. Network plots for efficacy outcomes, by risk of bias.

The following figures were generated through the Confidence in Network Meta-Analysis Software (CINeMA). Graphical representation of the network of included trials for efficacy outcome. The thickness of lines between nodes is proportional to the number of trials comparing the treatments. The color of each line represents the majority of risk of bias for each comparison. In nodes, the proportion of low/unclear/high risk of bias studies is represented by pie charts. Low risk of bias is in green, unclear risk of bias in yellow and high risk of bias in red.

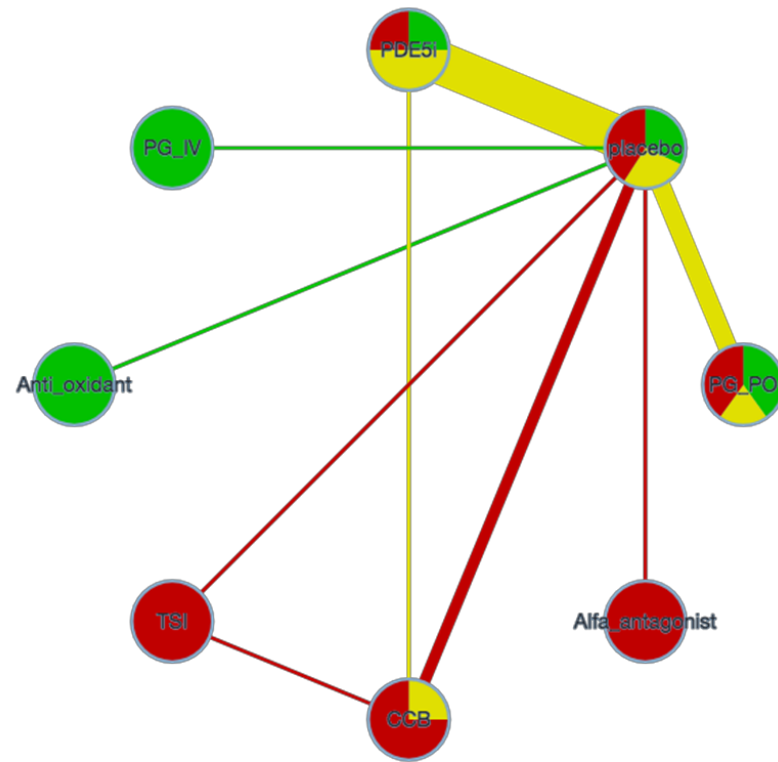
A. Frequency of RP attacks



B. Severity of RP attacks



C. Duration of RP attacks



Appendix Table 7. Result of GRADE evaluation for each drug class versus placebo.

- Daily frequency of RP attacks

Drug classes versus placebo	Study limitations	Imprecision	Heterogeneity and Inconsistency	Indirectness	Publication bias	Overall quality of evidence
PDE5i	Some concerns	Optimal information size not reach in TSA but effect size significant with adjusted threshold	No heterogeneity (0%) in direct comparisons. No node-splitting inconsistency	Treatment effects were not significantly influenced by clinical effect modifiers in meta-regressions, and the point estimate did not widely vary in subgroup analyses.	Egger regression test for funnel plot asymmetry non-significant in pairwise meta-analysis. The meta-regression on sample size is not suggestive of any dominant publication bias	Moderate (downgraded by one level due to study limitations)
CCBs	Some concerns	Optimal information size not reach in TSA but effect size significant with adjusted threshold	No heterogeneity (0%) in direct comparison. No node splitting inconsistency	The treatment effects were not significantly influenced by clinical modifiers in the meta-regression. However, the point estimate widely varied in subgroup analysis.	Egger regression test for funnel plot asymmetry non-significant in pairwise meta-analysis. The meta-regression on sample size is not suggestive of any dominant publication bias	Low (downgraded by two levels due to study limitations and indirectness)
IV PGI2 analogues	Some concerns	Optimal information size not reach in TSA	No significant heterogeneity (0%) in direct comparison. No node splitting inconsistency.	The treatment effects were not significantly influenced by clinical modifiers in the meta-regression. However, the point estimate widely varied in subgroup analysis.	Undetectable by the routine method. The meta-regression on sample size is not suggestive of any dominant publication bias	Very Low (downgraded by three levels due to study limitations, imprecision and indirectness)
α adrenoreceptors antagonists	Major concerns	Optimal information size not reach in TSA	Significant heterogeneity according to I2 (74%) in direct	The treatment effects were not significantly influenced by clinical	Undetectable by the routine method. The meta-regression on	Very Low (downgraded by five levels due to study limitations,

			comparisons. No node splitting inconsistency.	modifiers in the meta-regression. However, the point estimate widely varied in subgroup analysis.	sample size is not suggestive of any dominant publication bias	imprecision, heterogeneity and indirectness)
Oral IP agonists	Some concerns	Optimal information size not reach in TSA	Significant heterogeneity according to I2 (73%) in direct comparison. No node splitting inconsistency	The treatment effects were not significantly influenced by clinical modifiers in the meta-regression. However, the point estimate widely varied in subgroup analysis.	Undetectable by the routine method. The meta-regression on sample size is not suggestive of any dominant publication bias	Very low (downgraded by four levels due to study limitations, imprecision, heterogeneity and indirectness)
Anti-oxidants	No concerns	Optimal information size not reach in TSA	No heterogeneity (0%) in direct comparison. No node splitting inconsistency	The treatment effects were not significantly influenced by clinical modifiers in the meta-regression. However, the point estimate widely varied in subgroup analysis.	Undetectable by the routine method. The meta-regression on sample size is not suggestive of any dominant publication bias	Low (downgraded by two levels due to imprecision and indirectness)
Topical NO	Major concerns	Optimal information size not reach in TSA	No heterogeneity (0%) in direct comparison. No node splitting inconsistency	The treatment effects were not significantly influenced by clinical modifiers in the meta-regression. However, no trial with only secondary RP patients was available in sensitivity analysis.	Undetectable by the routine method. The meta-regression on sample size is not suggestive of any dominant publication bias	Very Low (downgraded by four levels due to study limitations, imprecision and indirectness)
PDE3i	Major concerns	Optimal information size not reach in TSA	No heterogeneity (0%) in direct comparison. No node splitting inconsistency	The treatment effects were not significantly influenced by clinical modifiers in the meta-regression. However, no trial with baseline data was available in sensitivity analysis.	Undetectable by the routine method. The meta-regression on sample size is not suggestive of any dominant publication bias	Very Low (downgraded by four levels due to study limitations, imprecision and indirectness)

Thromboxane Synthase Inhibitors	Major concerns	Optimal information size not reach in TSA	No heterogeneity (0%) in direct comparison. No node splitting inconsistency	The treatment effects were not significantly influenced by clinical modifiers in the meta-regression. However, no trial with secondary RP patients and baseline data were available in sensitivity analysis.	Undetectable by the routine method. The meta-regression on sample size is not suggestive of any dominant publication bias	Very Low (downgraded by four levels due to study limitations, imprecision and indirectness)
SSRI	Major concerns	Optimal information size not reach in TSA	No placebo controlled trial. Only indirect comparison and no node-splitting inconsistency.	The treatment effects were not significantly influenced by clinical modifiers in the meta-regression. However, no trial with secondary RP patients available in sensitivity analysis.	Undetectable by the routine method. The meta-regression on sample size is not suggestive of any dominant publication bias	Very Low (downgraded by five levels due to study limitations, imprecision, no placebo controlled trial and indirectness)
sGC stimulators	Some concerns due to small sample size	Optimal information size not reach in TSA	No heterogeneity (0%) in direct comparison. No node splitting inconsistency	The treatment effects were not significantly influenced by clinical modifiers in the meta-regression. However, the point estimate widely varied in subgroup analysis.	Undetectable by the routine method. The meta-regression on sample size is not suggestive of any dominant publication bias	Very Low (downgraded by three levels due to study limitations, imprecision and indirectness)

- Severity of attacks

Drug classes versus placebo	Study limitations	Imprecision	Heterogeneity and Inconsistency	Indirectness	Publication bias	Overall quality of evidence
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PDE5i	Some concerns	Optimal information size not reach in TSA but result significant with adjusted threshold	No heterogeneity ($I^2=0\%$) in direct comparison. No node splitting inconsistency	The treatment effects were not influenced by clinical effect modifiers in meta-regressions and the point estimate did not widely varied in subgroup analysis.	Egger regression test for funnel plot asymmetry non-significant in pairwise meta-analysis. The meta-regression on sample size is not suggestive of any dominant publication bias	Moderate (downgraded by one level due to study limitations)
CCBs	Some concerns	Optimal information size reach in TSA	No significant heterogeneity ($I^2=28\%$) in direct comparison. No node splitting inconsistency	The treatment effects were not influenced by clinical effect modifiers in meta-regressions but the point estimate widely varied in subgroup analysis.	Undetectable by the routine method. The meta-regression on sample size is not suggestive of any dominant publication bias	Low (downgraded by two levels due to study limitations and indirectness)
IV PGI2 analogues	No concerns	Optimal information size not reach in TSA	Significant heterogeneity ($I^2= 64\%$) in direct comparison. No node splitting inconsistency.	The treatment effects were not influenced by clinical effect modifiers in meta-regressions but the point estimate widely varied in subgroup analysis and no trial with RCS outcome was available in sensitivity analysis.	Undetectable by the routine method. The meta-regression on sample size is not suggestive of any dominant publication bias	Very Low (downgraded by three levels due to imprecision, heterogeneity and indirectness)
α adrenoreceptors antagonists	Major concerns	Optimal information size not reach in TSA	No heterogeneity ($I^2=0\%$) in direct comparison. No node splitting inconsistency.	The treatment effects were significantly influenced by clinical modifiers in the meta-regression and no trial with only secondary RP patients and RCS outcome was available in sensitivity analysis.	Undetectable by the routine method. The meta-regression on sample size is not suggestive of any dominant publication bias	Very Low (downgraded by four levels due to study limitations, imprecision and indirectness)
Oral IP agonists	Some concerns	Optimal information size not reach in TSA but result significant	No heterogeneity (0%) in direct comparison. No node splitting	The treatment effects were not influenced by clinical effect modifiers	Undetectable by the routine method. The meta-regression on	Low (downgraded by two levels due to study limitations and

		with adjusted threshold	inconsistency	in meta-regressions and the point estimate did not widely varied in subgroup analysis.	sample size is not suggestive of any dominant publication bias	imprecision)
Anti-oxidants	No concerns	Optimal information size not reach in TSA	No heterogeneity (0%) in direct comparison. No node splitting inconsistency	The treatment effects were significantly influenced by clinical modifiers in the meta-regression and no trial with RCS outcome was available in sensitivity analysis.	Undetectable by the routine method. The meta-regression on sample size is not suggestive of any dominant publication bias	Low (downgraded by two levels due to imprecision and indirectness)
Topical NO	Major concerns	Optimal information size not reach in TSA	No heterogeneity (0%) in direct comparison. No node splitting inconsistency	The treatment effects were not significantly influenced by clinical modifiers in the meta-regression but no trial with only secondary RP patients was available in sensitivity analysis.	Undetectable by the routine method. The meta-regression on sample size is not suggestive of any dominant publication bias	Very Low (downgraded by four levels due to study limitations, imprecision and indirectness)
PDE3i	Major concerns	Optimal information size not reach in TSA	No heterogeneity (0%) in direct comparison. No node splitting inconsistency	The treatment effects were significantly influenced by clinical modifiers in the meta-regression and no trial with RCS outcome and baseline data was available in sensitivity analysis.	Undetectable by the routine method. The meta-regression on sample size is not suggestive of any dominant publication bias	Very Low (downgraded by four levels due to study limitations, imprecision and indirectness)
Thromboxane Synthase Inhibitors	Major concerns	Optimal information size not reach in TSA	No heterogeneity (0%) in direct comparison. No node splitting inconsistency	The treatment effects were significantly influenced by clinical modifiers in the meta-regression and no trial with only secondary RP patients, RCS outcome and baseline data was available in sensitivity	Undetectable by the routine method. The meta-regression on sample size is not suggestive of any dominant publication bias	Very Low (downgraded by four levels due to study limitations, imprecision and indirectness)

				analysis.		
SSRI	Major concerns	Optimal information size not reach in TSA	No placebo controlled trial. Only indirect comparison and no node-splitting inconsistency	The treatment effects was not significantly influenced by clinical modifiers in the meta-regression but no trial with only secondary RP patients and RCS outcome was available in sensitivity analysis.	Undetectable by the routine method. The meta-regression on sample size is not suggestive of any dominant publication bias	Very Low (downgraded by five levels due to study limitations, imprecision, no placebo controlled trial and indirectness)
Endothelin Receptor Antagonist	No concerns	Optimal information size not reach in TSA and result non-significant with adjusted thresholds	No heterogeneity (0%) in direct comparison. No node splitting inconsistency	The treatment effects were significantly influenced by clinical effect modifiers in meta-regressions and the point estimate widely varied in subgroup analysis.	Undetectable by the routine method. The meta-regression on sample size is not suggestive of any dominant publication bias	Low (downgraded by two levels due to imprecision and indirectness)
Botulinum Toxin type A	No concerns	Optimal information size not reach in TSA	No heterogeneity (0%) in direct comparison. No node splitting inconsistency	The treatment effects were significantly influenced by clinical modifiers in the meta-regression and no trial with baseline data was available in sensitivity analysis.	Undetectable by the routine method. The meta-regression on sample size is not suggestive of any dominant publication bias	Low (downgraded by two levels due to imprecision and indirectness)
Anti-IL6	No concerns	Optimal information size not reach in TSA	No heterogeneity (0%) in direct comparison. No node splitting inconsistency	The treatment effects were significantly influenced by clinical modifiers in the meta-regression and no trial with RP primary outcome, RCS outcome and baseline data was available in sensitivity analysis.	Undetectable by the routine method. The meta-regression on sample size is not suggestive of any dominant publication bias	Low (downgraded by two levels due to imprecision and indirectness)

ACEi/ARB	No concerns	Optimal information size not reach in TSA	No placebo controlled trial. Only indirect comparison and no node-splitting inconsistency	The treatment effects were significantly influenced by clinical modifiers in the meta-regression and no trial with RP primary outcome, RCS outcome and baseline data was available in sensitivity analysis.	Undetectable by the routine method. The meta-regression on sample size is not suggestive of any dominant publication bias	Very Low (downgraded by three levels due to imprecision, no placebo controlled trial and indirectness)
sGC stimulators	Some concerns due to small sample size	Optimal information size not reach in TSA	No heterogeneity (0%) in direct comparison. No node splitting inconsistency	The treatment effects were not significantly influenced by clinical modifiers in the meta-regression. However, the point estimate widely varied in subgroup analysis.	Undetectable by the routine method. The meta-regression on sample size is not suggestive of any dominant publication bias	Very Low (downgraded by three levels due to study limitations, imprecision and indirectness)

- Duration of each RP attack

Drug classes versus placebo	Study limitations	Imprecision	Heterogeneity and Inconsistency	Indirectness	Publication bias	Overall quality of evidence
PDE5i	Some concerns	Optimal information size not reach in TSA but effect size significant with adjusted threshold	No heterogeneity (0%) in direct comparison. No node splitting inconsistency	The treatment effects were not significantly influenced by clinical modifiers in the meta-regression. However, the point estimate widely varied in subgroup analysis.	Egger regression test for funnel plot asymmetry non-significant in pairwise meta-analysis. The meta-regression on sample size is not suggestive of any dominant publication bias	Low (downgraded by two levels due to study limitations and imprecision)
CCBs	Major concerns	Optimal information size not reach in TSA	No heterogeneity (0%) in direct comparison. No node splitting inconsistency	The treatment effects were not significantly influenced by clinical modifiers in the meta-	Undetectable by the routine method. The meta-regression on sample size is not	Very Low (downgraded by three levels due to study limitations and indirectness)

				regression and the point estimate did not widely varied in subgroup analysis.	suggestive of any dominant publication bias	
IV PGI2 analogues	No concerns	Optimal information size not reach in TSA	No heterogeneity (0%) in direct comparison. No node splitting inconsistency.	The treatment effects were not significantly influenced by clinical modifiers in the meta-regression. However, the point estimate widely varied in subgroup analysis and no trial with baseline data was available in sensitivity analysis.	Undetectable by the routine method. The meta-regression on sample size is not suggestive of any dominant publication bias	Low (downgraded by two levels due to imprecision and indirectness)
α adrenoreceptors antagonists	Major concerns	Optimal information size not reach in TSA	No heterogeneity (0%) in direct comparisons. No node splitting inconsistency.	The treatment effects were not significantly influenced by clinical modifiers in the meta-regression. However, no trial with only secondary RP patients was available in sensitivity analysis.	Undetectable by the routine method. The meta-regression on sample size is not suggestive of any dominant publication bias	Very Low (downgraded by five levels due to study limitations, imprecision, heterogeneity and indirectness)
Oral IP agonists	Major concerns	Optimal information size not reach in TSA	No significant heterogeneity (29%) in direct comparison. No node splitting inconsistency	The treatment effects were not significantly influenced by clinical modifiers in the meta-regression. However, the point estimate widely varied in subgroup analysis.	Undetectable by the routine method. The meta-regression on sample size is not suggestive of any dominant publication bias	Very Low (downgraded by three levels due to study limitations, imprecision and indirectness)
Anti-oxidants	No concerns	Optimal information size not reach in TSA	No heterogeneity (0%) in direct comparison. No node splitting inconsistency	The treatment effects were not significantly influenced by clinical modifiers in the meta-regression. However, no trial with baseline data	Undetectable by the routine method. The meta-regression on sample size is not suggestive of any dominant publication	Low (downgraded by two levels due to imprecision and indirectness)

				was available in sensitivity analysis.	bias	
Thromboxane Synthase Inhibitors	Major concerns	Optimal information size not reach in TSA	No heterogeneity (0%) in direct comparison. No node splitting inconsistency	The treatment effects were not significantly influenced by clinical modifiers in the meta-regression. However, no trial with secondary RP patients and baseline data was available in sensitivity analysis.	Undetectable by the routine method. The meta-regression on sample size is not suggestive of any dominant publication bias	Very Low (downgraded by four levels due to study limitations, imprecision and indirectness)

Appendix Table 8. Results of meta-regressions. Selected model, number of studies, and beta coefficient (95% CrI) are presented.

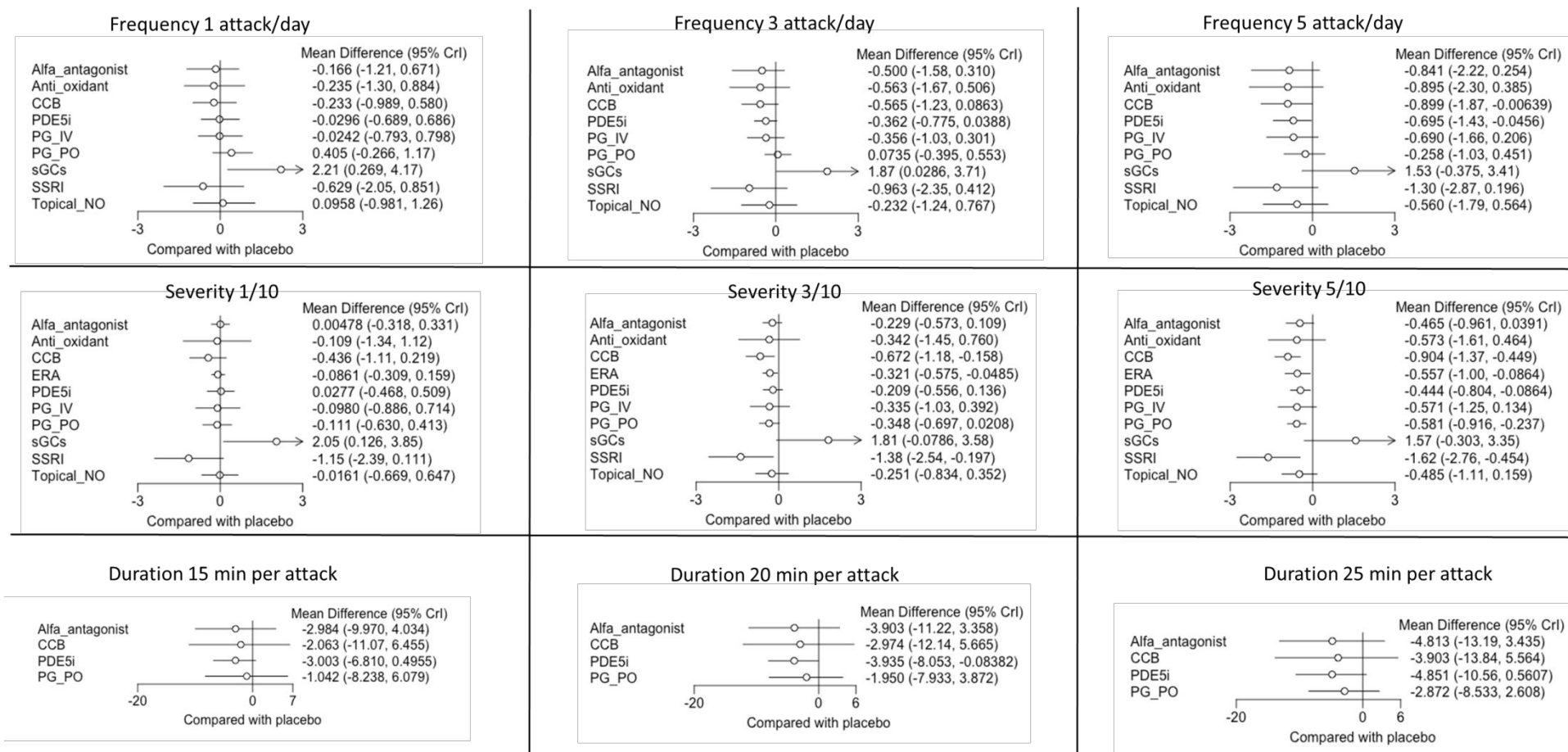
Covariate	Frequency	Severity	Duration
Latitude	n=35 0.06 (-0.45, 0.54)	n=35 0.44 (-0.25, 1.14)	n=18 3.02 (-2.92, 9.02)
Age	n=36 0.31 (-0.17, 0.79)	n=41 <u>0.59 (0.02, 1.16)</u>	n=21 2.56 (-3.13, 8.41)
Proportion of females	n=41 -0.03 (-0.45, 0.38)	n=46 -0.06 (-0.58, 0.45)	n=23 -3.28 (-10.86, 4.03)
Duration of disease	n=30 0.12 (-0.29, 0.53)	n=30 0.41 (-0.28, 1.10)	n=16 4.32 (-3.21, 11.88)
Follow up period	n=42 -0.04 (-0.45, 0.33)	n=46 -0.07 (-0.67, 0.69)	n= 24 -1.25 (-8.09, 5.37)
Sample size	n=42 -0.05 (-0.35, 0.27)	n=46 0.09 (-0.22, 0.38)	n= 24 2.83 (-2.30, 7.81)
Baseline	n=31 -0.34 (-0.95, 0.20)	n=36 -0.44 (-0.90, 0.03)	n= 17 -2.88 (-10.88, 4.90)
Industry sponsorship (yes relative to no-)	n=42 -0.08 (-0.47, 0.28)	n=46 -0.26 (-0.70, 0.19)	n= 24 -0.15 (-5.66, 5.30)
Design (parallel relative to crossover)	n=42 -0.02 (-0.51, 0.544)	n=46 0.15 (-0.49, 0.77)	n= 24 8.58 (-0.26, 17.87)
Treatment used as “add on” therapy relative to no	n=40 -0.01 (-0.58, 0.57)	n=46 0.12 (-0.22, 0.45)	n=22 -0.28 (-7.18, 6.16)

Smokers percentage	n=20 0.19 (-0.70, 1.07)	n=26 -0.05 (-0.58,0.49)	n=16 0.17 (-8.20; 8.62)
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Appendix Table 9. Results of the bayesian network meta-analysis adjusted on significant meta-regression and baseline for each drug classes are also presented.

Drug classes versus placebo	Frequency	Severity			Duration
	Adjusted on baseline (baseline = 3.0)	Adjusted on age (age = 47.5)	Adjusted on age (age = 52)	Adjusted on baseline (baseline = 4.2)	Adjusted on baseline (baseline = 19.8)
PDE5i	-0.37 (-0.78, 0.04)	<u>-0.36 (-0.70, -0.04)</u>	-0.08 (-0.49, 0.31)	<u>-0.35 (-0.69, -0.02)</u>	<u>-3.87 (-8.12, -0.08)</u>
CCBs	-0.57 (-1.23, 0.09)	<u>-0.86 (-1.35, -0.37)</u>	-0.58 (-1.19, 0.01)	<u>-0.81 (-1.30, -0.30)</u>	-2.96 (-12.24, 5.72)
IV PGI2 analogues	-0.36 (-1.03, 0.30)	-0.54 (-1.25, 0.17)	-0.26 (-1.02, 0.50)	-0.48 (-1.19, 0.25)	
α adrenoreceptors antagonists	-0.50 (-1.58, 0.31)	-0.02 (-0.33, 0.29)	0.25 (-0.19, 0.69)	-0.38 (-0.81, 0.05)	-3.92 (-11.18, 3.38)
Oral IP agonists	0.07 (-0.39, 0.55)	<u>-0.53 (-0.93, -0.14)</u>	-0.25 (-0.68, 0.17)	<u>-0.48 (-0.81, -0.15)</u>	-1.89 (-7.94, 3.97)
Anti-oxidants	-0.56 (-1.67, 0.51)	-0.85 (-1.81, 0.16)	-0.56 (-1.55, 0.47)	-0.43 (-1.44, 0.62)	
Topical NO donor	-0.23 (-1.24, 0.77)	-0.17 (-0.77, 0.44)	0.10 (-0.59, 0.80)	-0.38 (-1.01, 0.23)	
PDE3i		0.86 (-0.96, 2.76)	1.09 (-0.88, 3.05)		
Thromboxane Synthase Inhibitors		-0.10 (-3.90, 3.74)	0.30 (-3.57, 4.37)		
ACEi/ARB		-0.47 (-1.27, 0.32)	-0.15 (-0.82, 0.53)		
Endothelin Receptor Antagonist		<u>-0.32 (-0.57, -0.07)</u>	-0.05 (-0.29, 0.19)	<u>-0.47 (-0.83, -0.08)</u>	
Botulinum Toxin type A		-0.32 (-1.37, 0.77)	0.01 (-1.03, 1.06)		
Anti-IL6		-0.69 (-2.04, 0.73)	-0.29 (-1.69, 1.12)		
SSRI	-0.97 (-2.35, 0.41)			<u>-1.51 (-2.68, -0.36)</u>	
sGCs	1.87 (0.30, 3.71)	1.38 (-0.47, 3.21)	1.78 (-0.02, 3.66)	<u>1.70 (-0.15, 3.52)</u>	

Appendix Figure 11. Results of the Bayesian network meta-analysis meta-regressions adjusted on several baseline levels for efficacy outcomes.



Appendix Table 10. Number of studies, heterogeneity and selected models for subgroup network meta-analyses.

Covariate	Frequency	Severity	Duration
Only secondary	N=29, I ² =0%, random	N=35, I ² =0%, random	N=18, I ² =0%, random
Excluding high risk of bias trials	N=25, I ² =0%, random	N=28, I ² =2%, random	N=20, I ² =0%, random
Only RCS	NA	N=17, I ² =0%, random	NA

Appendix Table 11. Subgroup network meta-analyses of each drug class compared with placebo for efficacy outcomes.

Results are expressed as mean differences (95% CrI).

Drug classes versus placebo	Frequency		Severity			Duration	
	Only secondary	Excluding high risk of bias trials	Only secondary	Excluding high risk of bias trials	Only RCS	Only secondary	Excluding high risk of bias trials
PDE5i	-0.33 (-0.72, 0.05)	<u>-0.41 (-0.83, -0.00)</u>	-0.29 (-0.65, 0.06)	-0.33 (-0.68, 0.01)	-0.27 (-0.67, 0.12)	-3.52 (-7.51, 0.01)	-2.44 (-6.42, 1.30)
CCBs	-0.20 (-0.64, 0.25)	-0.21 (-0.83, 0.40)	-0.48 (-0.99, 0.05)	-0.50 (-1.09, 0.07)	-0.21 (-1.70, 1.30)	-3.08 (-11.12, 4.37)	-1.56 (-10.49, 7.06)
IV PGI2 analogues	-0.21 (-0.82, 0.39)	-0.34 (-1.22, 0.55)	-0.28 (-1.10, 0.51)	-0.29 (-1.09, 0.52)		-2.21 (-103.00, 102.20)	-3.26 (-110.21, 102.89)
α adrenoreceptors antagonists	<u>-3.01 (-5.17, -0.80)</u>						
Oral IP agonists	0.12 (-0.25, 0.52)	0.19 (-0.36, 0.72)	<u>-0.39 (-0.72, -0.06)</u>	-0.37 (-0.81, 0.07)	<u>-0.62 (-1.30, -0.05)</u>	-3.41 (-9.80, 2.20)	-7.42 (-16.14, 1.50)
Anti-oxidants	-0.37 (-1.07, 0.33)	-0.37 (-1.13, 0.40)	-0.73 (-1.75, 0.28)	-1.96 (-2.06, 0.12)		0.75 (-18.73, 20.00)	0.43 (-19.00, 20.09)
Topical NO donor					-0.28 (-1.10, 0.50)		
PDE3i	0.00 (-0.97, 0.97)		0.47 (-1.37,				

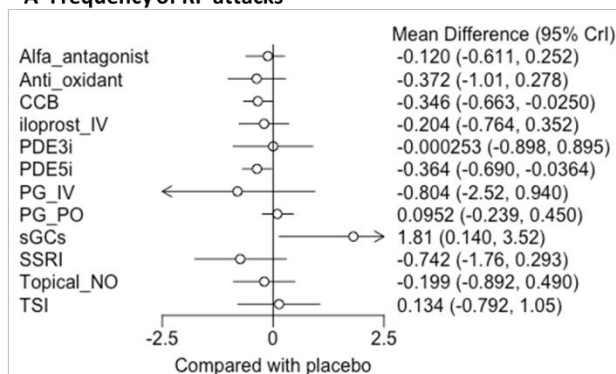
			2.26)				
Thromboxane Synthase Inhibitors							
ACEi/ARB			0.02 (-0.66, 0.69)	0.02 (-0.68, 0.69)			
Endothelin Receptor Antagonist			-0.17 (-0.38, 0.05)	-0.17 (-0.39, 0.07)	0.17 (-1.60, 2.00)		
Botulinum Toxin type A			-0.00 (-1.04, 1.03)	0.02 (-1.04, 1.06)	0.00 (-1.20, 1.20)		
Anti-IL6			-0.44 (-1.85, 0.97)	-0.45 (-1.84, 0.93)			
SSRI sGC stimulators	1.81 (0.07, 3.54)	1.82 (0.07, 3.60)	1.67 (-0.21, 3.48)	1.72 (-0.22, 3.55)	1.70 (-0.30, 3.60)		

Appendix Figure 12. Sensitivity analyses separating IV iloprost from other IV PGI₂ analogs.

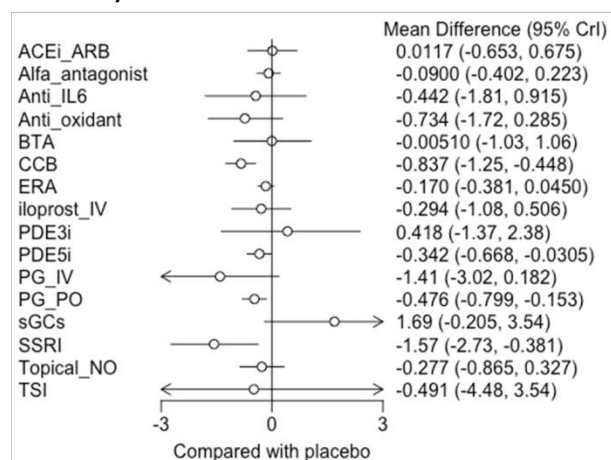
Mean posterior deviance (Dbar), the effective number of data points (pD) and the Deviance Information Criterion (DIC) are presented.

Outcome	Model	Dbar	pD	Deviance Information Criterion (DIC)
Frequency	Random	82.73	59.74	142.47
Severity	Random	89.44	63.17	152.61
Duration	Random	41.42	32.16	73.572

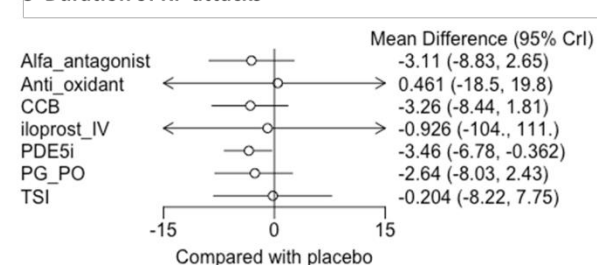
A- Frequency of RP attacks



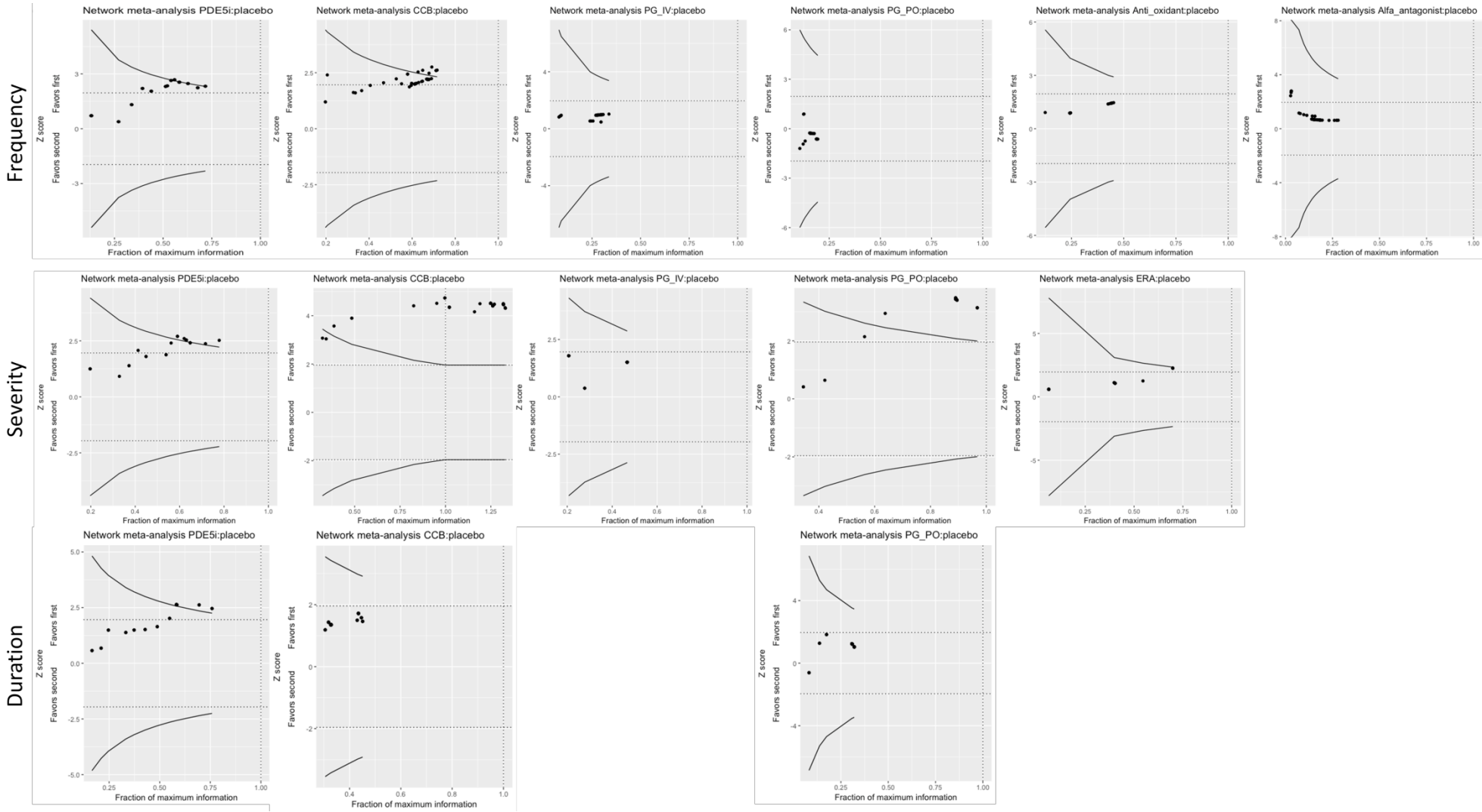
B- Severity of RP attacks



C- Duration of RP attacks



Appendix Figure 13. Result of trial sequential analysis for the network meta-analysis



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**Drug repurposing in Raynaud's phenomenon through adverse event signature matching in the WHO
pharmacovigilance database**

Supplementary Material

Supplementary Table 1. Excluded drugs and reasons for exclusion. EM: erythromelalgia; RP Raynaud’s phenomenon

Drugs	Reason for exclusion
Acetylsalicylic acid	EM treatment
Ascorbic acid;Riboflavin;Tocopherol	Multiple treatments
Bisoprolol	RP induction
Bromocriptine	RP induction
Cabergoline	RP induction
Doxorubicin	RP induction
Droxidopa	RP induction
Etoposide	RP induction
Hepatitis b vaccine	vaccine
HPV vaccine	vaccine
Influenza A(H1N1)pdm09 vaccine	vaccine
Influenza vaccine	vaccine
Interferon alfa-2b	RP induction
Interferon beta-1a	RP induction
Interferon beta-1b	RP induction
Investigational drug	NA
Lidocaine	EM treatment
Meningococcal vaccine	vaccine
Methylphenidate	RP induction
Nebivolol	RP induction
Pergolide	RP induction
Pramipexole	RP induction
Prednisone	EM treatment
Pregabalin	EM treatment
Propranolol	RP induction
Rabies vaccine	vaccine
Reboxetine	RP induction
Ropinirole	RP induction
Tick-borne encephalitis vaccine	vaccine
Typhoid vaccine	vaccine
Vincristine	RP induction

Comparative safety of drugs targeting the nitric oxide pathway in pulmonary hypertension: a mixed approach combining a meta-analysis of clinical trials and a disproportionality analysis from the WHO pharmacovigilance database.

Supplementary material

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e-Appendix 1. Building of adverse events/effects subgroups in the meta-analysis and in pharmacovigilance Vigibase database.

MedDRA classifies the medical terms into five hierarchical levels arranged from the very general the System Organ Classes, High Level Group Terms, High Level Terms, Preferred Terms, to the very specific Lowest Level Terms comprising from than 70000 terms. MedDRA also includes more than 100 “Standardized Medical Queries” (SMQ) which are a collection of MedDRA terms grouped together to a clinical syndrome, developed to facilitate retrieval of data in investigating drug safety. We constructed adverse events subgroups as follow: we used the corresponding SMQ when available (Cardiac arrhythmias, Cardiac failure, Ischaemic heart disease, Retinal disorders, Hearing and vestibular disorders, Gastrointestinal disorders, Oedema, Haemorrhages and if not we constructed suitable groups using different MedDRA terms (Musculoskeletal disorders, Vascular disorders).

Cardiac arrhythmias

All terms included in the SMQ Cardiac arrhythmias

Terms found in the included studies: arrest cardiac; arrhythmia; arrhythmia supraventricular; atrial fibrillation; atrial flutter; atrial tachycardia; atrioventricular block; atrioventricular block; bradycardia; bundle branch block right; cardiac disorder; cardiac disorder; cardiac flutter; cardiac other; chest discomfort; chest pain; extrasystoles; palpitations; sick sinus syndrome; supraventricular extrasystoles; supraventricular tachycardia; syncope; tachyarrhythmia; tachycardia; ventricular arrhythmia; ventricular extrasystoles.

Ischaemic heart disease

All terms included in the SMQ Ischaemic heart disease

Terms found in the included studies: acute coronary syndrome; angina pectoris; cardiac arrest; coronary artery disease; coronary artery stenosis; coronary artery occlusion myocardial infraction.

Visual disorders

All terms included in the SMQ Retinal disorders

Terms found in the included studies: blurred vision; eye pain; visual disturbance; light hypersensitivity; retinal artery occlusion; visual impairment.

Musculoskeletal disorders

All MedDRA terms included in Joint disorders (HLGT) + Muscle disorders(HLGT) +Musculoskeletal and connective tissue pain and discomfort (HLT).

Terms found in the included studies: arthralgia; arthritis; back pain; body aches; bone pain; bone swelling; flank pain; groin pain; joint stiffness joint swelling; leg pain; limb pain; muscle pain; muscle rupture; muscle spasm; muscle strain; muscular weakness; musculoskeletal; musculoskeletal chest pain; musculoskeletal pain; musculoskeletal stiffness; myalgia; neck pain; non cardiac chest pain; pain in extremity; pain in jaw; rib pain.

Hearing and vestibular disorders

All terms included in the SMQ Hearing and vestibular disorders

Terms found in the included studies: deafness; deafness neurosensory; dizziness; ear disorder; giddiness; hearing impaired; hypoacusis; sudden hearing loss; tinnitus; vertigo; vertigo positional.

Gastrointestinal disorders

All terms included in the Gastrointestinal nonspecific inflammation and dysfunctional conditions (SMQ)

Terms found in the included studies: abdominal discomfort; abdominal distension; abdominal pain; abdominal pain upper; abdominal tenderness; chest pain; constipation; diarrhoea; duodenal ulcer; dyspepsia; dysphagia; epigastric discomfort; flatulence; gastric disorder; gastric ulcer; gastritis; gastroenteritis; gastrointestinal; gastrointestinal haemorrhage; gastrointestinal motility disorder; gastrointestinal pain; gastroesophageal reflux disease; haemorrhoidal haemorrhage; intestinal functional disorder; intestinal obstruction; irritable bowel syndrome; ischemic colitis; melena nausea; nausea and vomiting; oesophageal haemorrhage; rectal haemorrhage; rgo; vomiting; vomiting projectile.

Oedema

All MedDRA terms included in the Haemodynamic oedema, effusions and fluid overload (SMQ)

Terms found in the included studies: Catheter site oedema Fluid overload; Fluid retention; Generalised oedema; Oedema; Oedema peripheral; Pericardial effusion; Peripheral oedema; Pleural effusion; Pulmonary oedema.

Haemorrhages

All terms included in the SMQ Haemorrhages

Terms found in the included studies: activated partial thromboplastin time prolonged; bleeding time prolonged; blood shot eyes; catheter site haematoma; catheter site haemorrhage; cerebral haemorrhage; conjunctival haemorrhage; diarrhoea haemorrhagic; dysfunctional uterine bleeding; ear haemorrhage; enterocolitis haemorrhagic; epistaxis; eye haemorrhage; gastrointestinal haemorrhage; gingival bleeding; haemarthrosis; hematemesi; haematoma; haematuria; haemoglobin decreased; haemoptysis; haemoptysis/pulmonary haemorrhage; haemorrhage; haemorrhoidal haemorrhage; hematemesi; haemoptysis; increase INR; INR increased; intra-abdominal haemorrhage; lower gastrointestinal haemorrhage; major bleeding; mean cell haemoglobin decreased; melena ; metrorrhagia; oesophageal haemorrhage; peptic ulcer haemorrhage; periorbital haematoma; post procedural haemorrhage; pulmonary alveolar haemorrhage; rectal haemorrhage; retinal haemorrhage; skin ulcer haemorrhage; subdural haematoma; upper gastrointestinal haemorrhage; uterine haemorrhage; vaginal haemorrhage.

Vascular disorders

All MedDRA terms included in the Blood pressure diastolic decreased(PT)+ Blood pressure diastolic decreased(PT)+ Decreased and nonspecific blood pressure disorders and shock (HLGT)+ Conjunctival hyperaemia (PT)+ Epistaxis (PT)+ Feeling hot(PT) + Peripheral vascular disorders NEC (HLT)+ Headaches(HLGT)+ Nasal congestion and inflammations (HLT) + Ocular hyperaemia(PT) Priapism(PT) + Vasodilatation(PT).

Terms found in the included studies: Blood pressure decrease; Circulatory collapse; Conjunctival hyperaemia; Dizziness; Dizziness exertional; Dizziness postural; Epistaxis; Feeling hot; Flush; Flushing; Giddiness; Headache; Hot flush; Hypotension; Nasal congestion; Ocular hyperaemia; Orthostatic hypotension; pre syncope; Priapism; Rhinorrhoea; Sinus congestion; Sinus headache; Symptomatic hypotension; Syncope; Vascular other; Vasodilatation

e-Appendix 2. Detailed search strategy

Medline

Riociguat n=13

((("riociguat"[Supplementary Concept] OR "riociguat"[All Fields]) AND ("hypertension, pulmonary"[MeSH Terms] OR ("hypertension"[All Fields] AND "pulmonary"[All Fields]) OR "pulmonary hypertension"[All Fields] OR ("pulmonary"[All Fields] AND "hypertension"[All Fields]))) AND (Clinical Trial[ptyp] AND "humans"[MeSH Terms] AND "adult"[MeSH Terms])

Sildenafil + clinical trial + adult n=136

((("sildenafil citrate"[MeSH Terms] OR ("sildenafil"[All Fields] AND "citrate"[All Fields]) OR "sildenafil citrate"[All Fields] OR "sildenafil"[All Fields]) AND ("hypertension, pulmonary"[MeSH Terms] OR ("hypertension"[All Fields] AND "pulmonary"[All Fields]) OR "pulmonary hypertension"[All Fields] OR ("pulmonary"[All Fields] AND "hypertension"[All Fields]))) AND (Clinical Trial[ptyp] AND "humans"[MeSH Terms] AND "adult"[MeSH Terms])

Tadalafil n=16

((("tadalafil"[MeSH Terms] OR "tadalafil"[All Fields]) AND ("hypertension, pulmonary"[MeSH Terms] OR ("hypertension"[All Fields] AND "pulmonary"[All Fields]) OR "pulmonary hypertension"[All Fields] OR

("pulmonary"[All Fields] AND "hypertension"[All Fields])) AND (Clinical Trial[ptyp] AND "humans"[MeSH Terms] AND "adult"[MeSH Terms])

Clinicaltrial.gov

sildenafil and pulmonary hypertension n=119

tadalafil and pulmonary hypertension n=33

riociguat and pulmonary hypertension n=32

Cochrane library

Sildenafil and pulmonary hypertension +clinical trial n=236

Tadalafil and pulmonary hypertension +clinical trial n=57

Riociguat and pulmonary hypertension +clinical trial n=86

e-Appendix 3. Randomized controlled trials eligibility criteria in the meta-analysis

We included only randomized controlled trial assessing efficacy on sildenafil, tadalafil or riociguat on pulmonary hypertension. Studies were excluded if retrospective or observational design, non-chronic use (<4 weeks), less than 10 volunteers in each group, men or women under 18 and animal studies. Two reviewers have screened the titles and abstracts independently (CK and ML). A paired consensus process was used to select relevant citations. Disagreements between reviewers were discussed until consensus was achieved. Then, full-text articles were reviewed and assessed for eligibility. Paired consensus was repeated to confirm article eligibility. Any disagreement between the two reviewers was resolved through discussion.”

e-Appendix 4. Risk of bias assessment

The risk of bias was rated as low, unclear or high for the following items: randomization; allocation concealment; blinding; incomplete outcome data; selective reporting. The overall risk of bias for each trial was defined as high-risk if more than three high-risk criteria were met, moderate-risk if two to three high-risk criteria were met and low-risk if one or less high-risk criterion was met. A study that did not detailed strategy to report adverse events could not be considered at low-risk of bias even if no other high risk criterion was found.

Then, the same two reviewers appraised the quality and content of included studies using the Grading of Recommendation Assessment, Development and Evaluation (GRADE) recommendations for meta-analysis (8). Randomized controlled trials begin as high quality evidence, but can be rated down because of risk of bias (10), imprecision (11), inconsistency (12), indirectness (13) and publication bias (13). Finally, we rated their quality as very low, low, moderate or high. We paid special attention to the way of adverse event were reported.

e-Appendix 5. Selected indications for riociguat, sildenafil, tadalafil in Vigibase.

Associated with pulmonary arterial hypertension
Breast disorder
Chronic thromboembolic pulmonary hypertension
Disease of pulmonary vessels, unspecified
Dyspnoea
Familial pulmonary arterial hypertension
Hypertension pulmonary
Idiopathic pulmonary arterial hypertension
Idiopathic pulmonary fibrosis
Interstitial lung disease
Lung disease
Lung disorder

Obstructive airways disorder
Other diseases of pulmonary vessels
Other pulmonary heart diseases
Portopulmonary hypertension
Primary pulmonary hypertension
Pulmonary arterial hypertension
Pulmonary arterial pressure
Pulmonary arteriolar resistance within normal range
Pulmonary congestion
Pulmonary disorder
Pulmonary embolism
Pulmonary fibrosis
Pulmonary heart disease
Pulmonary heart disease, unspecified
Pulmonary heart disease, unspecified
Pulmonary hypertension
Pulmonary hypertension NOS
Pulmonary hypertension primary
Pulmonary hypertension secondary
Pulmonary sarcoidosis
Pulmonary thrombosis
Pulmonary vascular disorder
Pulmonary vascular resistance abnormality
Pulmonary veno-occlusive disease
Respiratory disorder
Respiratory distress
Secondary pulmonary arterial hypertension
Systemic sclerosis pulmonary
Vascular resistance pulmonary increased

e-Table 1. GRADE assessment of overall and subgroup analysis

Subgroup	Number of studies	Population (patient-year) (T/C)	Heterogeneity	LogIRR (95 CI)	High quality studies	Small number of studies with events	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Overall quality of evidence
Cardiac arrhythmias												
Riociguat	6	167,0 / 77,4	30%	-0.05 (-0.49,0.38)	6/6	no	no serious risk of bias	no serious inconsistency	serious	no serious imprecision	undetected	MODERATE ○⊗⊗⊗
Sildenafil	8	132,4 / 101,4	0%	0.04 (-0.24,0.33)	3/8	no	no serious risk of bias	no serious inconsistency	serious	no serious imprecision	undetected	MODERATE ○⊗⊗⊗
Tadalafil	6	257,2 / 115,1	0%	-0.03 (-0.77,0.72)	1/6	no	serious	no serious inconsistency	no serious indirectness	no serious imprecision	undetected	MODERATE ○⊗⊗⊗
Ischaemic heart disease												
Riociguat	6	167 / 77.4	0%	-0.42 (-1.95,1.12)	6/6	yes	no serious risk of bias	no serious inconsistency	serious	serious	undetected	VERY LOW ○○○⊗
Sildenafil	7	128,5 / 97,5	27.5%	0.31(-0.46,1.08)	3/7	no	no serious risk of bias	no serious inconsistency	serious	serious	undetected	LOW ○○⊗⊗
Tadalafil	7	271,1 / 128,9	0%	0.03(-1.01,1.07)	1/7	yes	serious	no serious inconsistency	no serious indirectness	serious	undetected	VERY LOW ○○○⊗
Visual disorder												
Riociguat	6	167 / 77.4	0	-0.15 (-1.50,1.21)	6/6	no	no serious risk of bias	no serious inconsistency	serious	serious	undetected	LOW ○○⊗⊗
Sildenafil	7	128,5 / 97,5	0	0.51(0.10,0.92)	3/7	no	no serious risk of bias	no serious inconsistency	serious	no serious imprecision	undetected	MODERATE ○⊗⊗⊗
Tadalafil	6	30,0 / 95,4	0	0.13 (-0.53,0.80)	1/6	no	serious	no serious inconsistency	no serious indirectness	serious	undetected	LOW ○○⊗⊗
Musculoskeletal disorder												
Riociguat	6	167,0 / 77,4	0	-0.15 (-0.48,0.19)	6/6	no	no serious risk of bias	no serious inconsistency	serious	no serious imprecision	undetected	MODERATE ○⊗⊗⊗
Sildenafil	8	132,4 / 101,4	7.5	0.05 (-0.15,0.24)	3/8	no	no serious risk of bias	serious	serious	no serious imprecision	undetected	LOW ○○⊗⊗

Tadalafil	6	257,2 / 115,1	0	0.25 (0.03,0.47)	1/6	no	serious	no serious inconsistency	no serious indirectness	no serious imprecision	undetected	MODERATE ○⊗⊗⊗
Hearing and vestibular disorders												
Riociguat	6	167 / 77,4	0	0.38 (0.02,0.74)	6/6	no	no serious risk of bias	serious	serious	no serious imprecision	undetected	LOW ○○⊗⊗
Sildenafil	7	80,7 / 81,3	0	0.37 (0.06,0.68)	3/7	no	no serious risk of bias	no serious inconsistency	serious	no serious imprecision	undetected	MODERATE ○⊗⊗⊗
Tadalafil	6	139,4 / 70,2	NA	-0.05 (-0.46,0.35)	1/6	no	serious	no serious inconsistency	serious	no serious imprecision	undetected	LOW ○○⊗⊗
Gastrointestinal disorders												
Riociguat	6	167,0 / 77,4	0	0.57 (0.36,0.78)	6/6	no	no serious risk of bias	no serious inconsistency	serious	no serious imprecision	undetected	MODERATE ○⊗⊗⊗
Sildenafil	8	132,4 / 101,4	0	0.22 (0.07,0.37)	3/8	no	no serious risk of bias	no serious inconsistency	serious	no serious imprecision	undetected	MODERATE ○⊗⊗⊗
Tadalafil	7	271,1 / 128,9	19.9	0.11 (-0.08,0.29)	1/7	no	serious	no serious inconsistency	no serious indirectness	no serious imprecision	undetected	MODERATE ○⊗⊗⊗
Haemorrhages												
Riociguat	6	167,0 / 77,4	0	0.59 (0.00,1.19)	6/6	no	no serious risk of bias	no serious inconsistency	serious	no serious imprecision	undetected	MODERATE ○⊗⊗⊗
Sildenafil	8	132,4 / 78,2	0	0.14 (-0.14,0.42)	3/8	no	no serious risk of bias	no serious inconsistency	serious	no serious imprecision	undetected	MODERATE ○⊗⊗⊗
Tadalafil	7	257,2 / 115,1	0	0.11 (-0.38,0.59)	1/7	no	serious	no serious inconsistency	no serious indirectness	no serious imprecision	undetected	MODERATE ○⊗⊗⊗
Vasodilator related disorders												
Riociguat	6	167,0 / 77,4	0	0.39 (0.19,0.60)	6/6	no	no serious risk of bias	no serious inconsistency	serious	no serious imprecision	undetected	MODERATE ○⊗⊗⊗
Sildenafil	8	132,4 / 101,4	0	0.38 (0.24,0.53)	3/8	no	no serious risk of bias	no serious inconsistency	serious	no serious imprecision	undetected	MODERATE ○⊗⊗⊗
Tadalafil	7	271,1 / 128,9	66.1	0.68 (0.26,1.10)	1/7	no	serious	serious	no serious indirectness	no serious imprecision	undetected	LOW ○○⊗⊗

We downgraded for risk of bias -1 if less than 25% of high quality studies, -2 if 0 high quality studies. We downgraded for inconsistency, -1 if substantial heterogeneity ($I^2 > 50$) or if sensibility analysis modified substantially the effect size (if sensibility analysis modified the direction of the result) and -2 if two criteria were present. We downgraded for indirectness if HTP aetiology/population were very different. We rate down for imprecision if CI > 2 . We downgraded one more if subgroup contained less than 3 studies. Publication bias was not assessed because of a too small number of studies in each treatment subgroup.

We downgraded one more if a small number of studies reported 0 event in both arms (more than 50%). *IRR: Incidence Rate Ratio; CI: Confidence Interval; HTP: pulmonary hypertension*

e-Appendix 6. Cumulative incidence of adverse drug reactions reported for sildenafil, tadalafil and riociguat in Vigibase

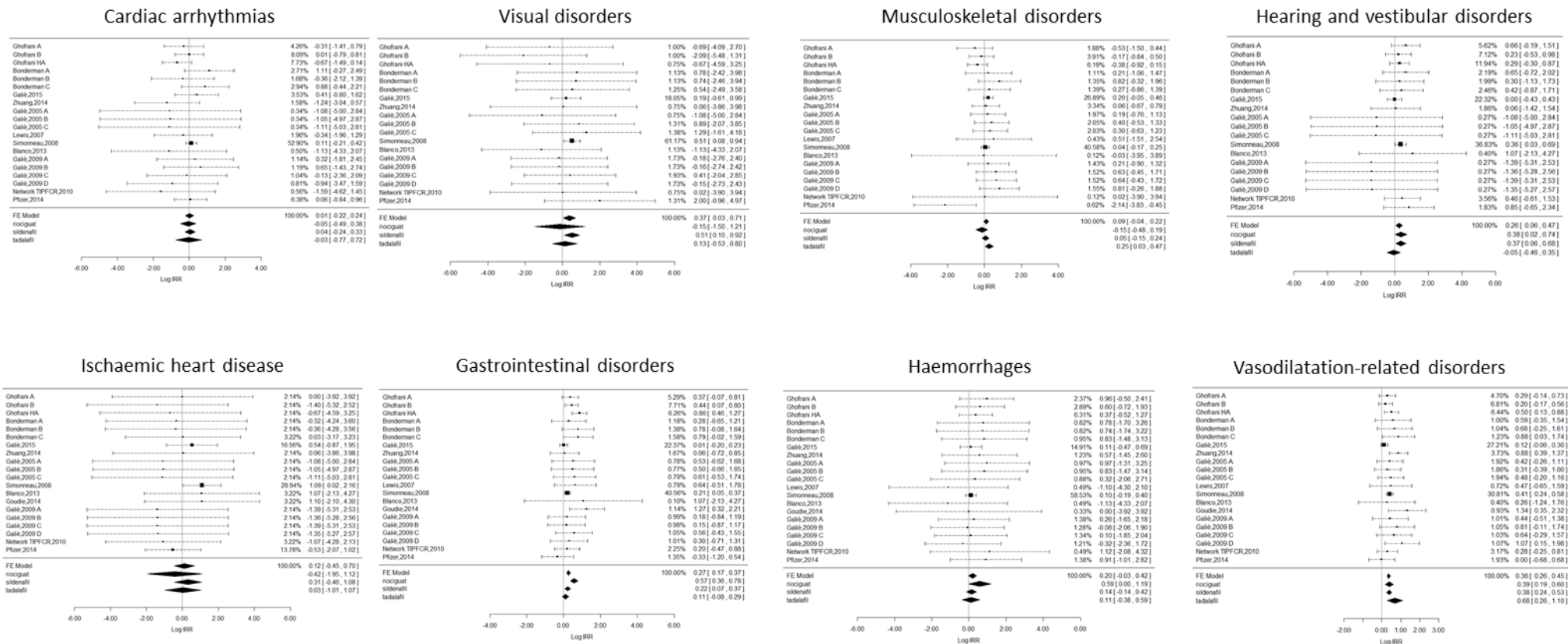
Sildenafil	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Revatio®	0	0	0	2	3	84	201	415	1011	1707	2218	3512	5007	5904
Sildenafil (subs)	2	7	30	46	56	102	172	254	315	353	389	471	592	738
Total	2	7	30	48	59	186	373	669	1326	2060	2607	3983	5599	6642
Cardiac arrhythmia	0	0	0	0	0	2	3	4	14	16	24	29	40	47
Ischaemic heart disease	0	0	0	1	1	1	4	7	12	14	17	22	34	45
Visual disorders	0	0	0	2	3	22	27	42	71	97	113	147	211	235
Hearing and vestibular disorders	0	0	4	4	5	19	32	42	88	124	149	186	291	347
Gastrointestinal disorders	0	0	6	12	13	34	50	81	127	176	214	283	465	595
Oedema	2	3	8	10	10	18	26	41	87	132	171	261	401	538
Haemorrhages	0	1	8	9	10	28	42	66	119	160	200	311	442	530
Musculoskeletal disorders	0	0	0	2	3	19	22	30	65	89	113	172	277	336
Vasodilatation-related disorders	0	3	11	21	25	109	140	188	302	442	507	682	988	1151
Competitors														
Pulmonary hypertension	1	1	6	13	13	35	68	106	225	343	457	766	1040	1255
Cardiac failure	2	3	9	11	12	28	45	71	140	217	293	480	656	805

Tadalafil	2009	2010	2011	2012	2013	2014	2015	2016
Adcirca®		14	89	185	139	318	1445	1076
Tadalafil	8	6	12	16	9	33	43	27
Total	8	28	129	330	478	829	2317	3420
Cardiac arrhythmia	0	1	1	1	1	2	11	16
Ischaemic heart disease	0	1	3	5	5	7	10	12
Visual disorders	1	3	19	39	47	71	123	163
Hearing and vestibular disorders	0	1	5	16	24	38	180	273
Gastrointestinal disorders	1	5	16	49	59	96	391	616
Oedema	1	1	9	35	59	99	341	514
Haemorrhages	0	1	12	28	46	82	181	286

Musculoskeletal disorders	2	7	16	41	51	77	342	482
Vasodilatation-related disorders	1	7	36	78	104	166	727	1121
Competitors								
Pulmonary hypertension		2	17	26	28	67	265	235
Cardiac failure	2	1	14	25	24	51	193	180

Riociguat			2013	2014	2015	2016
Adempas®			4	71	669	786
Total			13	84	753	1539
Cardiac arrhythmia			0	0	2	7
Ischaemic heart disease			0	0	2	12
Visual disorders			0	1	21	46
Hearing and vestibular disorders			2	5	125	294
Gastrointestinal disorders			4	16	319	680
Oedema			1	19	206	416
Haemorrhages			1	9	158	349
Musculoskeletal disorders			0	8	109	239
Vasodilatation-related disorders			3	36	526	1049
Competitors						
Pulmonary hypertension			6	17	224	491
Cardiac failure			2	16	188	367

Vigibase®	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Total	3 559 570	3 854 646	3 888 107	4 518 754	5 043 959	5 891 481	7 004 317	7 863 389	8 531 296	10 303 339	12 369 779	13 734 630
Cardiac arrhythmia	197 154	217 445	219 631	259 095	291 499	341 299	396 856	436 155	465 929	539 860	620 985	672 962
Ischaemic heart disease	47 853	62 800	64 542	86 948	98633	111639	133375	149478	158419	175429	215915	228109
Visual disorders	56942	64610	71304	85976	96834	113223	134701	150053	161494	188242	222740	241788
Hearing and vestibular disorders	162986	175718	177203	205447	228806	274533	325815	365017	393427	472926	571470	638753
Gastrointestinal disorders	499075	538484	542796	635040	706094	832652	994442	1125248	1218184	1474551	1786100	1990832
Oedema	118447	128869	130102	154984	177223	251833	298548	333858	361922	428942	510301	557224
Haemorrhages	210517	229861	231793	274163	306945	351999	411215	463051	506131	616966	735642	814003
Musculoskeletal disorders	187583	209392	211609	258658	298081	373715	454230	519459	565660	676887	815527	906839
Vasodilatation-related disorders	294114	316320	318825	366500	408814	493967	581089	646220	696037	825300	972703	1064963
Competitors												
Pulmonary hypertension	113 816	125 197	126 571	149 829	169 779	201 347	238 384	266 380	287 239	343 067	408 900	449 846
Cardiac failure	105 784	115 640	116 834	136 902	153 474	185 563	219 898	244 983	261 663	295 021	357 150	387 051

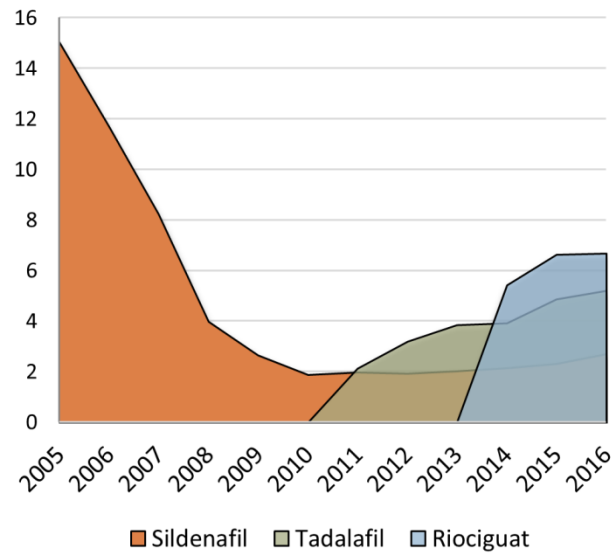
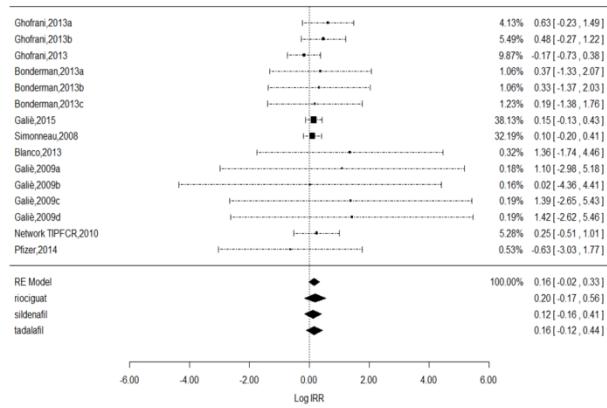


e-Figure 1. Results of separate meta-analysis

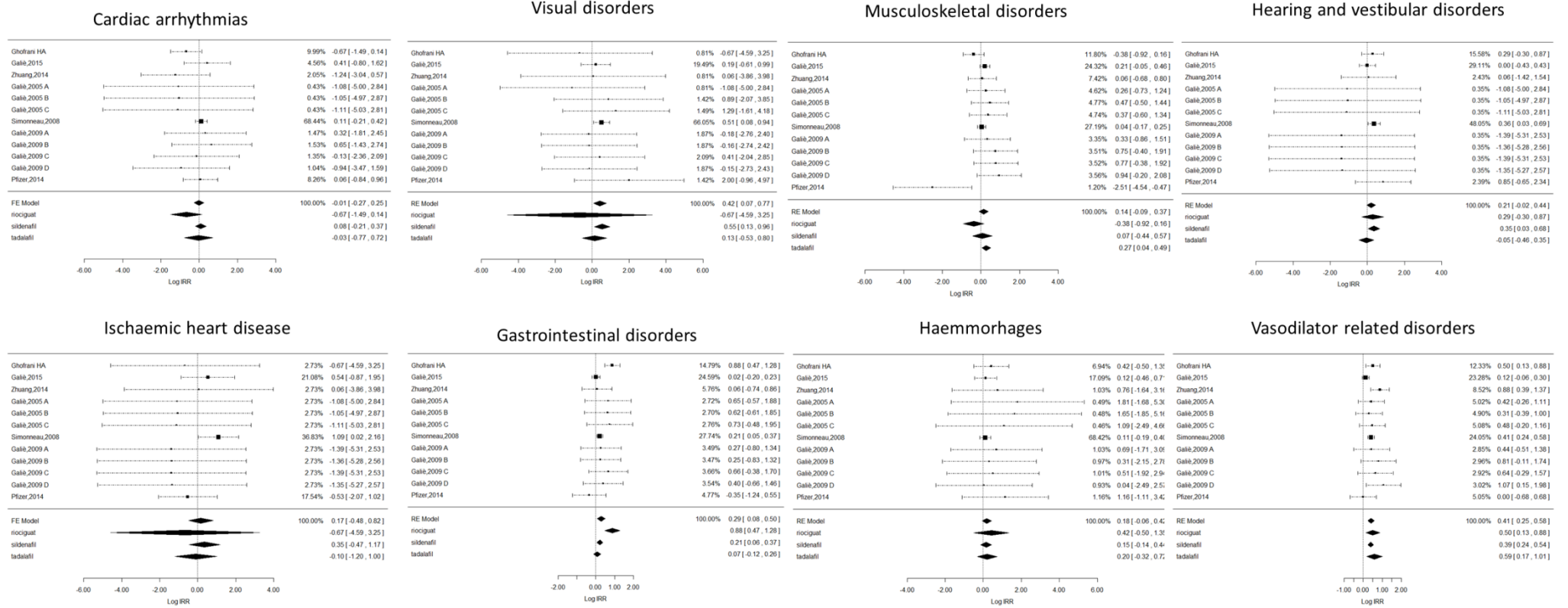
	Sildenafil						Tadalafil						Riociguat					
	WHO pharmacovigilance database			Clinical trials			WHO pharmacovigilance database			Clinical trials			WHO pharmacovigilance database			Clinical trials		
	N=6642 ADRs cases (n,%)			n=529 patients (n,%)			N=3420 ADRs cases (n,%)			n=745 patients (n,%)			N=1539 ADRs cases (n,%)			n=622 patients (n,%)		
Cardiac arrhythmia	Total	47	0.7%	Total	85	16.1%	Total	16	0.5%	Total	27	3.6%	Total	7	0.5%	Total	75	12.1%
	Arrhythmia	28	0.4%	Palpitations	39	7.4%	Heart rate irregular	10	0.3%	Palpitations	17	2.3%	Arrhythmia	3	0.2%	Palpitations	36	5.8%
	Heart rate irregular	12	0.2%	Tachycardia	12	2.3%	Arrhythmia	4	0.1%	Atrial flutter	3	0.4%	Heart rate irregular	3	0.2%	Atrial fibrillation	12	1.9%
	Pulseless electrical activity	7	0.1%	Atrial fibrillation	8	1.5%	Pulseless electrical activity	2	0.1%	Supraventricular tachycardia	2	0.3%	Pulseless electrical activity	1	0.1%	Tachycardia	9	1.4%
				Arrest cardiac	8	1.5%				Arrest cardiac	2	0.3%				Atrial flutter	3	0.5%
			Supraventricular tachycardia	5	0.9%				Atrial fibrillation	1	0.1%				Bradycardia	3	0.5%	
Ischaemic heart disease	Total	147	2.2%	Total	15	2.8%	Total	42	1.2%	Total	8	1.1%	Total	23	1.5%	Total	1	0.2%
	Myocardial infarction	83	1.2%	Cardiac arrest	8	1.5%	Myocardial infarction	27	0.8%	Angina pectoris	2	0.3%	Myocardial infarction	10	0.6%	Coronary artery disease	1	0.2%
	Coronary artery disease	15	0.2%	Coronary artery disease	3	0.6%	Coronary artery disease	7	0.2%	Cardiac arrest	2	0.3%	Angina pectoris	9	0.6%			
	Angina pectoris	9	0.1%	Angina pectoris	2	0.4%	Coronary artery occlusion	2	0.1%	Coronary artery stenosis	2	0.3%	Angina unstable	2	0.1%			
	Acute myocardial infarction	7	0.1%	Acute coronary syndrome	1	0.2%	Acute myocardial infarction	1	0.0%	Myocardial infarction	2	0.3%	Coronary arterial stent insertion	1	0.1%			
	Exercise test abnormal	6	0.1%	Coronary artery stenosis	1	0.2%	Blood CPK increased	1	0.0%	Coronary artery occlusion	1	0.1%	Myocardial ischaemia	1	0.1%			
Visual disorders	Total	235	3.5%	Total	65		Total	163	4.8%	Total	30	4%	Total	46	3.0%	Total	4	0.6%
	Visual impairment	75	1.1%	Blurred vision	14	2.6%	Vision blurred	70	2.1%	Blurred vision	29	4%	Vision blurred	16	1.0%	Blurred vision	2	0.3%
	Vision blurred	57	0.9%	Visual disturbance	8	1.5%	Visual impairment	57	1.7%	Retinal vein occlusion	1	0%	Visual impairment	11	0.7%	Visual impairment	2	0.3%
	VA reduced	38	0.6%	Light hypersensitivity	7	1.3%	Blindness	16	0.5%				VA reduced	6	0.4%			
	Blindness	26	0.4%	Visual impairment	6	1.1%	VA reduced	9	0.3%				Eye haemorrhage	4	0.3%			
	Eye disorder	23	0.4%	Chromatopsia	4	0.8%	Eye disorder	7	0.2%				Photophobia	4	0.3%			
Musculoskeletal disorders	Total	336	5.1%	Total	247	46.7%	Total	482	14.1%	Total	319	42.8%	Total	239	15.5%	Total	106	17.0%
	Pain in extremity	76	1.1%	Back pain	50	9.5%	Back pain	129	3.8%	Back pain	73	9.8%	Pain in extremity	62	4.0%	Back pain	24	3.9%
	Arthralgia	44	0.7%	Pain in extremity	45	8.5%	Pain in extremity	129	3.8%	Pain in extremity	73	9.8%	Joint swelling	44	2.9%	Arthralgia	19	3.1%
	Myalgia	47	0.7%	Myalgia	37	7.0%	Myalgia	85	2.5%	Myalgia	59	7.9%	Muscle signs and symptoms	32	2.1%	Pain in extremity	16	2.6%
	Back pain	41	0.6%	arthralgia	26	4.9%	Arthralgia	63	1.8%	arthralgia	58	7.8%	Back pain	30	2.0%	muscle spasm	14	2.3%
	Muscle spasms	31	0.5%	Pain in jaw	22	4.2%	Muscle spasms	38	1.1%	muscle spasm	44	5.9%	Muscle pains	27	1.8%	Myalgia	11	1.8%
Hearing and vestibular	Total	347	5.2%	Total	143	27.0%	Total	273	8.0%	Total	68	9.1%	Total	294	19.1%	Total	134	21.5%
	Dizziness	155	2.3%	Dizziness	101	19.1%	Dizziness	191	5.6%	Dizziness	65	8.7%	Dizziness	260	16.9%	Dizziness	125	20.1%
	Hypoacusis	56	0.8%	Vertigo	18	3.4%	Deafness	26	0.8%	Sudden hearing loss	2	0.3%	Balance disorder	15	1.0%	Vertigo	9	1.4%
	Deafness	52	0.8%	Tinnitus	6	1.1%	Tinnitus	23	0.7%	Vertigo	1	0.1%	Tinnitus	12	0.8%			
	Tinnitus	30	0.5%	Ear pain	6	1.1%	Hypoacusis	19	0.6%				Vertigo	8	0.5%			
	Balance disorder	19	0.3%	Vertigo positional	3	0.6%	Balance disorder	10	0.3%				Hypoacusis	4	0.3%			
Gastrointestinal disorders	Total	595	9.0%	Total	427	80.7%	Total	616	18.0%	Total	383	51.4%	Total	680	44.2%	Total	465	74.8%
	Diarrhoea	154	2.3%	Nausea	69	13.0%	Nausea	192	5.6%	Diarrhoea	100	13.4%	Nausea	238	15.5%	Dyspepsia	101	16.2%
	Nausea	140	2.1%	Diarrhoea	67	12.7%	Diarrhoea	186	5.4%	Nausea	80	10.7%	Diarrhoea	204	13.3%	Nausea	84	13.5%
	Vomiting	94	1.4%	Vomiting	47	8.9%	Chest pain	101	3.0%	Dyspepsia	73	9.8%	Vomiting	132	8.6%	Diarrhoea	75	12.1%
	Chest pain	85	1.3%	Diarrhoea haemorrhagic	44	8.3%	Vomiting	92	2.7%	Vomiting	50	6.7%	Dyspepsia	96	6.2%	Vomiting	57	9.2%
	Dyspepsia	48	0.7%	Dyspepsia	32	6.0%	Abdominal pain upper	42	1.2%	GERD	24	3.2%	Constipation	75	4.9%	GERD	30	4.8%
Hemorrhages	Total	530	8.0%	Total	112	21.2%	Total	286	8.4%	Total	56	7.5%	Total	349	22.7%	Total	51	8.2%
	Epistaxis	126	1.9%	Epistaxis	50	9.5%	Epistaxis	64	1.9%	Epistaxis	47	6.3%	Epistaxis	86	5.6%	Epistaxis	18	2.9%
	Haemorrhage	54	0.8%	Haemoptysis	19	3.6%	Hb decreased	25	0.7%	Gl hemorrhage	3	0.4%	Haemoptysis	46	3.0%	Activated PTT prolonged	11	1.8%
	Gl haemorrhage	51	0.8%	Gl hemorrhage	5	0.9%	Gl haemorrhage	23	0.7%	Vaginal hemorrhage	2	0.3%	Gl haemorrhage	29	1.9%	Haemoptysis	5	0.8%
	Hb decreased	38	0.6%	Haematoma	4	0.8%	Haemoptysis	21	0.6%	Haemorrhoidal haemorrhage	1	0.1%	Hb decreased	25	1.6%	Haematoma	4	0.6%
	Contusion	26	0.4%	Haematuria	4	0.8%	Haemorrhage	16	0.5%	Subdural haematoma	1	0.1%	Haematochezia	24	1.6%	Hb decreased	3	0.5%
Vasodilator related disorders	Total	1151	17.3%	Total	529	99.0%	Total	1121	33.0%	Total	617	82.8%	Total	1049	68.2%	Total	410	65.9%
	Headache	243	3.7%	Headache	220	41.6%	Headache	455	13.3%	Headache	282	37.9%	Dizziness	260	16.9%	Headache	147	23.6%
	Hypotension	220	3.3%	Flushing	75	14.2%	Dizziness	191	5.6%	Nasal congestion	72	9.7%	Hypotension	246	16.0%	Dizziness	114	18.3%
	Dizziness	155	2.3%	Dizziness	69	13.0%	Hypotension	115	3.4%	Flushing	69	9.3%	Flushing	244	15.9%	Headache	60	9.6%
	Epistaxis	126	1.9%	Epistaxis	50	9.5%	Nasal congestion	108	3.2%	Dizziness	65	8.7%	Epistaxis	86	5.6%	Nasal congestion	23	3.7%
	Flushing	79	1.2%	Hypotension	27	5.1%	Flushing	71	2.1%	Epistaxis	47	6.3%	BP decreased	70	4.6%	Flushing	19	3.1%

-Table 2. Proportion of 5 most frequent adverse events recorded in clinical trials and reported in the WHO pharmacovigilance database for sildenafil, tadalafil and iociguat. In the pharmacovigilance database N=number of reported ADRs and % rate of the total reported. In clinical trials n=number of patients who developed one ADE of interest and % rate of the total reported. ADE: adverse drug event; ADR: adverse drug reaction; BP: blood pressure; GERD: Gastroesophageal reflux disease; GI: gastrointestinal; Hb: haemoglobin; PTT: partial thromboplastin time VA: visual acuity.

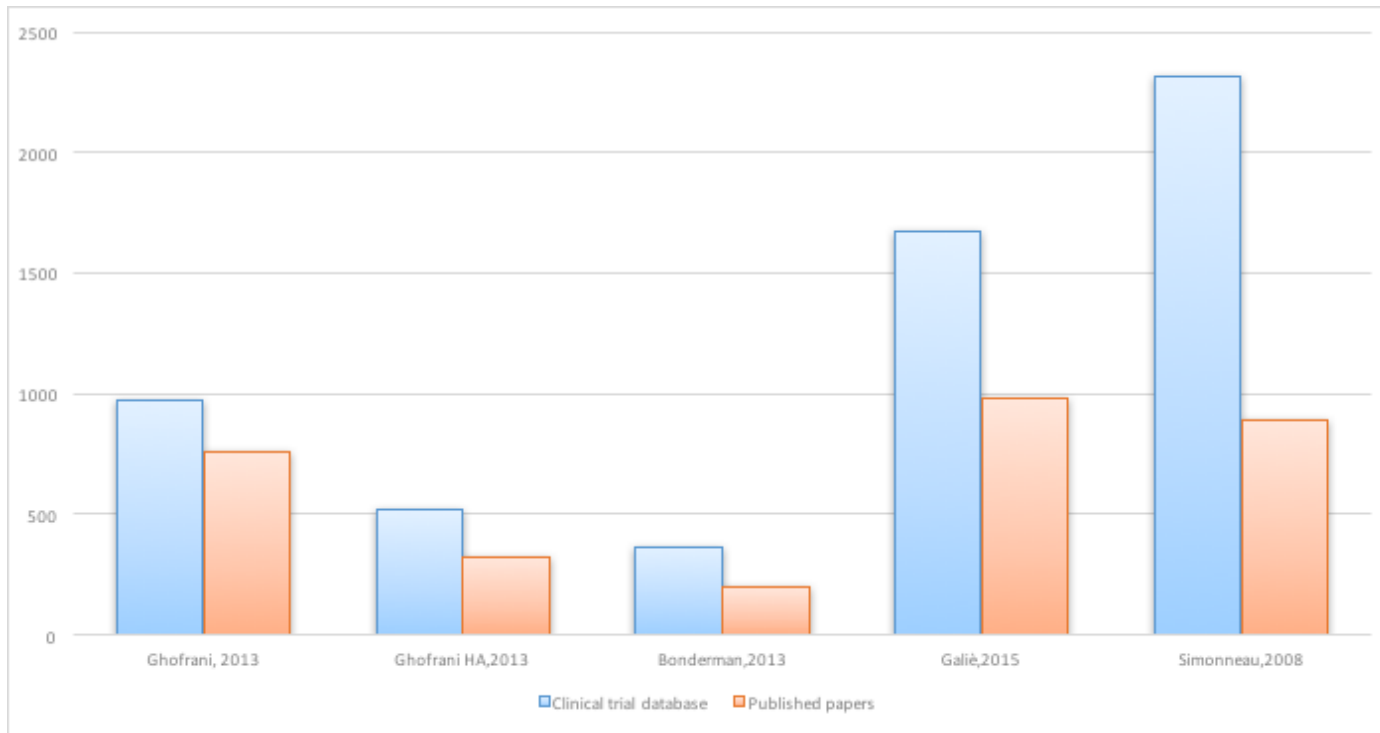
Oedema



e-Figure 2. Results of meta-analysis and disproportionality analysis of oedema adverse events subgroups



e-Figure 3. Results of sensibility analysis of the meta-analysis excluding non-type 1 pulmonary hypertension and low-quality studie



e-Figure 4. Number of adverse drug event of interest reported in published studies respectively to the clinical trial results published in clinicaltrial.gov.

Pulmonary Arterial Hypertension associated with Protein Kinase Inhibitors: A pharmacovigilance-pharmacodynamic study

SUPPLEMENTARY MATERIAL

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Appendix 1. Details of co-reported MedDRA term excluded from the analysis

-cardiac disorders (from MedDRA classification: Cardiac disorders SOC - Cardiac and vascular disorder congenital HGLT - Cardiac and vascular investigation HGLT).

- pulmonary disorders (respiratory and mediastinal neoplasms malignant and unspecified HGLT - bronchial disorders (excl neoplasms) HGLT - lower respiratory tract inflammatory and immunologic conditions HLT– parenchymal lung disorders HLT - pulmonary thrombotic and embolic conditions HLT - respiratory tract disorders NEC HLT – tumour embolism / tumour thrombosis PT) and

-thrombotic disorders (embolism and thrombosis HGLT).

Figure S1. Flow chart of PAH cases selection for analysis

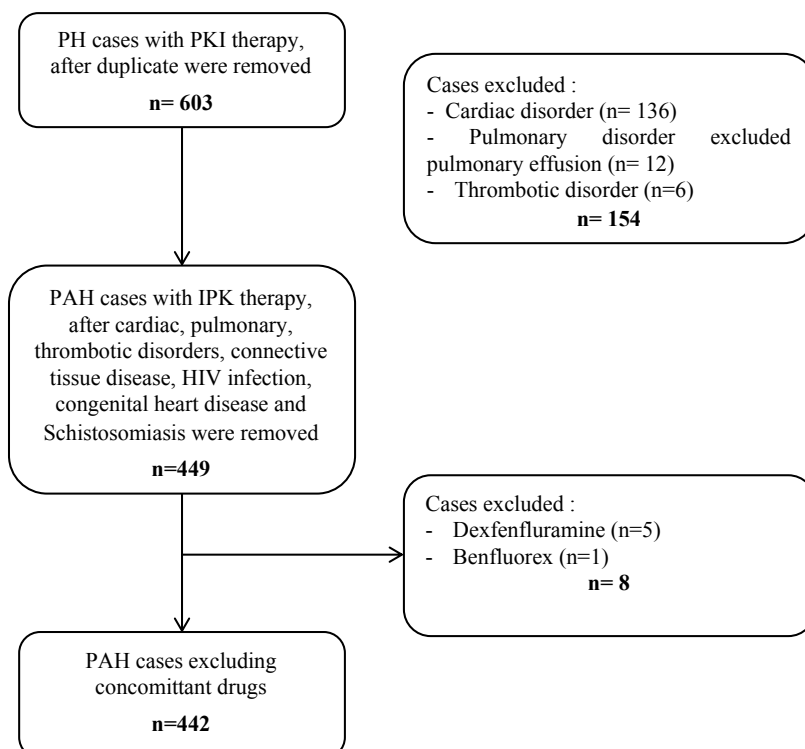
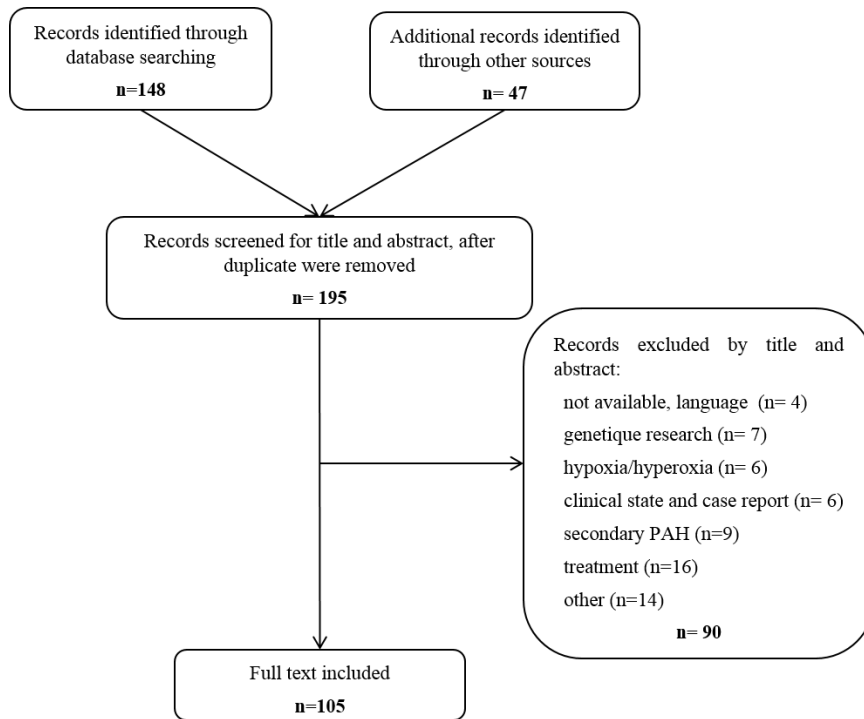


Figure S2. Flow chart of the literature review aiming to identify protein kinases involved in pulmonary function.



Appendix 2. Selected protein kinases and most relevant references.

Target involved in pulmonary pathophysiology	Sources
ALK1 Activin receptor-like kinase-1	(Star et al., 2010) ; (Girerd et al., 2017) ; (Gore et al., 2014)
ALK5 transforming growth factor-β1 (TGFβ1)	(Tojais et al., 2017) (Upton and Morrell, 2013)
AMPKa1 (AMP-activated protein kinase)	(Ibe et al., 2013) (Omura et al., 2016)
AMPKa2	(Ibe et al., 2013)
BMPR-1 = ALK6	(Chida et al., 2012)
BMPR-2	(Tojais et al., 2017)
B-Raf (Rapidly Accelerated Fibrosarcoma)	(Awad et al., 2016)
C-Raf = Raf1	(Hopper et al., 2015)
DDR1 Discoidin domain receptor	(Sakamoto et al., 2001)
EIF2AK4 eukaryotic translation initiation factor 2 alpha kinase 4	(Tenorio et al., 2015) (Eichstaedt et al., 2016) (Best et al., 2017)
ERB-b1 = EGFR = Her1	(Dahal et al., 2010)
ERB-b2 = HER2	(Dahal et al., 2010)
focal adhesion kinase FAK	(Paulin et al., 2014)
FGFR1	(Zheng et al., 2015) (Kim, 2014) (Izikki et al., 2009)
FGFR2	(Schermuly et al., 2011)
IGF-1R (insulin like growth factor)	(Sun et al., 2016) (Baumgart et al., 2017) (Dewachter et al., 2014)
JAK 1	(Lachmann et al., 2017)

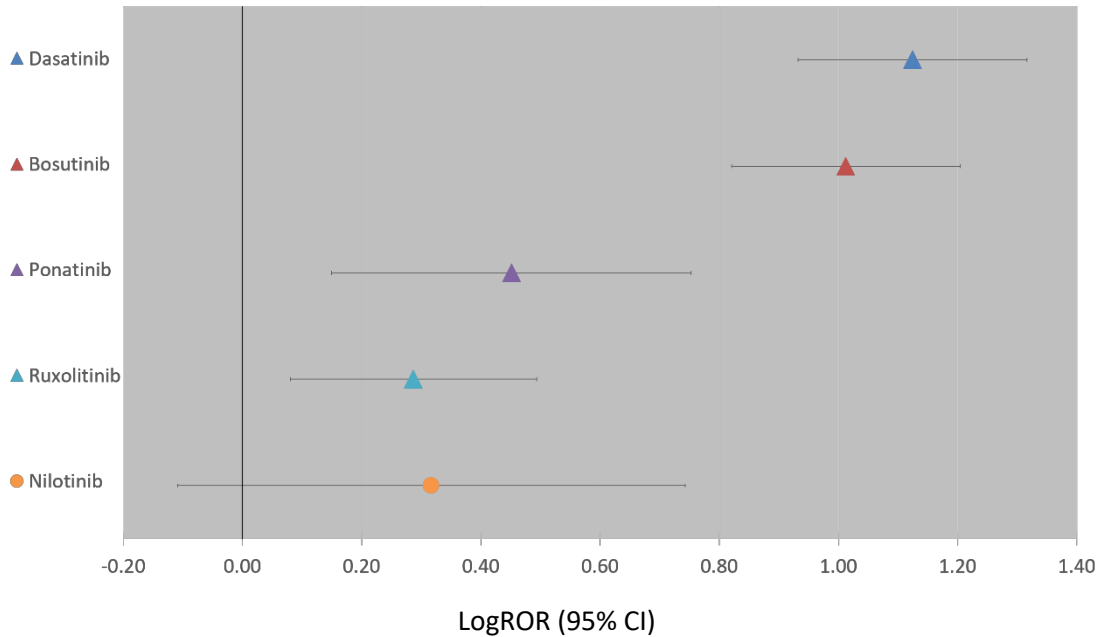
JAK2	(Mattar et al., 2016)
JNK1/2 (c-Jun N-terminal kinase) = mitogen-activated protein kinase 9	(Wilson et al., 2015) (Das et al., 2016)
c-kit = KIT stem cell growth factor receptor (SCFR)	(Montani et al., 2014) (Farha et al., 2014)
Lck Leukocyte C-terminal Src kinase	(Andruska et al., 2017)
lyn	(Pullamsetti et al., 2012a)
c MET = HGF	(Schermuly et al., 2011)
PDGFRα	(Berghausen et al., 2013) (Schermuly, 2005)
PDGFRβ	(Cai et al., 2017) (Weatherald et al., 2017)
PKG cGMP-dependent protein kinase	(Patel et al., 2014)
ROCK-2	(Shimizu et al., 2013)
Tyrosine-protein kinase c-Src	(Guignabert et al., 2016)
TEC	(de Lavallade et al., 2008)
TEK receptor tyrosine kinase TIE2	(Guignabert et al., 2016)
VEGFR-1	(Derrett-Smith et al., 2013)
VEGFR-2	(Nicolls et al., 2012)
VEGFR-3	(Hwangbo et al., 2017)
c-yes	(Pullamsetti et al., 2012b)

Appendix 3. Results of ROR sensitivity analysis, standardizing on time on the market.

Sensitivity analysis were performed to compare the proportion of PAH reported for each PKI with the proportion of PAH reported for all other PKI.

We performed an analysis using only reported cases from the first six years after the FDA approval. A positive disproportionality signal was found for dasatinib with a ROR of 13.32 (8.56; 20.72), bosutinib 10.30 (6.63; 16.00), ponatinib 2.83 (1.41; 5.66), ruxolitinib 1.94 (1.20; 3.12) and nilotinib 2.07 (0.78; 5.53). Logarithmic value are represented in Figure S3.

Figure S3. Disproportionality signal of PAH induced by PKI six years after FDA approval versus all medication in pharmacovigilance database. ROR and 95% CI were log transformed.



Appendix 4. Results of correlations sensitivity analysis standardizing on time on the market, including only PKI with more the 3 PAH cases and using affinity data from Davis et al.

Target	6 year after approval		More than 3 cases		Affinity data from Davis et al.	
	r	p-value	r	p-value	r	p-value
ALK1	0.47	0.079	0.9	0.0058	0.54	0.036
ALK5	0.42	0.12	0.98	0.000069	0.4	0.14
AMPKa1	-0.17	0.55	-0.26	0.57	-0.18	0.53
AMPKa2	-0.2	0.47	-0.3	0.52	-0.18	0.51
B_Raf	0.37	0.17	0.68	0.093	0.43	0.11
BMPR_1	0.3	0.28	0.85	0.015	0.56	0.031
BMPR_2	0.2	0.47	-0.12	0.8	0.095	0.74
c_yes	0.84	0.000079	0.87	0.0011	0.82	0.00018
c-src	0.89	0.000007	0.9	0.0054	0.86	0.000042
DDR1	0.48	0.068	0.66	0.11	0.47	0.075
EIF2K4	0.2	0.47	0.31	0.5	0.16	0.56
ERB_b1	0.053	0.85	0.044	0.93	0.052	0.85
FAK	0.084	0.76	-0.046	0.92	-0.0045	0.99
FGFR1	-0.2	0.48	0.0069	0.99	-0.17	0.54
FGFR2	-0.091	0.75	0.29	0.53	-0.044	0.88
HER2	-0.012	0.97	0.012	0.98	0.027	0.92
HGF	-0.2	0.47	-0.0081	0.99	-0.2	0.48
IGF_1R	-0.22	0.44	-0.3	0.52	-0.19	0.49
JAK1	-0.21	0.46	-0.31	0.49	-0.17	0.55
JAK2	0.014	0.96	-0.041	0.93	0.046	0.87
JNK1	-0.2	0.48	-0.2	0.67	-0.17	0.55
JNK2	-0.31	0.26	-0.41	0.37	-0.32	0.25
KIT	0.3	0.28	0.44	0.32	0.33	0.23
Lck	0.84	0.00016	0.86	0.028	0.78	0.00057
Lyn	0.83	0.00012	0.83	0.02	0.8	0.00036
PDGFRalfa	0.22	0.44	0.44	0.32	0.29	0.29
PDGFRbeta	0.25	0.38	0.39	0.39	0.27	0.32
PKG	-0.14	0.63	-0.15	0.75	NA	NA
RAF1	0.33	0.22	0.85	0.016	0.37	0.18
ROCK_2	0.038	0.89	-0.19	0.68	-0.02	0.94
TEC	0.77	0.00082	0.96	0.00069	0.88	0.000015
TIE2	-0.22	0.43	-0.056	0.91	-0.25	0.36

VEGFR_1	-0.32	0.24	-0.26	0.58	-0.32	0.24
VEGFR_2	-0.29	0.29	-0.26	0.58	-0.29	0.29
VEGFR_3	-0.37	0.18	-0.39	0.39	-0.37	0.18

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**Hierarchical evaluation of electrical stimulation protocols for chronic
wound healing: an effect size meta-analysis.**

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Supplementary Material

Table S1. Summary of studies and patient characteristics included in the meta-analysis.

Studies	Age in Year (Treated / Control)	No. of Subjects (Treated / Control)	No. of Ulcers (Treated / Control)	Type of Ulcers	Initial Ulcer Area Mean in cm ² (Treated / Control)	Initial wound duration Mean in month (Treated / Control)	Main outcome	Length of Follow Up (Weeks)
Adunsky et al., 2005	71.45 (71.1 / 71.8)	63 (35 / 28)	63 (35 / 28)	Pressure	7.5 (7.4 / 7.6)	0.15 (0.13 / 0.17)	NHU	21
Ahmad et al., 2008	38.9 (38.4 / 39.4)	30 (15 / 15)	30 (15 / 15)	Pressure	7.2 (7.12 / 7.21)	4.45 (4.41 / 4.48)	↘ WSA	5
Ahmad et al., 2008	38.935 (38.47 / 39.4)	30 (15 / 15)	30 (15 / 15)	Pressure	7.2 (7.12 / 7.21)	4.44 (4.40 / 4.48)	↘ WSA	5
Ahmad et al., 2008	39.4 (39.4 / 39.4)	30 (15 / 15)	30 (15 / 15)	Pressure	7.2 (7.14 / 7.21)	4.45 (4.41 / 4.48)	↘ WSA	5
Baker et al., 1996	33.5 (34 / 33)	80 (20 / 19)	192 (67 / 25)	Mixed	7.6 (6.6 / 8.6)	4.48 (6.1 / 2.9)	WHR	Until healing
Baker et al., 1996	36.5 (40 / 33)	80 (21 / 19)	192 (58 / 25)	Mixed	5.5 (2.4 / 8.6)	5.28 (7.7 / 2.9)	WHR	Until healing
Baker et al., 1996	34.5 (36 / 33)	80 (20 / 19)	192 (42 / 25)	Mixed	8.5 (8.5 / 8.6)	4.00 (5.1 / 2.9)	WHR	Until healing
Baker et al., 1997	55 (58 / 52)	80 (21 / 20)	114 (33 / 25)	Diabetic	NS	2.80 (3.6 / 2.0)	WHR	Until healing
Baker et al., 1997	51 (50 / 52)	80 (20 / 20)	114 (28 / 25)	Diabetic	NS	2.22 (2.4 / 2.0)	WHR	Until healing
Baker et al., 1997	51.5 (51 / 52)	80 (19 / 20)	114 (28 / 25)	Diabetic	NS	1.88 (1.8 / 2.0)	WHR	Until healing
Carley et al., 1985	71.95 (70.3 / 73.6)	30 (15 / 15)	30 (15 / 15)	Mixed	4.3 (4.74 / 3.92)	6.90 (8.6 / 5.2)	↘ WSA	5
Feedar et al., 1991	63.65 (66.6 / 60.7)	47 (NS)	50 (24 / 26)	Mixed	15.8 (14.65 / 16.93)	NS	%↘ WSA	4
Franek et al., 2000	67.35 (68.1 / 66.6)	65 (33 / 32)	65 (33 / 32)	Venous	23.3 (22.7 / 23.9)	60.00 (72 / 4)	↘ WSA	7
Franek et al., 2005	60.5	60	60	Venous	25.6	35.65	NHU	7

	(60 / 61)	(30 / 30)	(30 / 30)		(25.85 / 25.27)	(35.7 / 35.6)		
)			
Franek et al., 2006	66.5 (68 / 65)	55 (28 / 27)	55 (28 / 27)	Venous	19.0 (18.6 / 19.3)	52.90 (52.4 / 53.4)	NHU	7
Franek et al., 2006	61 (61 / 61)	55 (28 / 27)	55 (28 / 27)	Venous	20.6 (21.4 / 19.7)	35.35 (35.6 / 35.1)	NHU	7
Franek et al., 2011	59.95 (59.9 / 60)	58 (29 / 29)	58 (29 / 29)	Pressure	4.7 (4.45 / 4.93)	2.99 (3.2 / 2.8)	Score	6
Franek et al., 2012	NS	50 (26 / 24)	50 (26 / 24)	Pressure	4.3 (4.54 / 3.97)	3.00 (3.2 / 2.8)	%↘ WSA	6
Gentzkow et al., 1991	62.75 (63.3 / 62.2)	37 (NS)	40 (21 / 19)	Pressure	15.9 (19.2 / 12.5)	4.85 (NA / NA)	%↘ WSA	4
Houghton et al., 2003	64.35 (66.3 / 62.4)	27 (14 / 13)	27 (14 / 13)	Mixed	6.0 (6.39 / 5.53)	45.18 (35.2 / 54.8)	%↘ WSA	8
Houghton et al., 2010	50.55 (50.3 / 50.8)	34 (16 / 18)	34 (16 / 18)	Pressure	3.1 (3.38 / 2.73)	25.20 (14.4 / 36)	%↘ WSA	26
Janković et al., 2008	68.6 (66.7 / 70.5)	35 (20 / 15)	43 (24 / 19)	Mixed	6.0 (6.18 / 5.9)	11.10 (10.5 / 11.7)	↘WSA	8
Jerčinović et al., 1994	36 (36 / 36)	73 (42 / 31)	109 (61 / 48)	Pressure	13.9 (10.6 / 17.2)	4.72 (5.3 / 4.2)	WHR	4 (± 4 Cross-Over)
Jünger et al., 2008	67.2 (67.2 / 67.2)	39 (20 / 19)	39 (20 / 19)	Venous	8.9 (9.7 / 8)	42.00 (42 / 42)	↘WSA	16
Lundeberg et al., 1992	66.75 (66 / 67.5)	64 (32 / 32)	64 (32 / 32)	Diabetic	23.1 (22 / 24.2)	NS	%↘ WSA	12
Magnoni et al., 2013	65.5 (65.9 / 65.1)	60 (30 / 30)	60 (30 / 30)	Mixed	6.4 (5.6 / 7.1)	2.70 (1.6 / 3.8)	Score	52
Mohajeri-Tehrani et al., 2014	56.55 (57 / 56.1)	20 (10 / 10)	20 (10 / 10)	Diabetic	2.5 (2.48 / 2.43)	13.35 (12 / 14.7)	%↘ WSA	4
Mulder et al., 1991	NA	47 (NA)	50 (26 / 24)	Mixed	16 (15 / 17)	NS	%↘ WSA	14
Ogrin et al., 2009	75.65 (74.8 / 76.5)	29 (14 / 15)	29 (14 / 15)	Venous	8.3 (9.5 / 7)	34.85 (31.6 / 38.1)	WHR	12
Ortíz et al., 2014	59.3 (59.3 / 59.3)	114 (54 / 60)	114 (54 / 60)	Diabetic	30.8 (20 / 41.6)	7.55 (1.6 / 3.8)	NHU	NS
Peters et al., 2001	57.15	40	40	Diabetic	2.6	5.25	NHU	12

	(54.4 / 59.9)	(20 / 20)	(20 / 20)		(1.63 / 3.54)	(2.9 / 12.2)		
Petrofsky et al., 2010	48.4 (48.4 / 48.4)	20 (10 / 10)	20 (10 / 10)	Diabetic	26.2 (24.1 / 28.2)	38.90 (38.9 / 38.9)	% ↘ WSA	4
Santamato et al., 2012	72.9 (73.1 / 72.7)	20 (10 / 10)	20 (10 / 10)	Mixed	9.5 (9.11 / 9.89)	3.62 (6 / 1.2)	↘ WSA	3
Taradaj et al., 2011	64.6 (68.1 / 61.1)	59 (32 / 27)	59 (32 / 27)	Venous	21.5 (22.77 / 20.18)	36.39 (42.2 / 30.6)	NHU	7
Ullah et al., 2007	69.5 (69 / 70)	60 (30 / 30)	114 (54 / 60)	Pressure	NS	NS	WHR	12
Wood et al., 1993	75.25 (75.6 / 74.9)	74 (43 / 31)	74 (43 / 31)	Pressure	2.3 (2.61 / 1.91)	5.20 (5.5 / 4.9)	NHU	8

NHU: number of healed ulcer. ↘ WSA: decrease of wound size area. WHR: wound healing rate. Score: wound score. %↘ WSA: percentage of decrease of initial wound size area. NS: not specified.

Table S2. Summary of the electrical stimulation protocols.

Study	Electrode placement	Electrical stimulation direction	Current Type	Control Type	Treatment Duration (Weeks)	Electrical stimulation Cumulated Duration (Hours)	Electrical stimulation protocol	Electrode Polarity
Adunsky et al., 2005	peri	uni	DC	SS	8	42	NA	NA
Ahmad et al., 2008	over	uni	HVPC	SS	5	26.25	Monophasic twin-pulsed generator – frequency of 120Hz – interphase interval of 50µsec – voltage of 100-175 V	Cathodal followed by anodal
Ahmad et al., 2008	over	uni	HVPC	SS	5	35	Monophasic twin-pulsed generator – frequency of 120Hz – interphase interval of 50µsec – voltage of 100-175 V	Cathodal followed by anodal
Ahmad et al., 2008	over	uni	HVPC	SS	5	70	Monophasic twin-pulsed generator – frequency of 120Hz – interphase interval of 50µsec – voltage of 100-175V	Cathodal followed by anodal
Baker et al., 1996	peri	uni	AC (Asymmetric)	SS	4	51	Asymmetric biphasic generator – intensity of 64.9mA – 100µs phase duration – frequency of 50pps – ON:OFF ratio of 7:7 sec.	Cathodal
Baker et al., 1996	peri	bi	AC (Symmetric)	SS	4	63	Symmetric biphasic generator – intensity of 62.8mA – phase duration of 300µs – frequency of 50pps – ON:OFF ratio of 7:7 sec.	.
Baker et al., 1996	peri	bi	AC (Symmetric)	SS	4	57	Symmetric biphasic generator Intensity of 4mA – phase duration of 10µs – frequency of 1pps – ON:OFF ratio of 7:7 sec.	.
Baker et al., 1997	peri	uni	LVPC (Asymmetric)	SS	4	42	Asymmetric biphasic generator – intensity of 64.9mA – 100µs phase duration – frequency of 50pps; ON:OFF ratio of 7:7 sec.	Cathodal
Baker et al., 1997	peri	bi	AC (Symmetric)	SS	4	42	Symmetric biphasic generator – intensity of 62.8mA – phase duration of 300µs – frequency of 50pps - ON:OFF ratio of 7:7 sec.	.
Baker et al., 1997	peri	bi	AC (Symmetric)	SS	4	42	Symmetric biphasic generator Intensity of 4mA – phase duration of 10µs – frequency of 1pps - ON:OFF ratio of 7:7 sec.	.

Carley et al., 1985	over	uni	DC	SWC	5	100	Intensity of 300-700 μ A	Cathodal followed by anodal
Feedar et al., 1991	over	uni	LVPC	SS	4	28	Pulse duration of 132 μ s – intensity of 29.2mA – frequency of 128Hz (followed by 64 Hz from stage II)	Cathodal followed by anodal
Franek et al., 2000	over	uni	HVPC	SWC	7	35	Double-peak monophasic impulses generator – phase duration of 100 μ s – frequency of 100Hz – voltage of 100V	Cathodal followed by anodal
Franek et al., 2005	over	uni	HVPC	SWC	7	35	Double-peak monophasic impulses generator – phase duration of 100 μ s – frequency of 100Hz – voltage of 100V	Cathodal followed by anodal
Franek et al., 2006	over	uni	HVPC	SWC	7	35	Double-peak monophasic impulses generator – phase duration of 100 μ s – frequency of 100Hz – voltage of 100V	Cathodal followed by anodal
Franek et al., 2006	over	uni	HVPC	SWC (with surgery)	7	35	Double-peak monophasic impulses generator – phase duration of 100 μ s – frequency of 100Hz – voltage of 100V	Cathodal followed by anodal
Franek et al., 2011	over	uni	HVPC	SWC	6	25	Double-peak monophasic impulses generator – phase duration of 100 μ s – frequency of 100Hz – voltage of 100V	Cathodal followed by anodal
Franek et al., 2012	over	uni	HVPC	SWC	6	35	Double-peak monophasic impulses generator – phase duration of 100 μ s – frequency of 100Hz – voltage of 100V	Cathodal followed by anodal
Gentzkow et al., 1991	over	uni	LVPC	SS	4	28	Pulse duration of 110 μ s – intensity of 35mA – frequency of 128Hz (followed by 64Hz from stage II)	Cathodal followed by anodal
Houghton et al., 2003	over	uni	HVPC	SS	4	9	Pulse duration of 100 μ s – frequency of 100Hz – voltage of 150V	Cathodal
Houghton et al., 2010	mix	uni	HVPC	SWC	13	728	Twin-peaked monophasic pulsed current generator – pulse duration of 50 μ s – voltage of 50 - 150V – frequency of 100Hz during 20min followed 10Hz during 20min and 20min off	Cathodal followed by anodal
Janković et al., 2008	peri	bi	FREMS	SWC	3	10	Voltage amplitude from 0 to 300V – frequency of 1 000Hz – pulse duration of 10 to 40 μ s – intensity from 100 to 170 μ A	.
Jerčinović et al., 1994	peri	bi	AC	SWC	4	70	Biphasic asymmetric generator – pulse duration of 250 μ s – intensity of 35mA max – frequency of 40Hz – ON:OFF ratio of 4:4 sec.	.

Jünger et al., 2008	over	uni	HVPC	SS	16	112	NA	Cathodal followed by anodal
Lundeberg et al., 1992	peri	bi	AC	SS	12	56	NA	.
Magnoni et al., 2013	peri	bi	FREMS	SWC	NA	36	Voltage amplitude from 0 to 300V – frequency of 1 000Hz – pulse duration of 10 to 40 μ s – intensity from 100 to 170 μ A	.
Mohajeri-Tehrani et al., 2014	peri	uni	DC	SS	4	12	Intensity of 1.48mA max	Cathodal
Mulder et al., 1991	peri	uni	HVPC	SS	4	28	Frequency of 128Hz – intensity of 35mA	Cathodal followed by anodal
Ogrin et al., 2009	peri	bi	TENS	SS	12 (Minimum)	14	Frequency of 5Hz – intensity of 4mA	.
Ortíz et al., 2014	over	uni	HVPC	SWC	NA	36	Pulse duration of 100 μ s – frequency of 100Hz	Cathodal followed by anodal
Peters et al., 2001	peri	uni	HVPC	SS	12	224	Pulse duration of 100 μ s – frequency of 80Hz during 10min followed by 8Hz during 10min – Voltage amplitude of 50V	NA
Petrofsky et al., 2010	peri	bi	LVPC	SWC	4	6	Biphasic generator – pulse duration of 250 μ s – frequency of 30Hz – intensity of 20mA	.
Santamato et al., 2012	peri	bi	FREMS	SWC	3	6.25	NA	.
Taradaj et al., 2011	over	uni	HVPC	SWC	7	35	Pulse duration of 100 μ s – frequency of 100Hz – voltage of 100V	Cathodal followed by anodal
Ullah et al., 2007	over	uni	LVPC	SS	12	NA	NA	Cathodal followed by anodal
Wood et al., 1993	peri	uni	LVPC	SS	8	NA	Frequency of 0.8Hz – intensity of 600 μ A	NA

ES: electrical stimulation; SS: sham stimulation; SWC: standard wound care; direct current (DC), high voltage pulsed current (HVPC), low voltage pulsed current (LVPC), or alternating current (AC). In addition, the frequency, intensity, duration and amplitude of electrical stimulation differed. FREMS: Frequency rhythmic electrical modulation system; TENS: Transcutaneous Electrical Nerve Stimulation

Table S3. GRADE assessment of overall and subgroup analysis.

	Number of studies	Number of ulcers	Heterogeneity	SMD	CI	High quality studies (%)	Large effect	Small number of studies	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Overall quality of evidence
Overall	29	1753	78.5%***	0.72	0.48-1	7 (25%)	no large effect size	no	serious	serious	serious	no serious imprecision	undetected	LOW ○○⊗⊗
Electrode placement														
Electrode over the wound	17	820	78.5%***	0.84	0.48 to 1.19	3 (18%)	large effect size	No	serious	serious	serious	no serious imprecision	undetected	LOW ○○⊗⊗
Unidirectional current	17	820	78.5%***	0.84	0.48 to 1.19	3 (18%)	large effect size	No	serious	serious	serious	no serious imprecision	undetected	LOW ○○⊗⊗
DC	2	144	92%***	1.42	-0.42 to 3.26	0	large effect size	Small number of studies	very serious	serious	serious	serious	undetected	VERY LOW ○○○⊗
HVPC	14	626	78.6%***	0.80	0.38 to 1.21	2 (14%)	large effect size	No	serious	serious	no serious indirectness	no serious imprecision	undetected	MODERATE ○⊗⊗⊗
LVPC	1	50	NA	0.58	0.03 to 1.13	1 (100%)	no large effect size	Small number of studies	no serious risk of bias	no serious inconsistency	no serious indirectness	no serious imprecision	undetected	MODERATE ○⊗⊗⊗
Electrode nearby the wound	18	500	78.9%***	0.63	0.31 to 0.94	4 (22%)	no large effect size	No	serious	serious	serious	no serious imprecision	undetected	VERY LOW ○○○⊗
Bidirectional current	7	489	84.1%***	0.4	-0.11 to 0.91	0	no large effect size	Small number of studies	very serious	serious	serious	no serious imprecision	undetected	VERY LOW ○○○⊗

Nervous stimulation	4	152	81.2%***	1.30	0.43 to 2.18	2 (50%)	large effect size	Small number of studies	no serious risk of bias	serious	no serious indirectness	serious	undetected	LOW ○○⊗⊗
Unidirectional current	7	397	68.4%***	0.55	0.14 to 0.96	2 (8%)	no large effect size	No	serious	serious	serious	serious	undetected	VERY LOW ○○○⊗
DC	2	83	78.1%*	0.28	-0.88 to 1.44	1 (50%)	no large effect size	Small number of studies	no serious risk of bias	serious	no serious indirectness	serious	undetected	VERY LOW ○○○⊗
HVPC	2	90	0%	0.6	0.16 to 10.3	0	no large effect size	Small number of studies	serious	no serious inconsistency	no serious indirectness	no serious imprecision	undetected	LOW ○○⊗⊗
LVPC	3	224	82%***	0.7	-0.04 to 1.44	1 (33%)	no large effect size	Small number of studies	no serious risk of bias	serious	no serious indirectness	no serious imprecision	undetected	LOW ○○⊗⊗
Electrode polarity														
Electrode switch	16	713	79.7%***	0.87	0.47 to 1.26	4 (24%)	large effect size	No	serious	serious	no serious indirectness	no serious imprecision	undetected	MODERATE ⊗⊗⊗⊗
Cathode only	3	97	0%	0.67	0.26 to 1.07	1 (33%)	no large effect size	Small number of studies	no serious risk of bias	no serious inconsistency	no serious indirectness	no serious imprecision	undetected	MODERATE ⊗⊗⊗⊗
Control type														
Sham stimulation	22	1081	80.8%***	0.60	0.31 to 0.90	5 (23%)	no large effect size	No	serious	serious	serious	no serious imprecision	undetected	VERY LOW ○○○⊗
SWC	14	672	70.8%***	0.92	0.57 to 1.26	3 (21%)	large effect size	No	serious	serious	serious	no serious imprecision	undetected	LOW ○○⊗⊗
Ulcer etiology														
Pressure ulcer	11	632	84.1%***	1.00	0.54 to 1.46	2 (18%)	large effect size	No	serious	serious	serious	no serious imprecision	undetected	LOW ○○⊗⊗
Venous ulcer	7	362	0%	0.29	0.04 to	2 (29%)	no large	No	no serious	no serious inconsistency	no serious indirectness	no serious imprecision	undetected	HIGH ⊗⊗⊗⊗

					0.55		effect size		risk of bias					
Diabetic ulcer	8	336	72.5%***	0.67	0.21 to 1.12	2 (25%)	no large effect size	No	no serious risk of bias	serious	serious	no serious imprecision	undetected	LOW ○○⊗⊗
Outcome														
NHU	8	434	65.6%**	0.38	-0.07 to 0.83	2 (25%)	no large effect size	No	no serious risk of bias	serious	no serious indirectness	no serious imprecision	undetected	MODERATE ○⊗⊗⊗
WSA	17	642	80.1%***	1.21	0.82 to 1.60	5 (29%)	large effect size	No	no serious risk of bias	serious	no serious indirectness	no serious imprecision	undetected	HIGH ⊗⊗⊗⊗
Score	2	118	29.1%	0.87	0.42 to 1.32	0	large effect size	Small number of studies	Very serious	no serious inconsistency	serious	no serious imprecision	undetected	VERY LOW ○○○⊗
WHR	9	658	49.9%*	0.21	-0.02 to 0.45	1 (11%)	no large effect size	No	serious	no serious inconsistency	serious	serious	undetected	VERY LOW ○○○⊗

We upgraded for large effect size if $d > 0.8$. We downgraded for risk of bias -1 if less than 25% of high quality studies, -2 if 0 high quality studies. We downgraded for inconsistency if substantial heterogeneity ($I^2 > 50\%$) or if CI of effect size overlap 0. We downgraded for indirectness if outcomes used majority of intermediate criteria (score, healing rate) or ulcer etiology/population were very different. We rate down for impression if $CI > 1.6$. We downgraded one more if subgroup contained less than 5 studies.

SMD: Standardized mean difference; CI: Confidence Interval.

	Random sequence generation	Allocation concealment	Blinding participant/personnel	Blinding outcome assessment	Incomplete outcome data	Selective reporting	Other biases
Adunsky, 2005	+	+	+	+	-	-	+
Ahmad, 2008	-	?	-	-	+	+	+
Baker, 1996	-	?	-	-	+	+	-
Baker, 1997	-	?	-	-	+	+	+
Carley, 1985	-	-	-	-	+	+	+
Feedar, 1991	+	+	+	+	+	+	+
Franeek, 2000	?	-	-	-	+	+	+
Franeek, 2005	?	?	-	-	+	-	+
Franeek, 2006	?	-	-	-	+	+	+
Franeek, 2011	+	+	-	-	+	+	+
Franeek, 2012	+	+	-	-	-	+	+
Gentzkow, 1991	?	?	+	+	-	-	+
Houghton, 2003	?	+	+	+	-	+	+
Houghton, 2010	+	+	+	-	+	+	+
Janković, 2008	-	-	-	-	+	+	+
Jerčinović, 1994	?	?	-	-	+	+	+
Jünger, 2008	+	+	+	+	+	+	+
Lundeberg, 1992	-	-	-	-	+	+	+
Magnoni, 2013	+	+	-	-	+	+	+
Mohajeri-Tehrani, 2014	+	+	+	+	+	+	+
Mulder, 1991	?	?	+	+	+	-	+
Ogrin, 2009	+	+	+	+	+	+	+
Ortíz, 2014	+	+	+	+	-	+	+
Peters, 2001	?	?	+	+	+	+	+
Petrofsky, 2010	?	?	-	-	+	+	+
Santamoto, 2012	+	+	+	+	+	+	+
Taradaj, 2011	+	+	-	-	+	+	+
Ullah, 2007	?	?	-	-	+	+	+
Wood, 1993	+	+	+	+	+	+	+

FigS1. Cochrane risk of bias of included studies.

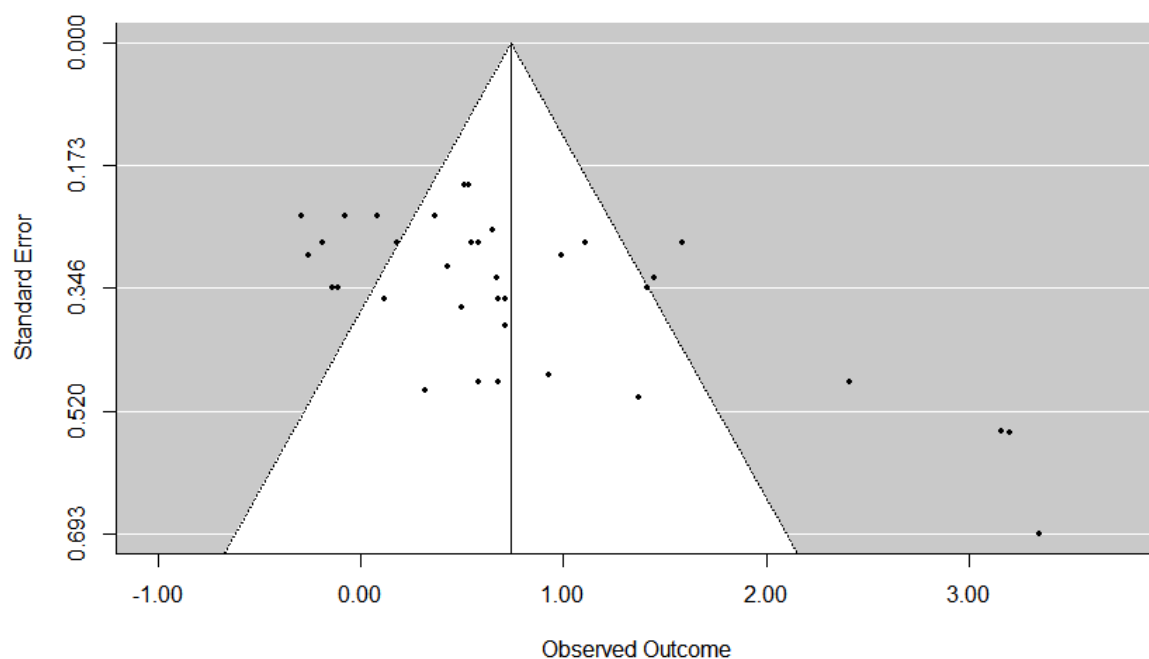


Fig S2. Funnel plot.

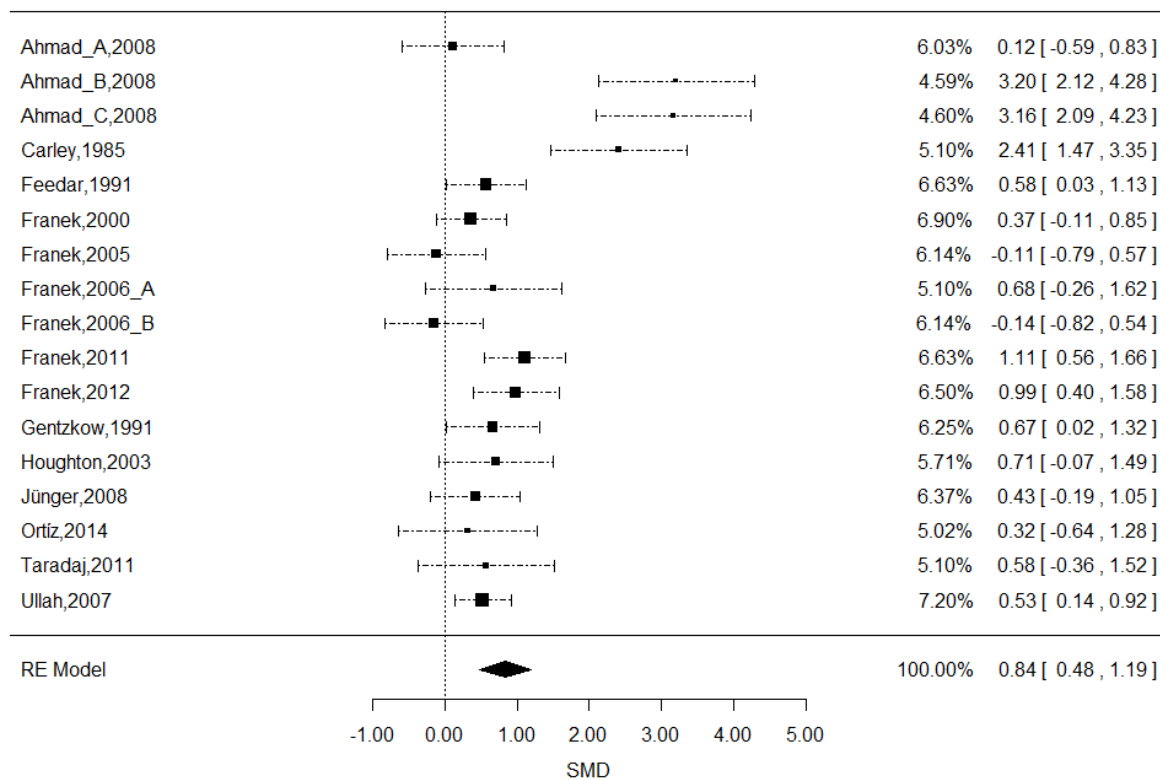


Figure S3. Separate meta-analysis of studies using an active electrode over the wound

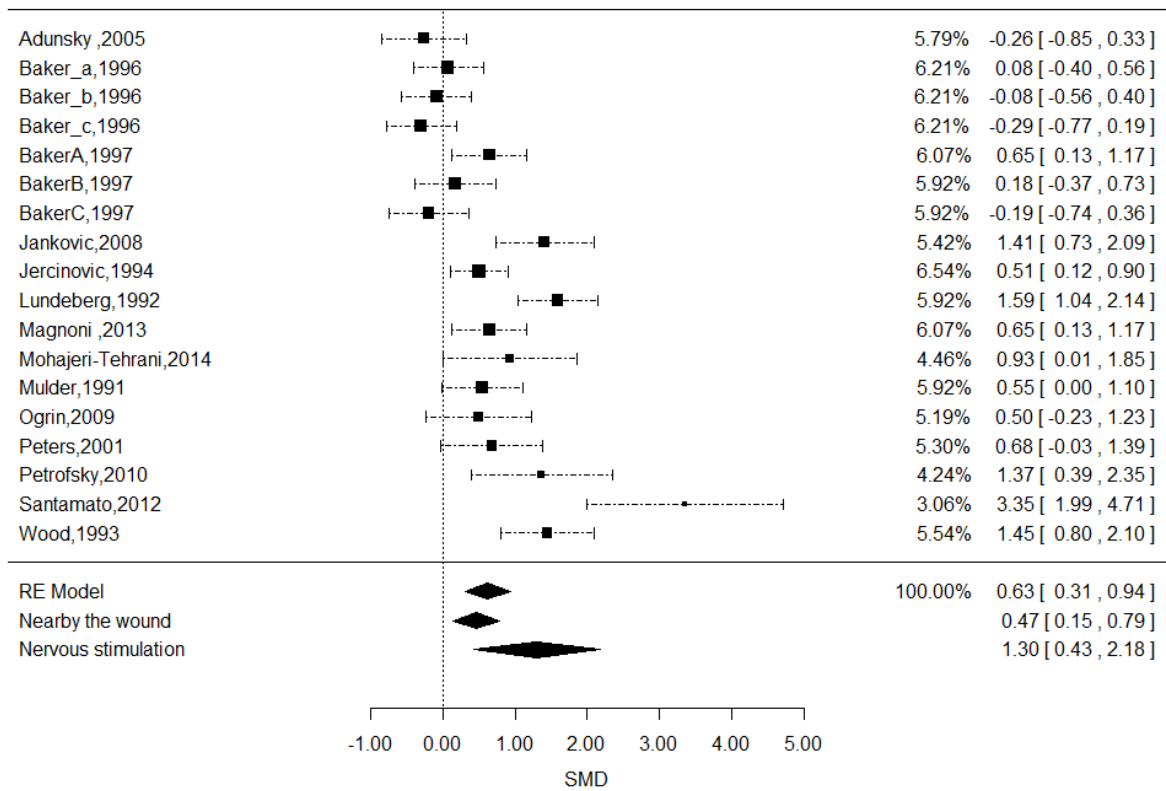


Figure S4. Separate meta-analysis of studies using an active electrode around the wound and subgroup analysis according to electrode placement.

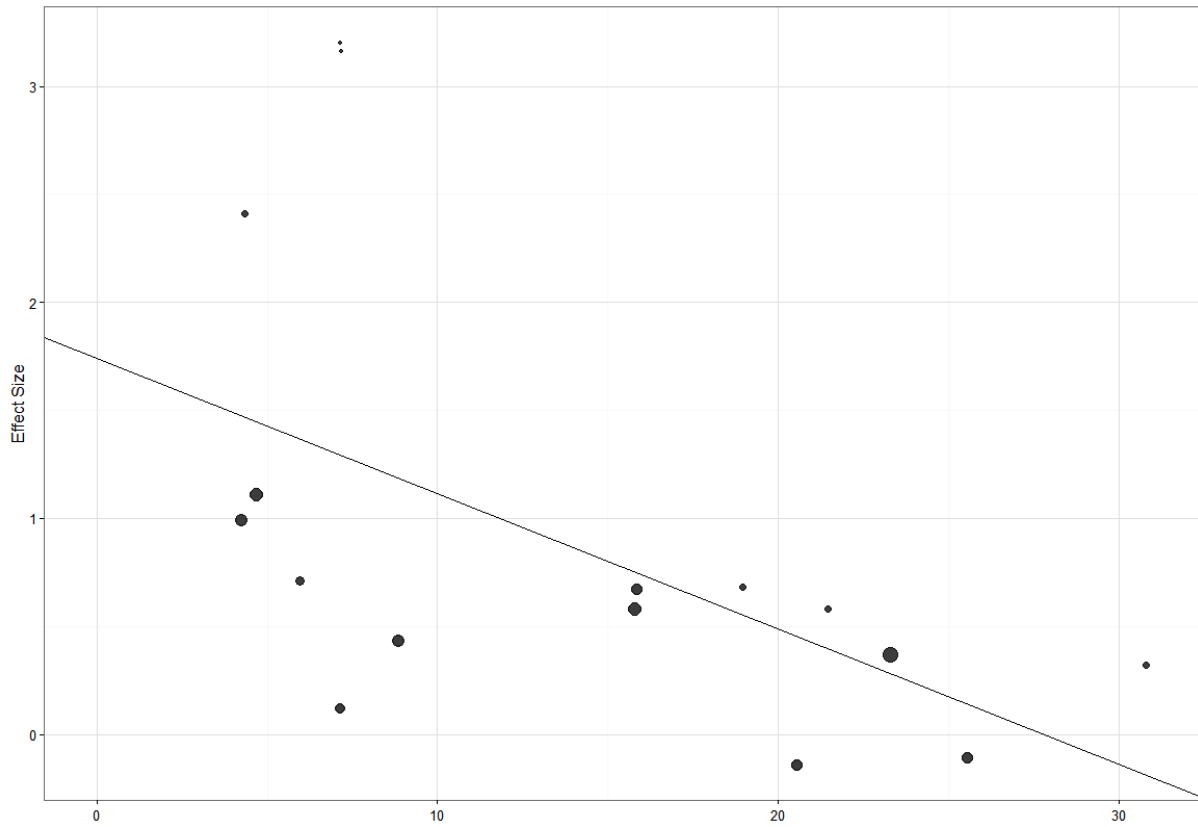


Figure S5. Metaregression plot of effect size depending of initial wound size area (cm²)

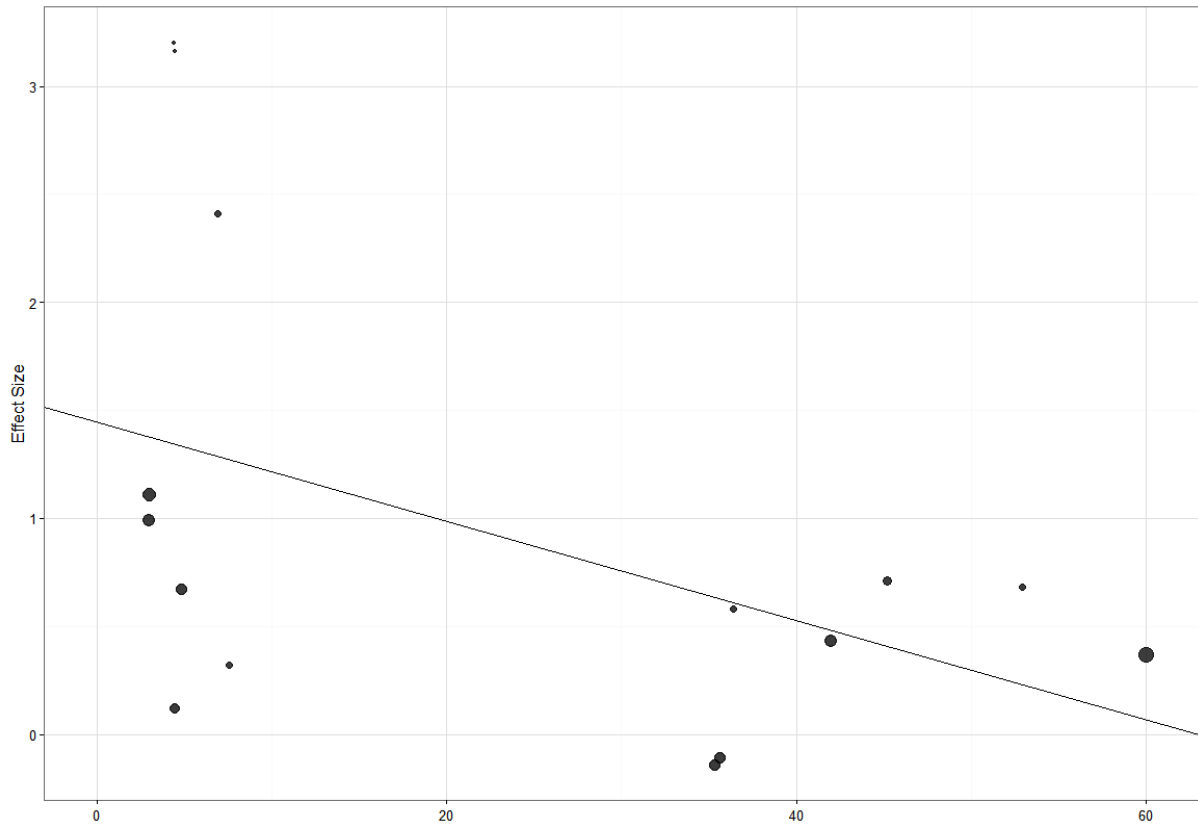


Figure S6. Metaregression plot of effect size depending of duration of the wound (months)

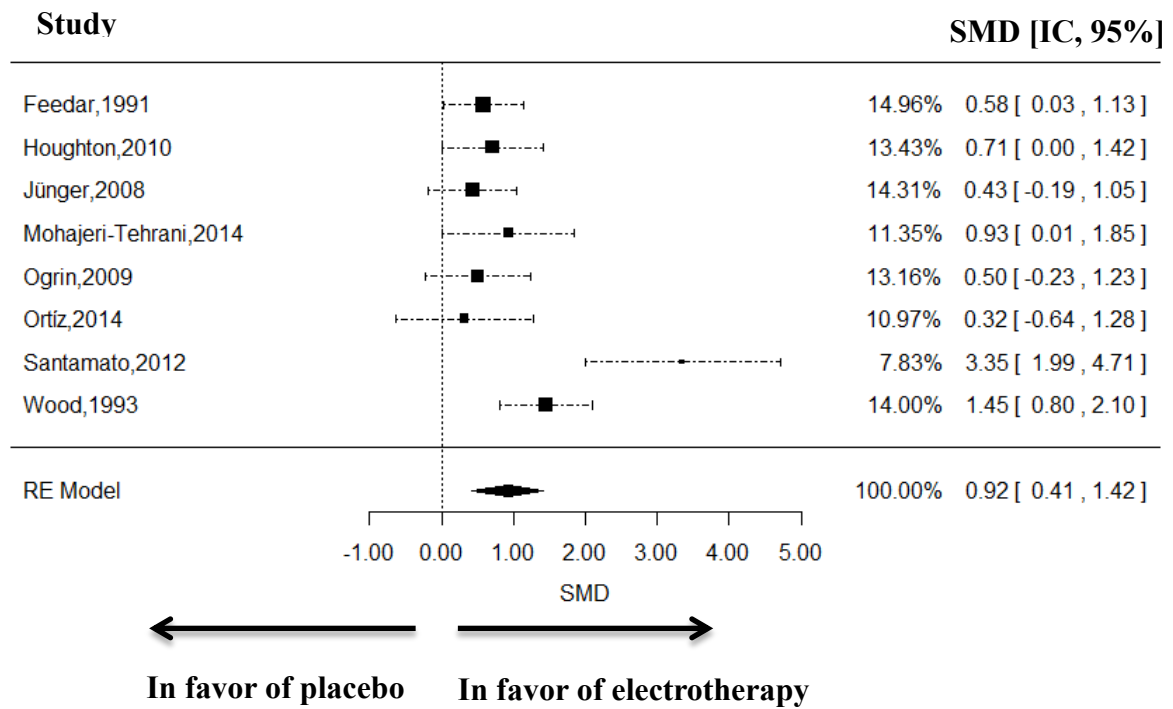


Figure S7. Forest plot of low risk of bias studies.

SMD: Standardized mean difference

SGLT2 inhibitors and the risk of amputation: class effect or media bias?

Charles Khouri, Jean-Luc Cracowski, Matthieu Roustit

SUPPLEMENTARY MATERIAL

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Supplementary Figure S1. Annual proportion of lower limb amputation reports (n/1000 reports) and annual PRR (with lower boundary 95% confidence intervals) of lower-limb amputations for the different lipid lowering drug classes. The red arrows symbolize the first FDA safety communication, issued in May 2016.8

Supplementary Table S1. Detailed characteristics of 79 lower limb amputation cases associated with a SGLT-2 inhibitor listed as suspect or concomitant drug.

Id number	Country of primary source	Gender	Age	Drug	Concomitant medication	Dose (mg/day)	Start date	Onset date	Reported term
DE-BFARM-17414040	Germany	Male	82	Empagliflozin	Insulin human Insulin glargine Metformin	10 mg		2017-08-10	Foot amputation
GB-MHRA-ADR 24229509	United Kingdom	Female	71	Empagliflozin	Insulin human Insulin lispro Irbesartan Lercanidipine Linagliptin Metformin Paracetamol Simvastatin	10 mg			Toe amputation
DE-AstraZeneca-2017SE39636	Germany	Male		Dapagliflozin					Toe amputation
ES-AEMPS-306440	Spain	Male	60	Dapagliflozin		10 mg	2017-05-18	2017-09-19	Toe amputation
ES-AstraZeneca-2017SE22081	Spain	Male		Dapagliflozin					Foot amputation
FI-FIMEA-20172008	Finland	Male	60	Empagliflozin	Metformin	25 mg	2016 -	2017	Toe amputation
ES-BoehringerIngelheim-2017-BI-062159	Spain	Male	5	Empagliflozin					Toe amputation
DK-DKMA-ADR 24203709	Denmark	Male	66	Canagliflozin		300 mg	2015-05-18	2017-09-05	Toe amputation
FI-FIMEA-20171485	Finland	Male	83	Empagliflozin	Metformin;Sitagliptin Atorvastatin Levothyroxine Prednisolone		2016-09-20	2017-08-23	Toe amputation
ES-AGEMED-408290340	Spain	Female	67	Dapagliflozin	Sitagliptin Amlodipine Omeprazole Glimepiride		2017-01-07	2017-04-12	Toe amputation
SE-MPA-2017-005904	Sweden	Female	93	Empagliflozin	Colecalciferol Amoxicillin Sodium picosulfate Macrogol 3350;Potassium;Sodium bicarbonate;Sodium chloride Oxycodone Metoprolol Levothyroxine Rivaroxaban Furosemide Losartan Insulin aspart Glycerol Oxycodone Paracetamol	10 mg	2017-02-12	2017	Leg amputation
13705503	United States (USA)	Unknown		Canagliflozin					Toe amputation
13697137	United States (USA)	Unknown		Canagliflozin					Toe amputation
13486479	United States (USA)	Unknown		Canagliflozin					Toe amputation
13404082	United States (USA)	Male	53	Canagliflozin	Evolocumab Sotalol Rosuvastatin				Leg amputation
13683492	United States (USA)	Male		Canagliflozin					Toe amputation

13637961	United States (USA)	Male	45	Canagliflozin						Toe amputation
13608385	United States (USA)	Male	65	Canagliflozin	Insulin	100 mg	2016-10 -			Toe amputation
13594759	United States (USA)	Male		Canagliflozin						Leg amputation
13674932	United States (USA)	Unknown		Canagliflozin						Toe amputation
13654562	United States (USA)	Male	64	Canagliflozin	Insulin lispro Metformin Exenatide	300 mg	2016-01 -			Toe amputation
13654554	United States (USA)	Unknown		Canagliflozin						Toe amputation
13627609	United States (USA)	Unknown		Canagliflozin		100 mg	2017 -			Leg amputation
13705506	United States (USA)	Unknown		Canagliflozin						Toe amputation
13668503	United States (USA)	Male		Canagliflozin	Metformin Colesevelam	300 mg				Toe amputation
13627156	United States (USA)	Unknown		Canagliflozin						Toe amputation
13678015	United States (USA)	Male		Canagliflozin			2015-01 -			Foot amputation
13664755	United States (USA)	Female	77	Dapagliflozin	Becaplermin Bumetanide Estradiol Tizanidine Metoprolol Celecoxib Tramadol Amoxicillin;Clavulanic acid Hydralazine Esomeprazole Hydrocodone Levothyroxine Atorvastatin Amitriptyline Acetylsalicylic acid Losartan Gabapentin Temazepam	10 mg	2017-03-23			Toe amputation
13705500	United States (USA)	Male		Canagliflozin	Insulin					Toe amputation
CA-HEALTHCANVIG-000692673	Canada	Male	53	Canagliflozin			2015-10-22			Toe amputation
GB-MHRA-ADR 24138490	United Kingdom	Male		Canagliflozin						Toe amputation
GB-MHRA-ADR 24107205	United Kingdom	Male	43	Canagliflozin	Atorvastatin Gliclazide Metformin Ramipril Trimethoprim	100 mg	2015-11	2017-07-09		Toe amputation
PH-PHFDA-2017-02266	Philippines	Male		Canagliflozin		300 mg				Toe amputation
GB-MHRA-ADR 24087231	United Kingdom	Male	36	Canagliflozin	Ciprofloxacin Codeine;Paracetamol Urea Flucloxacillin Gliclazide Potassium citrate Metformin Silver nitrate Sitagliptin		2017-01-30 -	2017-07-14		Toe amputation
13292125	United States (USA)	Male	69	Canagliflozin	Glimepiride Insulin glargine Quinapril Apixaban Metformin;Sitagliptin Clopidogrel	300 mg				Toe amputation
ES-AGEMED-505195241	Spain	Male	47	Canagliflozin		300 mg	2016-11-15 -	2017-02		Toe amputation

GB-MHRA-ADR 23988081	United Kingdom	Male	59	Canagliflozin	Amiodarone Acetylsalicylic acid Atorvastatin Bisoprolol Doxycycline Insulin glargine Metformin Omeprazole Quinine Sertraline Spirolactone Ticagrelor Umeclidinium; Vilanterol	100 mg		2017-04-28	Toe amputation
SE-MPA-2017-003532	Sweden	Male	54	Dapagliflozin	Insulin detemir Metformin Glipizide Amlodipine Candesartan Atorvastatin Citalopram Tadalafil	10 mg	2017-04-03 -	2017-04-18	Toe amputation
GB-MHRA-ADR 23944778	United Kingdom	Male	59	Empagliflozin	Metformin Ramipril Simvastatin Sitagliptin	10 mg	2016-02-02	2017-03-20	Toe amputation
ES-AGEMED- 806074440	Spain	Male		Empagliflozin	Insulin				Leg amputation
ES-AGEMED- 043702432	Spain	Female	62	Canagliflozin	Insulin				Leg amputation
ES-AGEMED- 843619332	Spain	Male	5	Canagliflozin	Dulaglutide Insulin Pioglitazone Atorvastatin Enalapril Metformin		2015-09-11	2017-02-15	Leg amputation
CA- HEALTHCANVIG- E2B_01063527	Canada	Male	71	Canagliflozin	Gliclazide Rosuvastatin Perindopril Sitagliptin	300 mg	2016-02-29		Leg amputation
13064804	United States (USA)	Male		Canagliflozin			2014-12-04		Leg amputation
12759079	United States (USA)	Female	43	Canagliflozin	Sertraline Medroxyprogesterone Cod- liver oil Colecalciferol	100 mg	2014-08-10		Foot amputation
12807390	United States (USA)	Male		Canagliflozin					Toe amputation
12949837	United States (USA)	Female	56	Canagliflozin Empagliflozin			2016-05-08 2016-06-28		Toe amputation
13064392	United States (USA)	Unknown		Canagliflozin			2014-12 -		Leg amputation
13022799	United States (USA)	Male	60	Canagliflozin	Clopidogrel Glimepiride Metformin; Sitagliptin Quinapril Insulin glargine Apixaban	300 mg			Toe amputation
12836281	United States (USA)	Female		Canagliflozin					Foot amputation
ES-AGEMED- 407300340	Spain	Male	72	Empagliflozin			2016-08 -	2017-01	Toe amputation
GB-MHRA-ADR 23839107	United Kingdom	Male	55	Canagliflozin	Amitriptyline Acetylsalicylic acid Exenatide Ezetimibe Insulin lispro Insulin glargine Levothyroxine Losartan Omeprazole Pravastatin Salbutamol Fluticasone; Salmeterol	300 mg	2016-03-01	2016-04-04	Toe amputation
12780748	United States (USA)	Male	55	Canagliflozin	Acetylsalicylic acid Warfarin Methadone Insulin detemir Clopidogrel	300 mg	2014-11-06		Leg amputation
12699526	United States (USA)	Female		Canagliflozin		100 mg			Toe amputation
12580818	United States (USA)	Female	57	Canagliflozin		300 mg			Leg amputation

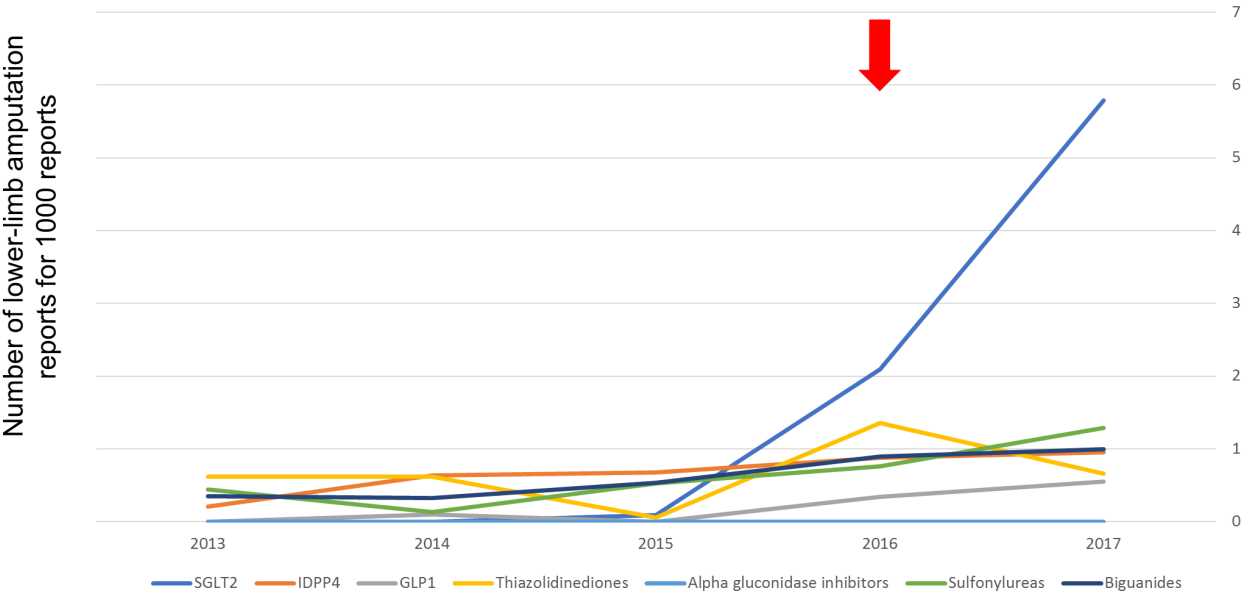
12504878	United States (USA)	Male	51	Empagliflozin	Becaplermin Glibenclamide				Toe amputation
12561865	United States (USA)	Male		Canagliflozin		300 mg			Toe amputation
12677950	United States (USA)	Male	54	Empagliflozin					Toe amputation
12724123	United States (USA)	Male	64	Canagliflozin	Metformin;Pioglitazone		2015-02-13		Toe amputation
GB-MHRA-ADR 23790226	United Kingdom	Male	51	Canagliflozin	Amoxicillin Clavulanic acid Acetylsalicylic acid Exenatide Calcipotriol Capsaicin Ceftriaxone Codeine Duloxetine Gabapentin Alginate acid;Potassium bicarbonate Gliclazide Pregabalin Metformin Metformin Metronidazole Naproxen Nortriptyline Omeprazole Paracetamol Pravastatin Sildenafil Simvastatin Sodium chloride Codeine;Paracetamol	100 mg	2014-10-22	2015-01-31	Toe amputation
GB-MHRA-ADR 23712672	United Kingdom	Male	61	Dapagliflozin	Insulin porcine Gliclazide	10 mg			Toe amputation
CH-SM-2016-04186	Switzerland	Male	57	Canagliflozin		300 mg	2014-09-04	2016-05-12	Toe amputation
DE-BFARM- 16222681	Germany	Male	75	Empagliflozin	Eplerenone Insulin Insulin Furosemide Eplerenone Torasemide Torasemide	25 mg			Toe amputation
12448174	United States (USA)	Female	58	Dapagliflozin	Insulin	10 mg	2015-05		Toe amputation
12311802	United States (USA)	Male	57	Dapagliflozin	Lisinopril Metformin Glipizide	10 mg	2013-09		Toe amputation
12411337	United States (USA)	Male	55	Canagliflozin		300 mg	2014-11-06		Leg amputation
12411772	United States (USA)	Male		Canagliflozin	Insulin lispro Insulin glargine Furosemide Acetylsalicylic acid Tamsulosin Atorvastatin Lisinopril Amoxicillin Glucagon	300 mg	2013 -		Toe amputation
12327883	United States (USA)	Male	56	Empagliflozin	Becaplermin Lisinopril Pantoprazole Atorvastatin Montelukast	25 mg			Toe amputation
12231649	United States (USA)	Male	73	Canagliflozin	Becaplermin Losartan Insulin detemir Metformin Nateglinide				Toe amputation
12355368	United States (USA)	Male	55	Canagliflozin	Becaplermin Metformin Carvedilol Lisinopril	300 mg			Toe amputation
12494898	United States (USA)	Male		Canagliflozin					Toe amputation
12398041	United States (USA)	Male	62	Canagliflozin	Indapamide Liraglutide Pioglitazone Sildenafil Varenicline Paroxetine Verapamil Fenofibrate Fenofibrate Atorvastatin Lisinopril	300 mg	2014-06-18		Toe amputation
12508406	United States (USA)	Male		Canagliflozin					Toe amputation

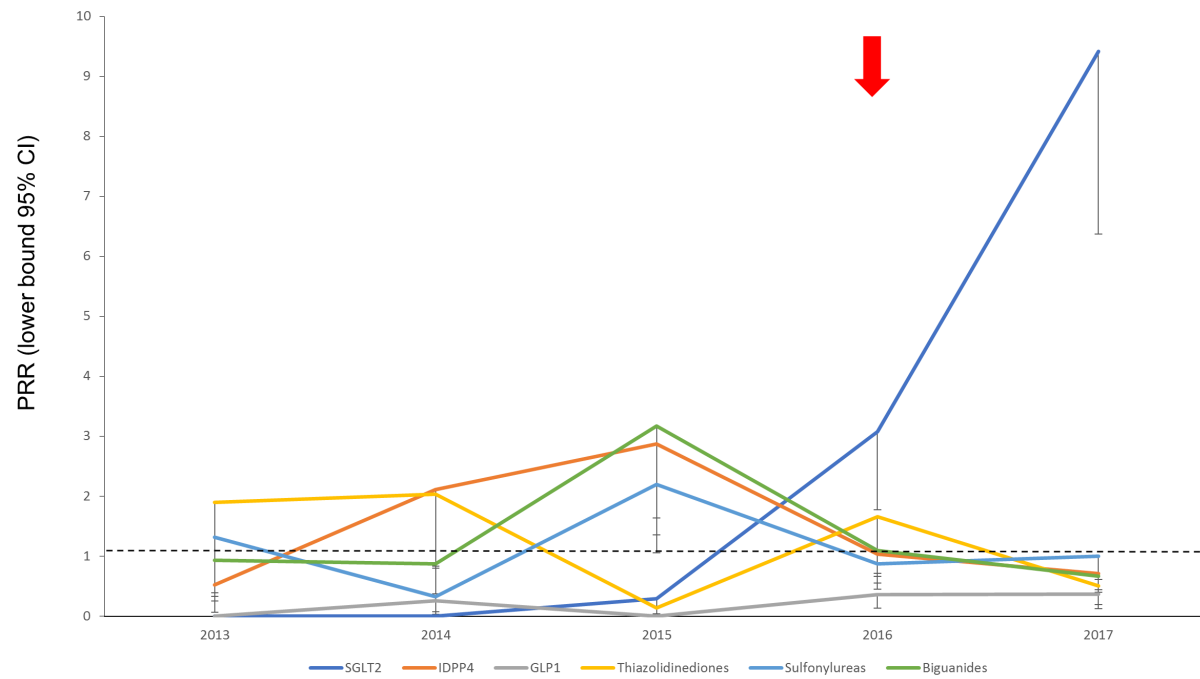
12355366	United States (USA)	Male	54	Canagliflozin	Becaplermin Metformin Insulin glargine Enalapril				Foot amputation
GB-MHRA-ADR 23518408	United Kingdom	Male	70	Canagliflozin	Metformin	300 mg	2015-01-15	2016-06-01	Toe amputation
GB-MHRA-ADR 23485793	United Kingdom	Unknown	65	Canagliflozin	Gliclazide Losartan Metformin Tamsulosin	300 mg		2016-05-03	Toe amputation
11694137	United States (USA)	Male	59	Canagliflozin	Becaplermin Amlodipine Metformin Gabapentin Furosemide Insulin detemir Losartan Ciprofloxacin		2015		Foot amputation
11860666	United States (USA)	Female	56	Dapagliflozin	Becaplermin Gemfibrozil Gabapentin Meloxicam Enalapril	5 mg			Toe amputation
11490270	United States (USA)	Male		Canagliflozin					Toe amputation

Supplementary Table S2. Pooled data retrieved from 79 Vigibase reports of SGLT2i-associated lower limb amputations compared to all other glucose lowering drugs. As not all reports are complete, the numbers (%) of reports providing each variable are reported. If a report was associated with several glucose lowering drugs it was counted in each drug class. Frequencies of reports between drug classes were compared using the χ^2 test and continuous outcomes using the Student test.

	Canagliflozin	Dapagliflozin	Empagliflozin	All SGLT-2 inhibitors	Other glucose lowering drugs (excluding insulin)	P-value
Total number of reports	15,873	8,980	4,728	31,495	369,543	NA
Number of lower limb amputation reports (notification rate/1000)	56 (3.5/1000)	10 (1.1/1000)	14 (3.0/1000)	79 (2.5/1000)	187(0.5/1000)	<0.001
Age (Mean \pm sd) (available reports %)	55.8 \pm 12.9 (55%)	61.3 \pm 7.5 (80%)	62.9 \pm 21.7 (93%)	58.4 \pm 15.1 (66%)	61.6 \pm 12.1 (73%)	0.148
Sex (M/F) (available reports %)	40/6 (82%)	6/4 (100%)	7/2 (100%)	57/13 (87%)	120/60 (96%)	0.021
Duration of treatment (Median (min-max)) (available reports %) (days)	503 (34-841) (16%)	95 (15-124) (30%)	153 (55-412) (30%)	165 (15-841) (19%)	NA	NA
Highest level of amputation						
Leg	11	0	2	13	63	0.004
Foot	5	1	1	6	21	0.370
Toe	40	9	11	61	103	<0.001

Supplementary Figure S1. Annual proportion of lower limb amputation reports (n/1000 reports) and annual PRR (with lower boundary 95% confidence intervals) of lower-limb amputations for the different lipid lowering drug classes. The red arrows symbolize the first FDA safety communication, issued in May 2016.





Supplementary Material

Drug-induced skin ulcer: a combined disproportionality analysis using data from Medline and Vigibase

Supplementary Table 1. Excluded drugs

Drugs	Reason for exclusion
Becaplermin	Ulcer treatment
Blood substitutes and perfusion solutions	Ulcer treatment
Bosentan	Ulcer treatment prevention
Calcium chloride;Glucose;Magnesium chloride;Sodium chloride;Sodium lactate	Ulcer treatment
Calcium chloride;Icodextrin;Magnesium chloride;Sodium chloride;Sodium lactate	Ulcer treatment
Canagliflozin	Protopathic bias
Canagliflozin;Metformin	Protopathic bias
Darbepoetin alfa	Protopathic bias
Epoprostenol	Ulcer treatment prevention
Erythropoietin human	Protopathic bias
Gadodiamide	Ulcer diagnostic product
Gadolinium	Ulcer diagnostic product
Gadoversetamide	Ulcer diagnostic product
Insulin detemir	Protopathic bias
Insulin glargine	Protopathic bias
Insulin human	Protopathic bias
Insulin lispro	Protopathic bias
Pregabalin	Protopathic bias
Riociguat	Ulcer treatment prevention
Tocilizumab	Protopathic bias
Alendronic acid;Colecalciferol	Combined substances
Albumin human;Glucose;Interferon beta	Combined substances
Mycophenolic acid	Pyoderma treatment
Tacrolimus	Pyoderma treatment
Rituximab	Pyoderma treatment
Adalimumab	Pyoderma treatment
Etanercept	Pyoderma treatment
Thalidomide	Pyoderma treatment
Warfarin	Calciphylaxis
Fluindione	Calciphylaxis
Doxorubicine	Extravasation
Daunorubicine	Extravasation
Aminopterin	Not labelled anymore (as methotrexate)
Hydralazine	Systemic lupus erythematosus
Anagrelide	Protopathic bias
Ergotamine	Gangrenous ergotism

Impact of pharmacovigilance disproportionality analysis study design on the vibration of effect and on the correlation with drug-related risks.

SUPPLEMENTARY MATERIAL

Text S1. Adverse drug reactions characterization

The frequency was determined based on Micromedex[®] database and ranked according to the scale recommended by the Council for International Organizations of Medical Sciences (CIOMS): Very common ($\geq 1/10$); common ($\geq 1/100$ to $< 1/10$); uncommon ($\geq 1/1,000$ to $< 1/100$); rare ($\geq 1/10,000$ to $< 1/1,000$); very rare ($< 1/10,000$)¹. The severity of an ADR was determined by calculating the proportion of serious cases over 1000 ICSRs reported with the drug class in Vigibase[®]. A serious adverse event was defined as any event that was fatal, life-threatening, caused hospitalization, resulted in persistent or significant disability or incapacity, required intervention to prevent permanent damage or caused congenital anomalies². An ADR was quoted as serious if more than 50% of reports were serious. An ADR was considered immediate if it occurred in the first month following drug exposure and delayed in other situations. ADRs were also distinguished into types A, B and C. Type A adverse effects are relatively common, dosage-related because they are related to the pharmacological effects of the drug. They are generally identified before marketing. As for those of type B, they are often allergic or idiosyncratic reactions and occur in few patients (e.g less than 1 per 1000). They are usually serious. Their unpredictable and unexpected character makes them difficult to detect during clinical trials. Finally, type C adverse effects are associated with a chronic intake of a drug. Occurring randomly, sometimes after a long period of latency, a causal relationship with drug is often difficult to identify³. The influence of media attention on the rate of reporting was assessed by comparing the proportion of the ADR of interest reported 6 months before and 6 months after the date of the first media alert using a Chi-2 test⁴.

Table S1. Details of excluded meta-analysis assessing the safety of several pharmacological interventions and reason for exclusion.

Journal/Meta-analysis title	Reason for exclusion
British Medical Journal	
Alpha blockers for treatment of ureteric stones: systematic review and meta-analysis	no specific adverse events
Antenatal corticosteroids for maturity of term or near term fetuses: systematic review and meta-analysis of randomized controlled trials	no safety
Renin angiotensin system inhibitors for patients with stable coronary artery disease without heart failure: systematic review and meta-analysis of randomized trials	pooled analysis only
Addition of dipeptidyl peptidase-4 inhibitors to sulphonylureas and risk of hypoglycaemia: systematic review and meta-analysis	pooled analysis only
Comparative effectiveness and tolerance of treatments for Helicobacter pylori: systematic review and network meta-analysis	pooled analysis only
ipeptidyl peptidase-4 inhibitors and risk of heart failure in type 2 diabetes: systematic review and meta-analysis of randomised and observational studies	pooled analysis only
Glibenclamide, metformin, and insulin for the treatment of gestational diabetes: a systematic review and meta-analysis	pooled analysis only
Comparative benefits and harms of second generation antidepressants and cognitive behavioral therapies in initial treatment of major depressive disorder: systematic review and meta-analysis	no safety
Efficacy and safety outcomes of oral anticoagulants and antiplatelet drugs in the secondary prevention of venous thromboembolism: systematic review and network meta-analysis.	no specific adverse events
Different combined oral contraceptives and the risk of venous thrombosis: systematic review and network meta-analysis.	treatment combination
Comparative effectiveness of renin-angiotensin system blockers and other antihypertensive drugs in patients with diabetes: systematic review and bayesian network meta-analysis.	no safety
Efficacy of recommended drugs against soil transmitted helminths: systematic review and network meta-analysis	no safety
Treatment strategies for women with WHO group II anovulation: systematic review and network meta-analysis.	no safety
Oral anticoagulants for prevention of stroke in atrial fibrillation: systematic review, network meta-analysis, and cost effectiveness analysis.	included
New England Journal of Medicine	
Journal of the American Medical Association	
Association Between Use of Sodium-Glucose Cotransporter 2 Inhibitors, Glucagon-like Peptide 1 Agonists, and Dipeptidyl Peptidase 4 Inhibitors With All-Cause Mortality in Patients With Type 2 Diabetes: A Systematic Review and Meta-analysis.	not enough groups

Association of Inhaled Corticosteroids and Long-Acting Muscarinic Antagonists With Asthma Control in Patients With Uncontrolled, Persistent Asthma: A Systematic Review and Meta-analysis.	no safety
Association of Inhaled Corticosteroids and Long-Acting β -Agonists as Controller and Quick Relief Therapy With Exacerbations and Symptom Control in Persistent Asthma: A Systematic Review and Meta-analysis.	no safety
Comparison of Clinical Outcomes and Adverse Events Associated With Glucose-Lowering Drugs in Patients With Type 2 Diabetes: A Meta-analysis.	included
Association of Pharmacological Treatments for Obesity With Weight Loss and Adverse Events: A Systematic Review and Meta-analysis.	no specific ei
Clinical and safety outcomes associated with treatment of acute venous thromboembolism: a systematic review and meta-analysis.	no specific ei
Blood pressure lowering in type 2 diabetes: a systematic review and meta-analysis.	no safety
The Lancet	
The risk of serious infection with biologics in treating patients with rheumatoid arthritis: A Systematic Review and Meta-analysis	no enough treatment groups
Mortality in patients treated with extended duration dual antiplatelet therapy after drug-eluting stent implantation: a pairwise and Bayesian network meta-analysis of randomised trials.	pooled analysis only
Comparative efficacy and safety of blood pressure-lowering agents in adults with diabetes and kidney disease: a network meta-analysis.	included
Comparison of the efficacy and safety of new oral anticoagulants with warfarin in patients with atrial fibrillation: a meta-analysis of randomised trial	no safety
Comparative efficacy and tolerability of antidepressants for major depressive disorder in children and adolescents: a network meta-analysis.	included
Blood pressure lowering for prevention of cardiovascular disease and death: a systematic review and meta-analysis.	no safety
Blood pressure-lowering treatment based on cardiovascular risk: a meta-analysis of individual patient data.	no safety
Comparative efficacy and tolerability of 15 antipsychotic drugs in schizophrenia: a multiple-treatments meta-analysis.	included
Annals of Internal Medicine	
Benefits and Harms of Intensive Blood Pressure Treatment in Adults Aged 60 Years or Older: A Systematic Review and Meta-analysis.	no enough groups
Benefits and Harms of Osteoporosis Medications in Patients With Chronic Kidney Disease: A Systematic Review and Meta-analysis.	no enough groups
Diabetes Medications as Monotherapy or Metformin-Based Combination Therapy for Type 2 Diabetes: A Systematic Review and Meta-analysis.	no enough groups
Benefits and Harms of Once-Weekly Glucagon-like Peptide-1 Receptor Agonist Treatments: A Systematic Review and Network Meta-analysis.	no enough groups
Leukotriene-receptor antagonists versus placebo in the treatment of asthma in adults and adolescents: a systematic review and meta-analysis.	no specific adverse events
Comparative effectiveness of pharmacologic interventions for knee osteoarthritis: a systematic review and network meta-analysis.	no specific adverse events

Biological agents for moderately to severely active ulcerative colitis: a systematic review and network meta-analysis.	pooled analysis only
Pharmacologic interventions for painful diabetic neuropathy: An umbrella systematic review and comparative effectiveness network meta-analysis.	no safety
Sodium-glucose cotransporter 2 inhibitors for type 2 diabetes: a systematic review and meta-analysis.	no enough groups
Plos Medicine	
Treatment and outcomes in children with multidrug-resistant tuberculosis: A systematic review and individual patient data meta-analysis.	no specific adverse events
Blood pressure-lowering treatment strategies based on cardiovascular risk versus blood pressure: A meta-analysis of individual participant data.	no safety
Benefits and safety of gabapentinoids in chronic low back pain: A systematic review and meta-analysis of randomized controlled trials.	no enough groups
JAMA Internal Medicine	
Association of Gastric Acid Suppression With Recurrent Clostridium difficile Infection: A Systematic Review and Meta-analysis.	pooled analysis only
Effect of angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers on all-cause mortality, cardiovascular deaths, and cardiovascular events in patients with diabetes mellitus: a meta-analysis.	no safety

Table S2. Values of slopes and intercept with standard errors (se) and Pearson production moment correlation coefficients for each adverse drug reaction and study design.

Adverse events	Suspect reports			Concomitant reports included			Time standardization			Regional standardization			Therapeutic area standardized			Competitor excluded			Lower CI			Robust regression			Median ROR		
	Intercept (se)	Slope(se)	Pearson r	Intercept (se)	Slope(se)	Pearson r	Intercept (se)	Slope(se)	Pearson r	Intercept (se)	Slope(se)	Pearson r	Intercept (se)	Slope(se)	Pearson r	Intercept (se)	Slope(se)	Pearson r	Intercept (se)	Slope(se)	Pearson r	Intercept (se)	Slope(se)	Pearson r	Intercept (se)	Slope(se)	Pearson r
1. Weight gain	-0.75 (1.65)	0.12 (0.98)	0.02	0.93 (0.24)	-0.13 (0.12)	-0.28	0.1 (0.88)	0.37 (0.51)	0.23	0.84 (0.18)	-0.21 (0.14)	-0.43	0.7 (0.13)	0.04 (0.14)	0.08	0.69 (0.3)	0.01 (0.17)	0.02	0.35 (0.2)	0.08 (0.13)	0.17	0.71 (0.21)	0 (0.13)	6.65E-06	0.7 (0.28)	0.00 (0.17)	0.05
1. Extrapyramidal syndrome	-1.68 (0.55)	0.92 (0.23)	0.75	-2.45 (0.72)	1.34 (0.32)	0.75	-0.36 (0.25)	0.33 (0.08)	0.93	-1.83 (0.62)	0.97 (0.25)	0.77	0.3 (0.14)	0.82 (0.19)	0.75	-1.75 (0.56)	0.9 (0.23)	0.75	-2.22 (0.56)	0.99 (0.25)	0.74	- (0.44)	0.72 (0.15)	0.56	-2.0 (0.56)	1.14 (0.25)	0.78
1. Prolactin increase	-2.39 (0.53)	0.55 (0.18)	0.77	-0.93 (0.42)	0.69 (0.16)	0.82	-1.11 (0.72)	0.8 (0.26)	0.81	-1.19 (0.3)	0.76 (0.1)	0.95	0.56 (0.19)	0.49 (0.11)	0.83	-0.67 (0.4)	0.5 (0.14)	0.82	-0.84 (0.26)	0.58 (0.11)	0.89	- (0.25)	0.78 (0.03)	0.93	-2.38 (0.53)	0.55 (0.18)	0.78
1. QTc prolongation	-0.26 (0.19)	0.33 (0.07)	0.87	-0.28 (0.19)	0.36 (0.07)	0.87	3.31 (1.45)	0.48 (0.4)	0.93	0.03 (0.13)	0.21 (0.06)	0.82	0.34 (0.1)	0.33 (0.07)	0.86				-0.43 (0.12)	0.32 (0.05)	0.93	- (0.17)	0.33 (0.06)	0.73	-0.38 (0.2)	0.38 (0.08)	0.85
1. Sedation	-1.36 (7.73)	1.45 (4.44)	0.1	7.71 (11.48)	-3.88 (6.48)	-0.18	0.24 (3.22)	0.46 (1.75)	0.09	1.79 (0.39)	-0.46 (0.24)	-0.54	0.84 (2.18)	1.11 (3.68)	0.09				-0.7 (1.38)	0.91 (0.93)	0.28	0.63 (0.94)	0.2 (0.43)	0.06	2.48 (5.71)	-0.95 (3.52)	-0.08
2. Hyperkalemia	-0.85 (0.35)	0.56 (0.16)	0.96	0.85 (0.35)	0.56 (0.16)	0.82				-0.61 (0.22)	0.36 (0.08)	0.88	0.22 (0.1)	0.34 (0.07)	0.89	-0.61 (0.2)	0.35 (0.07)	0.9	-3.15 (1.07)	0.84 (0.39)	0.89	- (0.2)	0.31 (0.06)	0.83	-0.56 (0.23)	0.35 (0.08)	0.87
2. Presyncope	2.61 (6.1)	-2.94 (5.91)	-0.16	-8.01 (15.45)	8.67 (16.11)	0.18				-0.29 (0.21)	0.29 (0.13)	0.65	-2.27 (4.1)	-1.55 (2.72)	-0.19				-0.39 (0.74)	-1.66 (0.83)	-0.2	0.16 (0.32)	- (0.36)	0.03	-5.2 (23.5)	6.6 (26.2)	0.08
2. Cough	0.35 (0.2)	0.33 (0.13)	0.65	-0.03 (0.4)	0.85 (0.42)	0.56				0.17 (0.27)	0.69 (0.27)	0.65	1.19 (0.31)	0.34 (0.14)	0.64	0.34 (0.21)	0.3 (0.1)	0.65	-1.15 (0.32)	0.72 (0.2)	0.64	0.33 (0.15)	0.3 (0.06)	0.43	0.36 (0.23)	0.49 (0.2)	0.62

				2)																							
2. Oedema	0.14 (0.5 4)	0.59 (0.4 2)	0.4 2	- 2.29 (4.1 5)	3.45 (4.1 7)	0.2 7				0.21 (1.56)	0.84 (1.6 4)	0.1 7	1.51 (0.8)	0.57 (0.4 2)	0.4 1				- 14.08 (133. 24)	18.1 2 (115. 29)	0.4 8	0.34 (0.2 5)	0.3 (0.2 9)	0.1 31 5	- 0.85 (1.8 4)	2.36 (2.1 7)	0.3 4
3. Suicide	-0.9 (1.5 9)	0.47 (0.5 9)	0.3 1	- 1.47 (0.9 8)	0.71 (0.4 0)	0.5 6				- 18.3 5 (50.6 4)	7.07 (18. 87)	0.1 7	0.18 (0.5 3)	0.44 (0.5 5)	0.3 1	- 1.14 (1.8 7)	0.4 6 (0.5 8)	0.3 1	9.7 (461. 09)	-5.11 (180. 91)	- 0.0 12	- 0.29 (0.1 1)	0.13 (0.0 4)	0.2 53 1	- 1.31 (0.9 3)	0.65 (0.3 7)	0.5 5
4. Intracranial bleeding	- 1.36 (0.9 3)	0.18 (0.3 1)	0.3 2	-1.6 (0.6 2)	0.25 (0.2)	0.3 2	- 0.83 (2.2 2)	0 (0.8)	0	-7 (21.9 8)	2.1 (7.4 2)	0.2	- 0.83 (0.1 5)	0.17 (0.3 5)	0.2 6	- 1.62 (1.1 4)	0.2 5 (0.3 4)	0.3 8	-1.69 (0.48)	0.47 (0.16)	0.5 2	- 1.23 (0.4 4)	0.14 (0.1 8)	0.0 81 29	- 1.59 (1.1 4)	0.27 (0.3 9)	0.3 7
4. GI bleeding	- 0.94 (0.0 5)	0.35 (0.0 2)	0.9 7	- 1.02 (0.0 6)	0.37 (0.0 2)	0.9 7	- 0.84 (1.7 7)	0.3 6 (0.6 7)	0.3 6	-0.03 (0.11)	0.07 (0.0 3)	0.7 6	- 0.06 (0.0 2)	0.36 (0.0 3)	0.9 9	- 1.03 (0.0 6)	0.3 7 (0.0 2)	0.9 7	-1.93 (0.69)	0.26 (0.24)	0.8 7	- 0.94 (0.0 4)	0.35 (0.0 2)	0.9 7	- 0.97 (0.0 4)	0.36 (0.0 1)	0.9 98
5. Hypoglycaemia	- 0.88 (0.7 4)	0.57 (0.2 1)	0.6 6	- 1.69 (0.9 9)	1.01 (0.3 6)	0.6 8				-0.82 (0.82)	0.64 (0.2 8)	0.6 1	1.16 (0.3 3)	0.55 (0.2 2)	0.6 3				-0.8 (0.48)	0.32 (0.14)	0.6 7	- 0.28 (0.2 3)	0.31 (0.0 7)	0.0 81 29	- 1.12 (0.8 5)	0.77 (0.3 0)	0.6 5

Table S3. Agreement results through Bland and Altman method.

Adverse events	Suspect reports			Concomitant reports included			Time standardization			Regional standardization			Therapeutic area standardized			Competitor excluded			Median ROR		
	CI inf	Mean dif	CI sup	CI inf	Mean dif	CI sup	CI inf	Mean dif	CI sup	CI inf	Mean dif	CI sup	CI inf	Mean dif	CI sup	CI inf	Mean dif	CI sup	CI inf	Mean dif	CI sup
1. Weight gain	-3.4	3.4	10.3	-62.3	12.8	87.9	-4.7	3.9	12.5	-3.6	1.0	5.7	-4.3	-0.5	3.4	-3.5	3.7	11.0	-0.06	8.00	16.05
1. Extrapyramidal syndrome	-3.4	10.3	24.0	-3.6	11.3	26.2	-384.9	80.0	544.9	-2.6	10.6	23.8	-2.0	-0.4	1.3	-3.6	11.3	26.2	-0.06	8.00	16.05
1. Prolactin increase	-47.7	23.2	94.1	-35.3	19.0	73.2	-45.0	21.2	87.3	-61.5	30.2	122.0	-20.2	3.5	27.2	-51.2	24.7	100.7	-42.09	20.91	83.90
1. QTc prolongation	-94.4	31.0	156.5	-90.7	27.2	145.1	-424.1	124.7	673.5	-47.2	18.2	83.6	-16.2	3.7	23.7				-85.73	25.04	135.81
1. Sedation	-5.9	2.7	11.3	-5.2	2.8	10.7	-5.0	3.3	11.7	-8.3	1.6	11.6	-6.9	-2.0	3.0				-6.16	2.08	10.32
2. Hyperkalemia	-86.7	31.0	148.7	-14.4	9.6	33.6				-77.6	29.1	135.8	-9.0	1.7	12.5	-92.7	33.2	159.0	-79.38	27.59	134.56
2. Presyncope	-1.0	1.7	4.5	-0.3	1.5	3.3				-2.9	2.3	7.5	-2.0	-0.8	0.4				-0.87	1.33	3.53
2. Cough	-13.8	3.7	21.2	-4.6	0.5	5.6				-3.3	0.3	3.9	-4.7	-1.8	1.1	-14.7	4.0	22.6	-4.85	0.50	5.84
2. Oedema	-6.7	0.9	8.4	-6.8	0.0	6.8				-7.1	-0.6	5.8	-8.6	-2.4	3.9				-6.88	-0.51	5.87
3. Suicide	-0.8	14.6	30.0	-2.0	11.7	25.3				1.7	13.2	24.7	-4.9	-0.2	4.5	0.3	27.0	53.7	0.38	2.12	3.85
4. Intracranial bleeding	3.5	20.0	36.6	-2.2	24.7	51.7	3.9	16.4	29.0	11.7	19.1	26.5	-0.2	0.7	1.6	6.6	28.4	50.2	2.90	3.69	4.47
4. GI bleeding	-4.9	24.7	54.4	-2.6	24.0	50.7	1.0	13.3	25.6	-20.0	30.4	80.8	-1.0	0.9	2.7	-3.2	26.5	56.1	2.16	2.96	3.76
5. Hypoglycaemia	-37.9	42.4	122.7	-10.0	14.6	39.1				-38.2	23.9	86.0	-20.1	-3.6	13.0				-0.06	1.75	3.56
Overall	-23.3	16.1	55.6	-18.5	12.3	43.0	-122.7	37.6	197.8	-19.9	13.8	47.5	-7.7	-0.1	7.6	-20.2	19.8	59.9	-17.0	8.0	32.9

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