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DESAFIOS METODOLÓGICOS EM EPIDEMIOLOGIA: UMA ABORDAGEM COM FOCO NA REAÇÃO ADVERSA DA TROMBOCITOPENIA INDUZIDA POR HEPARINA E NA CONDIÇÃO CLÍNICA DA DOR LOMBAR

Belo Horizonte - MG
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Tese como requisito parcial para obter o grau de doutor em Ciências Farmacêuticas, submetida ao Programa de Pós-Graduação em Ciências Farmacêuticas da Faculdade de Farmácia da Universidade Federal de Minas Gerais.

Orientadora: Maria das Graças Carvalho – UFMG

Co-orientadores: Edson Perini – UFMG

Paulo Ferreira – *The University of Sydney*

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FOLHA DE APROVAÇÃO

DANIELA REZENDE GARCIA JUNQUEIRA

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DEDICATÓRIA

Para Mamãe

You gave me a world where I could breathe
a sparkling place where I could feel the joy
You transmuted my survival into life
and my aching heart into a smooth wave
because God did you
since She could not be as You are, always there for me

Você me deu um mundo onde eu pudesse respirar
um lugar encantado onde eu podia sentir a alegria
Você transmutou minha sobrevivência em vida
e meu coração dolorido em uma onda suave
porque Deus fez você
já que Ela não poderia ser como você, sempre presente para mim

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Programa de Pós-graduação em Ciências Farmacêuticas, Centro de Estudo do Medicamento (Cemed) e Departamento de Análises Clínicas e Toxicológicas da Faculdade de Farmácia da Universidade Federal de Minas Gerais; *Arthritis and Musculoskeletal Research Group, Faculty of Health Sciences, The University of Sydney;* Fundação de Amparo à Pesquisa do Estado de Minas Gerais – FAPEMIG; Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq.

“Primum non nocere”

Hipócrates (460-370 AC)

RESUMO

O foco do modelo biomédico predominante das intervenções em saúde dos tempos contemporâneos omite nuances do processo da terapêutica com medicamentos, e de falhas desse processo. Assim, o adequado tratamento do processo de utilização de medicamentos depende de uma mudança de paradigma no sentido de garantir intervenções farmacológicas eficazes, mas também seguras, além de estratégias alternativas na ausência desses pilares fundamentais. Alguns dos desafios para essa mudança paradigmática são exemplificados por dois problemas de saúde pública: as reações adversas a medicamentos e os problemas de saúde complexos. Em ambos os contextos, as intervenções farmacológicas ora não oferecem a segurança necessária, ora não exercem o efeito terapêutico segundo a eficácia presumida. O caminho para o delineamento e aplicação de métodos epidemiológicos diferenciados para a abordagem desses problemas e desafios são percorridos nessa tese com foco em uma reação adversa a medicamentos (a trombocitopenia induzida por heparina) e em um problema de saúde complexo (a dor lombar). Nessa perspectiva, esse trabalho estrutura duas linhas de pesquisa: *Epidemiologia das reações adversas a medicamentos* e *Epidemiologia genética e estudo de gêmeos*. Os resultados estão organizados em cinco artigos originais, apresentados no formato de publicação ou de submissão. Considerando a linha de pesquisa *Epidemiologia das reações adversas*, podemos realçar como principais resultados: a demonstração da heterogeneidade nas heparinas comercializadas no Brasil, situação com impacto no perfil de segurança do medicamento e no risco de trombocitopenia induzida por heparina; a comprovação do menor risco de trombocitopenia induzida por heparina em pacientes expostos à heparina de baixo peso molecular em comparação aos expostos à heparina não fracionada; demonstração da escassez de ensaios clínicos controlados e aleatorizados abordando o aspecto da segurança sobre a utilização das heparinas; e a produção de informação em saúde para profissionais e para o sistema nacional e internacional de farmacovigilância acerca dos aspectos de segurança relacionados ao uso de heparinas no Brasil e sobre a trombocitopenia induzida por heparina. Considerando a linha de pesquisa *Epidemiologia genética e estudo de gêmeos*, devemos realçar

como principais resultados: demonstração do componente genético na dor lombar crônica, componente estimado com elevada influência na prevalência dessa condição crônica; e demonstração de uma associação exploratória entre atividades físicas leves e vigorosas com a dor lombar crônica. Os resultados contribuem para a compreensão do processo de utilização de medicamentos de forma abrangente além de representarem positiva contribuição para a produção de conhecimentos nas áreas de segurança de utilização de medicamentos e de determinantes de risco de doenças complexas. Acreditamos ainda que os conhecimentos produzidos poderão apoiar o delineamento de novos protocolos clínicos e estratégias de saúde pública com relação à utilização de heparinas e à dor lombar. Ressaltamos ainda que ambas as linhas de pesquisas são incipientes no Brasil e, nessa perspectiva, a tese materializa as expectativas de inovação científica e de colaboração internacional com vias ao desenvolvimento de pesquisas competitivas para o avanço científico do país.

Palavras-chave: epidemiologia, eventos adversos a medicamentos, estudo de gêmeos, trombocitopenia induzida por heparina, dor lombar.

ABSTRACT

The focus on the biomedical model which is a central aspect of the health interventions of the contemporary times omits nuances of the therapeutic process with medicines, and of failures of that process. Therefore, the appropriate managing of the use of medicines requires a paradigm shift regarding the guarantee of effect pharmacological interventions but also safe ones, in addition to alternative strategies in the absence of these fundamental pillars. Some of the challenges to this paradigm shift are exemplified by two public health problems: the adverse drug reactions and the complex health problems. In both contexts, the pharmacologic interventions sometimes do not provide the necessary safety and sometimes not exert a therapeutic effect according to the presumed effectiveness. This thesis follows the path for the design and application of unique epidemiological methods approaching these problems and challenges focusing on an adverse drug reaction (the heparin-induced thrombocytopenia) and on a complex health problem (the low back pain). Taking this perspective in account, this scientific work structures two research lines named: *Epidemiology of adverse drug reactions* and *Genetic epidemiology and twin study*. The results are organized in five original articles, presented in the form of publication or submission. Considering the research line *Epidemiology of adverse reactions* we should highlight as main results: our studies showed the heterogeneity of the heparins commercialised in Brazil, a situation with potential impact in the safety profile of the drug and in the risk of heparin-induced thrombocytopenia; we were able to ascertain the lower risk of heparin-induced thrombocytopenia in patients exposed to low molecular weight heparin when compared to patients exposed to unfractionated heparin; it was demonstrated a paucity of randomized controlled trials addressing the safety aspect of the use of heparins; and we were able to generate health information for professionals and for the national and international pharmacovigilance system about safety aspects related to the use of heparins in Brazil and about the heparin-induced thrombocytopenia. Considering the research line *Genetic epidemiology and twin study* we should highlight as main results: we were able to study the influence of the genetic component in chronic low back pain showing its major contribution in the prevalence of this chronic condition; and our

studies showed an important contribution of domestic physical workload but not leisure physical activity to chronic low back pain. These results contribute to a comprehensive understanding of the process of drug utilization and represent a positive contribution for the knowledge generation regarding the safe use of medicines and the risk assessment of complex diseases. We also believe that the knowledge behind these results may support the design of new clinical protocols and public health strategies considering the use of heparin and the low back pain problem. We also emphasize that both research lines are incipient in Brazil and, from this perspective, this thesis embodies the expectations of scientific innovation and of international partnerships as a strategy to the development of national researches with competitive calibre which may encourage the scientific advancement of the country.

Key-words: epidemiology, adverse drug events, twin studies, heparin-induced thrombocytopenia, low back pain.

SUMÁRIO

PREFÁCIO

Esta tese se desenvolveu sobre uma reflexão epidemiológica a respeito da segurança da utilização de medicamentos em dois contextos clínicos: da terapia de anticoagulação com heparinas e da dor lombar. Assim, duas linhas de pesquisa foram consolidadas, ambas possuindo em comum a utilização de diferentes métodos epidemiológicos na busca de resoluções para as limitações das intervenções farmacológicas de um importante problema de saúde pública.

A primeira linha de pesquisa é fruto de uma colaboração estabelecida entre o Departamento de Análises Clínicas e Toxicológicas da Faculdade de Farmácia da Universidade Federal de Minas Gerais (UFMG) e o Centro de Estudos do Medicamento (Cemed) da Faculdade de Farmácia da UFMG. Essa área de investigação se insere na linha de pesquisa “Uso racional do Medicamento, Farmacoepidemiologia e Farmacovigilância” do Cemed e na linha de pesquisa “Estudo dos estados de hipercoagulabilidade/trombofilia” do grupo de pesquisa em “Avanços em pesquisa hematológica: aspectos celulares, bioquímicos e moleculares” do Departamento de Análises Clínicas e Toxicológicas e as pesquisas desenvolvidas se baseiam na importância dos efeitos adversos induzidos por medicamentos nas diversas populações humanas. As reações adversas a medicamento são atualmente reconhecidas como importante problema de saúde pública nas diversas populações sendo responsáveis inclusive por significativo índice de internações hospitalares e mortalidade. A compreensão das características e do padrão de ocorrência desses efeitos é informação essencial para a segurança dos usuários de medicamentos, atividade intrínseca aos interesses da Farmacoepidemiologia.

Os estudos realizados nessa área de investigação dedicaram especial atenção às reações adversas hematológicas, eventos adversos de extrema importância, mesmo quando pouco comuns, dado o potencial de ameaça a saúde das alterações hematológicas induzidas por fármacos, como a agranulocitose, aplasia de medula óssea, trombocitopenias, entre outras. Particularmente, uma reação adversa

induzida pelo medicamento anticoagulante heparina, a trombocitopenia induzida por heparina, foi o objeto da primeira linha de pesquisa componente da tese em tela. Essa linha de pesquisa foi intitulada *Epidemiologia das reações adversas a medicamentos*.

A segunda linha de pesquisa que integra essa tese é fruto de uma colaboração estabelecida entre a autora e o *Arthritis and Musculoskeletal Research Group* da *Faculty of Health Sciences* da *The University of Sydney*. As condições musculoesqueléticas são altamente prevalentes em todas as populações mundiais e estão associadas a elevado índice de problemas associados, como dor e incapacidade funcional. A alta frequência de ocorrência e de danos associados ao diverso espectro de condições musculoesqueléticas, como osteoartrite, artrite reumatoide, osteoporose e dor lombar, determinam ainda um significativo impacto para os indivíduos e os sistemas de saúde. Nesse contexto, o *Arthritis and Musculoskeletal Research Group* dedica-se à compreensão de estratégias de prevenção, diagnóstico e tratamento de condições musculoesqueléticas com foco na melhoria dos desfechos clínicos dos pacientes. A dor lombar destaca-se dentre as condições musculoesqueléticas pela expressiva frequência em todos os sexos, idades e populações, sendo uma condição que poderá acometer qualquer indivíduo em algum momento de sua vida. Destaca-se ainda a relativa inefetividade dos tratamentos disponíveis para a dor lombar e o significante risco aos quais os pacientes estão expostos na utilização de intervenções farmacológicas que não apresentam efeitos significativos na resolução dos sintomas da dor lombar. Assim, a compreensão dos fatores de risco associados ao desenvolvimento da dor lombar adquire especial importância no contexto onde uma análise farmacoepidemiológica indica a necessidades de minimizar os riscos dos pacientes. A identificação de determinantes que possam ser individualmente modificados e utilizados nas políticas de saúde pública de forma a limitar o impacto social e financeiro da dor lombar e de seus desfechos associados se insere nessa perspectiva, sendo altamente desejável.

Os estudos realizados nessa segunda área de investigação dedicaram especial atenção à investigação de fatores de risco para a dor lombar, campo paradigmaticamente inserido na perspectiva do conhecimento epidemiológico. Em

especial, campos mais recentes de pesquisa em Epidemiologia, como a Epidemiologia Genética, oferecem modelos metodológicos inovadores para a compreensão da composição relativa das doenças pelos diferentes fatores de risco, ambientais e genéticos. A segunda linha de pesquisa dessa tese dedicou-se então à aplicação da Epidemiologia para o estudo dos determinantes genéticos e de estilo de vida da dor lombar, mais especificamente, na aplicação de um delineamento metodológico conhecido como Estudo de Gêmeos, e é intitulada *Epidemiologia genética e o estudo de gêmeos*. Essa pesquisa foi realizada durante o estágio de doutorado no exterior da autora (bolsa do Programa de Doutorado Sanduíche no Exterior do CNPq- SWE) na *Faculty of Health Sciences* da *The University of Sydney,* no período de fevereiro a julho de 2012.

Os resultados dos estudos desenvolvidos nas duas linhas de pesquisa que compõem essa tese são apresentados em duas partes, compreendendo a Parte I (um) a linha de pesquisa em *Epidemiologia das reações adversas a medicamentos* e a Parte II (dois) a linha de pesquisa em *Epidemiologia genética e o estudo de gêmeos.* Ambos os componentes, Parte I (um) e Parte II (dois), são constituídos de um capítulo referente a contextualização do objeto em estudo da(s) pesquisa(s) realizada(s) seguido dos artigos resultantes das investigações. Um capítulo final tece considerações gerais sobre os resultados das pesquisas constituintes dessa tese, contextualizando parcerias essenciais para a concepção e realização dos estudos concretizados e refletindo sobre desafios científicos futuros.

1 INTRODUÇÃO

A profissão farmacêutica é notável pela variedade de habilidades e possibilidades de atuação. Analisando a cadeia do medicamento desde sua gênese, que envolve as diversas fases de investigações químicas, farmacológicas, farmacotécnicas e clínicas necessárias para o desenvolvimento de um medicamento, até a disponibilização desse produto à população, comumente destacamos somente o processo de desenvolvimento e produção do fármaco. Por isso, não raramente, ao ser definido como profissional do medicamento, o farmacêutico é sistematicamente identificado somente com essa fase da cadeia do medicamento.

O medicamento é um produto tecnicamente produzido e comercializado para ser utilizado como objeto terapêutico principal ou secundário no tratamento de uma enfermidade, podendo também ser destinado para auxílio em processos diagnósticos. De fato, a grande maioria das ações de saúde culmina em uma intervenção medicamentosa e, muitas vezes, a sua resolutividade depende dessa terapia. Na verdade, mais que um insumo tecnológico, um medicamento é a interação do produto farmacoterapêutico com o indivíduo que o utiliza, tendo ainda, nessa interface, as situações de assistência médica e farmacêutica e apelo mercadológico. Em suma, o medicamento é o produto farmacêutico conjugado com os anseios do paciente e a informação, adequada ou não, disponível sobre este.

Os medicamentos interferem marcadamente na saúde, individual e coletiva, bem como nas organizações dos sistemas de saúde, sejam eles públicos ou privados. Portanto, a definição do farmacêutico como um profissional do medicamento compreendida e focada somente no produto químico é um tanto simples e, de fato, não demonstra a importância terapêutica e social desse insumo tecnológico. Essa definição omite no foco de atuação desse profissional um detalhe crucial: o indivíduo que necessita de medicamentos como objeto terapêutico. Além disso, a abordagem do produto e do profissional com foco somente no medicamento como produto exclui nuances do processo da terapêutica com medicamentos, e da falha desse processo. Assim, apesar das inovações tecnológicas aplicadas à produção de fármacos

constituírem aspecto essencial da medicina moderna, muitos problemas de saúde não são efetivamente tratados com intervenções farmacológicas. Ainda, problemas gerados pela utilização de medicamentos são causas comuns e importantes de adoecimento, deficiência e morte.[1; 2] Portanto, com relação ao uso de medicamentos, uma mudança de paradigma é necessária, pois não basta um medicamento ter eficácia e qualidade, o seu processo de utilização também deve ser efetivo e seguro.

Alguns dos desafios para essa mudança paradigmática na abordagem das intervenções farmacológicas são exemplificados pelo estudo de dois problemas de saúde pública: as reações adversas a medicamentos e os problemas de saúde complexos. Em ambos os contextos, as intervenções farmacológicas ora não oferecem a segurança necessária, ora não exercem o efeito terapêutico segundo a eficácia presumida. Métodos epidemiológicos diferenciados são necessários na busca de soluções para os desafios descritos e os caminhos para o delineamento e a aplicação desses métodos são percorridos nessa tese com foco no estudo de uma reação adversa a medicamentos (a trombocitopenia induzida por heparina) e de um problema de saúde complexo (a dor lombar).

PARTE I

EPIDEMIOLOGIA DAS REAÇÕES ADVERSAS A MEDICAMENTOS

2 OS EFEITOS ADVERSOS RELACIONADOS A MEDICAMENTOS

A consciência de que os efeitos adversos relacionados a medicamentos constituem um importante problema de saúde pública cresceu em paralelo à história dos desastres provocados pela ocorrência de graves reações adversas em grandes parcelas da população. A tragédia ocorrida com o medicamento talidomida na década de 1960 é um marco na história do estudo das reações adversas a medicamentos, tendo sido responsável por cerca de 10.000 casos de nascimentos de bebês com deformidades nos membros, além de deficiências e mortes devido a efeitos posteriormente atribuídos a talidomida, como cardiopatias congênitas, malformações da orelha interna e externa e alterações oculares.[3; 4]

Efeito adverso relacionado a medicamentos é qualquer efeito inesperado ou inconveniente causado ao paciente por uma intervenção plausivelmente relacionada à utilização de medicamentos.[5] Os efeitos adversos incluem uma série de situações como as interações medicamentosas, os erros de administração de medicamentos, os erros de prescrição, entre outros. Já as reações adversas a medicamentos são um tipo de efeito adverso relacionado a medicamentos definidas como uma resposta nociva e não intencional que ocorre após a administração de um medicamento em doses usualmente utilizadas no homem para profilaxia, diagnóstico ou tratamento de uma enfermidade.[6; 7]

Estimativas sobre a frequência de efeitos adversos relacionados a medicamentos são insuficientes devido a sub-notificação desses eventos nos prontuários médicos, nos certificados de óbito e mesmo para os órgãos reguladores. Contudo, as evidências demonstram a magnitude do problema ao identificarem que cerca de 3% a 14% das internações hospitalares são causadas por um efeito adverso a medicamento,[2; 8; 9; 10; 11] e que uma proporção de 6% a 20% dos pacientes hospitalizados experimentam um efeito adverso relacionado a medicamento durante a internação.[12; 13; 14] Além disso, uma substancial porcentagem desses efeitos é potencialmente prevenível.[15] Estudos considerando especificamente as reações adversas a medicamentos reportam ainda que entre 2% a 5% das internações

hospitalares relacionadas a medicamentos são diretamente relacionadas com uma reação adversa a medicamento.[11; 16] Ainda, um estudo de base populacional realizado na Suécia demonstrou que 6,4% dos óbitos hospitalares são associados a uma reação adversa a medicamento e 3,1% dos óbitos da população são suspeitos de terem sido induzidos por uma reação adversa.[17] As reações adversas são comumente induzidas por efeitos farmacológicos relacionados à ação do fármaco ou à dose (reações tipo A) [11; 18; 19] e, portanto, seriam em sua maioria previsíveis e passíveis de monitoramento e intervenção precoce. No entanto, em diversos países as reações adversas a medicamentos estão entre as 10 principais causas de mortalidade,[1] sendo a quarta causa de óbitos na Suécia[17] e estando entre a quarta e sexta causa de mortalidade nos Estados Unidos. [19]

Os desfechos negativos induzidos pelas reações adversas podem acometer os mais variados órgãos, induzindo anormalidades funcionais. As reações adversas hematológicas são reações de extrema importância, mesmo quando pouco comuns, dado o potencial de ameaça à saúde provocada por tais alterações induzidas por fármacos, como a agranulocitose aplasia de medula óssea, trombocitopenias, entre outras.[20; 21; 22; 23; 24] Dentre as reações adversas hematológicas, destaca-se a trombocitopenia induzida por heparina, uma reação adversa causada por uma resposta imunodependente à heparina cuja consequência mais importante é um aumento paradoxal do risco de complicações tromboembólicas.[25; 26] A trombocitopenia induzida por heparina apresenta elevada frequência em comparação com as demais reações adversas hematológicas e caracteriza-se por uma complexidade de fatores que influenciam o risco de sua ocorrência, fatores esses que desafiam seu reconhecimento e a determinação exata de sua incidência.

A estimativa da incidência (risco absoluto) da trombocitopenia induzida por heparina varia entre os estudos prospectivos. Diferentes estudos apresentam estimativas discordantes devido a diferentes definições da reação ou aos diferentes métodos laboratoriais para a confirmação da reação imunológica associada à trombocitopenia induzida por heparina. A incidência da reação é também difícil de ser precisamente estimada porque é dependente do tipo de heparina utilizada, se heparina não fracionada ou heparina de baixo peso molecular, bem como da população de

paciente exposta,[26; 27] sendo que o subgrupo de pacientes pós-cirúrgicos em tratamento com heparina não fracionada é considerado o grupo de pacientes de maior risco para o desenvolvimento da reação.[26; 28]

A determinação do risco de um evento adverso é essencial para o monitoramento dos pacientes e melhoria do cuidado com a tomada de ações rápidas e efetivas quando da suspeita do evento. Assim, as dificuldades da determinação do risco da trombocitopenia induzida por heparina estratificado de acordo com os diferentes tipos de heparina e as diversas populações de pacientes competem para o atraso no reconhecimento da reação e contribui para a morbi-mortalidade dos pacientes.[29]

Para a Epidemiologia, ciência que estuda a distribuição das doenças nas populações humanas e os fatores que influenciam essa distribuição,[30] modelos de riscos são idealmente baseados em medidas de incidência. Assim, a determinação precisa da incidência da trombocitopenia induzida por heparina é essencial para maior segurança nas decisões em relação à terapia de anticoagulação com heparina.

2.1 Métodos para o estudo das reações adversas a medicamentos

O registro de eventos adversos relacionados a medicamentos é antigo[31] e a necessidade de promover uma vigilância constante desses eventos determinou a consolidação das atividades de pós-comercialização ou Farmacovigilância, também conhecida como Fase IV de desenvolvimento clínico dos fármacos. A Farmacovigilância é definida como as atividades de identificação e avaliação dos efeitos do uso, agudo e crônico, dos tratamentos farmacológicos no conjunto da população ou em subgrupos de pacientes expostos a tratamentos específicos.[32] O estudo das reações adversas a medicamentos tem sido também o foco da atenção de um campo de pesquisas científicas que une conhecimentos da Farmacologia Clínica e da Epidemiologia: a Farmacoepidemiologia. A Farmacoepidemiologia busca compreender a utilização e os efeitos dos medicamentos em populações, com

especial interesse pelos efeitos adversos induzidos por medicamentos.[31] A Farmacoepidemiologia e a Farmacovigilância são, em conjunto, importantes na geração de informações sobre os riscos e benefícios dos medicamentos em diferentes populações e sob diferentes formas de uso. A produção de informação sobre as características e os padrões dos efeitos adversos das intervenções farmacológicas é essencial para dar suporte a conhecimentos e estratégias que aumentem a segurança da população que utiliza medicamentos e promovam o uso racional dos medicamentos.

Múltiplas estratégias metodológicas têm sido desenvolvidas na interface do estudo e da vigilância das reações adversas. Essa preocupação é inicialmente representada pela exigência de ensaios clínicos randomizados para comprovação da eficácia e segurança dos fármacos antes de sua comercialização. No entanto, 51% dos fármacos induzem reações adversas detectadas somente na fase pós-comercialização.[31] Além disso, há um crescente reconhecimento da necessidade de se estudar continuamente o balanço entre risco e benefício dos fármacos no intuito de se tomar decisões clínicas mais seguras. Nesse sentido, o método epidemiológico fornece ferramentas preciosas não somente na realização de ensaios clínicos de eficácia de medicamentos no período de pré-comercialização como também possui exaustiva aplicação na vigilância pós-comercialização dos medicamentos, em especial, no estudo das reações adversas a medicamentos.

Para a revisão contínua da eficácia dos medicamentos, um método que atualmente tem sido extensivamente utilizado é a revisão sistemática de ensaios clínicos. Revisões sistemáticas utilizando estudos experimentais possuem metodologia consolidada e amplamente utilizada. No entanto, a realização de revisões sistemáticas somente com ênfase no benefício do tratamento colabora para a omissão de informações sobre efeitos danosos dos medicamentos e impossibilita a tomada de decisões clínicas balanceadas em relação aos riscos e benefícios das opções terapêuticas.[33] Atualmente, tem se tornado óbvio que os efeitos prejudiciais das intervenções terapêuticas devem ser revisados com o mesmo rigor aplicado à investigação da eficácia dessas intervenções.

A revisão sistemática constitui um método de pesquisa que utiliza um processo rigoroso para identificar, selecionar, avaliar e reunir dados de estudos primários. Revisões sistemáticas de estudos experimentais (ensaios clínicos) possuem metodologia consolidada e são, atualmente, amplamente difundidas na literatura médica. No entanto, os ensaios clínicos de intervenções terapêuticas dão ênfase ao tratamento e buscam responder questões sobre eficácia. Além disso, a amostra utilizada é, em geral, insuficiente para a investigação de reações adversas e a duração dos estudos é insuficiente para a observação de reações de longo prazo. Tudo isso torna inapropriado extrapolar dados de reações adversas a medicamentos com base nos resultados de ensaios clínicos isolados, como exigido nos estudos de pré-comercialização.[31] O método de revisão sistemática tem o potencial de aumentar o poder estatístico para a investigação de reações adversas ao realizar análises secundárias com amostras advindas de vários estudos primários e a oportunidade de analisar efeitos diferenciados dos fármacos em subgrupos, algo difícil de ser realizado nos estudos individuais.

A Colaboração Cochrane, organização internacional sem fins lucrativos que prepara, mantém e promove o acesso a revisões sistemáticas, num empreendimento com potencial de alto impacto na medicina moderna[34] possui métodos consistentes que asseguram a qualidade de suas revisões sistemáticas, desenvolvidas em parceria com profissionais do mundo todo. Os métodos preconizados pela Colaboração Cochrane estão descritos em seu manual para a realização de revisões sistemáticas de intervenções em saúde (*Cochrane Handbook for Systematic Reviews of Interventions*).[35] Na última versão desse manual, *Handbook* 5, um capítulo original contempla a discussão da importância e dos métodos para se realizar o estudo sistemático de reações adversas a medicamentos em revisões sistemáticas de ensaios clínicos randomizados. Recentemente, além da atualização de seu manual, a Cochrane fundou um grupo metodológico dedicado a promover a inclusão de dados sobre efeitos adversos nas revisões da organização e desenvolver diretrizes para aspectos específicos da avaliação de eventos adversos em revisões sistemáticas.[33; 36]

3 OBJETIVOS

O objetivo geral da linha de pesquisa em *Epidemiologia das reações adversas a medicamentos* foi estudar aspectos da farmacoepidemiologia do medicamento anticoagulante heparina com foco na reação adversa da trombocitopenia induzida por heparina.

3.1 Objetivos específicos:

- Investigar aspectos que influenciam a segurança da heparina no Brasil gerando informações para o sistema de farmacovigilância do país;

- Determinar o risco (incidência) de trombocitopenia induzida por heparina em pacientes pós-cirúrgicos;

- Produzir informações para profissionais de saúde a nível nacional sobre as características e os padrões de ocorrência da trombocitopenia induzida por heparina.

4 RESULTADOS

Os resultados dos estudos realizados dentro da primeira linha de pesquisa que compõe essa tese, *Epidemiologia das reações adversas a medicamentos*, estão organizados em três artigos originais publicados em periódicos científicos.

4.1 Farmacovigilância da terapia de anticoagulação com heparina no Brasil

Esse artigo investigou aspectos que influenciam a segurança da terapia com heparina não fracionada no Brasil discutindo desafios determinados pela característica dos produtos disponíveis no mercado brasileiro. Os resultados do estudo representam informações relevantes para profissionais de saúde e o sistema de farmacovigilância do país. O artigo está publicado como:

- Daniela RG Junqueira, Thércia G Viana, Eliane RM Peixoto, Fabiana CR Barros, Maria das Graças Carvalho, Edson Perini. **Farmacovigilância da heparina no Brasil.** Revista da Associação Médica Brasileira 2011; 57(3):322-326

Farmacovigilância da heparina no Brasil

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Resumo

Objetivo: Investigar a origem das preparações de heparina, na forma farmacêutica injetável, disponíveis no mercado brasileiro, discutindo o impacto do perfil dos produtos comercializados e das alterações na monografia da heparina na segurança do fármaco. **Métodos:** Pesquisou-se o banco de dados de Produtos Registrados das Empresas de Medicamentos da Anvisa e o Dicionário de Especialidades Farmacêuticas (DEF 2008/2009). Foi realizado inquérito com as indústrias com autorização ativa para o comércio do fármaco no Brasil. **Resultados:** Cinco indústrias possuem autorização para o comércio de heparina não fracionada no Brasil. Três são de origem suína e duas de origem bovina, sendo que apenas uma possui essa informação explicitada na bula. A efetividade e a segurança da heparina, estudadas em populações estrangeiras, podem não representar a nossa realidade, já que a maioria dos países não produz a heparina bovina. A heparina atualmente comercializada tem, ainda, aproximadamente 10% menos atividade anticoagulante que a anteriormente produzida, e essa alteração pode ter implicações clínicas. **Conclusão:** Evidências acerca da ausência de intercambialidade de doses entre as heparinas de origem bovina e suína e o diferenciado perfil de segurança entre esses fármacos indicam necessidade de acompanhamento do tratamento e da resposta dos pacientes. Eventos que ameacem a segurança do paciente devem ser comunicados ao sistema da farmacovigilância do país.

Unitermos: Heparina; anticoagulantes; vigilância de produtos comercializados; monitoramento de medicamentos; toxicidade de drogas.

Summary

Heparin pharmacovigilance in Brazil

Objective: To investigate the biological origin of injectable unfractioned heparin available in Brazilian market by discussing the impact of the profile of commercial products and the changes in heparin monograph on the drug safety. **Methods:** The Anvisa data base for the Registered Products of Pharmaceutical Companies and the Dictionary of Pharmaceutical Specialties (DEF 2008/2009) were searched. A survey with industries having an active permission for marketing the drug in Brazil was conducted. **Results:** Five companies were granted a permission to market unfractioned heparin in Brazil. Three of them are porcine in origin and two of them are bovine in origin, with only one explicitly showing this information in the package insert. The effectiveness and safety of heparin studied in non-Brazilian populations may not represent the Brazilian reality, since most countries no longer produce bovine heparin. The currently marketed heparin has approximately 10% less anticoagulant activity than that previously produced and this change may have clinical implications. **Conclusions:** Evidence about the lack of dose interchangeability between bovine and porcine heparins and the unique safety profile of these drugs indicates the need to follow the treatment and the patients' response. Events threatening the patient's safety must be reported to the pharmacovigilance system in each particular country.

Keywords: Heparin; anticoagulants, product surveillance; drug monitoring; drug toxicity.

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Introdução

A heparina é um agente natural com ação anticoagulante. Os fármacos comercialmente disponíveis são isolados e extraídos da mucosa intestinal de suínos ou do tecido pulmonar bovino. O processo de isolamento e extração da heparina leva à degradação parcial das cadeias de glicosaminoglicanos que a compõem[1], produzindo um fármaco formado por fragmentos moleculares de pesos moleculares heterogêneos, variando de 3.000 a 30.000[2], conhecido como heparina não fracionada (HNF), heparina convencional ou simplesmente heparina. As propriedades farmacocinéticas e farmacodinâmicas da heparina também apresentam grande heterogeneidade devido às diferentes potências de ação anticoagulante apresentadas por frações com pesos moleculares distintos e também devido à ligação da heparina a células e proteínas plasmáticas.

Outro tipo de heparina comercialmente disponível, a heparina de baixo peso molecular (HBPM), é composta por fragmentos moleculares com peso molecular médio de 5.000 e é obtida por despolimerização ácida da heparina convencional[3]. Devido à existência de diferentes métodos de despolimerização, existem diferentes heparinas de baixo peso molecular[2]. As preparações de HBPM apresentam propriedades farmacocinéticas e farmacodinâmicas mais previsíveis, sendo então, mais convenientes que a HNF para utilização em diversas situações clínicas[2]. De fato, em diversos países as HBPM estão substituindo a HNF. No Brasil, no entanto, diversas intervenções clínicas ainda são dependentes da HNF[4]. Além disso, no Brasil parece permanecer a oferta de heparina obtida das duas fontes animais disponíveis, enquanto nos Estados Unidos e Europa a HNF de origem bovina não é mais produzida devido à epidemia de encefalite espongiforme bovina[5,6].

Em 2008, uma contaminação em lotes de HNF comercializada pela Baxter Healthcare®, importante produtora do fármaco em diversos países, fez eclodir uma grave crise mundial no mercado das heparinas[6]. O contaminante identificado, o sulfato de condroitina supersulfatado, é uma substância semelhante à heparina, e sua administração provocou reações caracterizadas por hipotensão, náuseas e dificuldades respiratórias, ocorrendo em até 30 minutos após a exposição[6]. Tais reações foram associadas a mais de 200 óbitos em vários países[7]. No Brasil, a crise internacional somou-se à inexplicável retirada do mercado da heparina não fracionada intravenosa do laboratório Roche (Liquemine®), em 2007[4].

A crise provocada pela contaminação da heparina culminou com diversas mudanças nas ações clínicas e de produção do fármaco. Em âmbito mundial, a monografia da heparina foi revisada pela Farmacopeia dos Estados Unidos (USP, do inglês *U.S. Pharmacopeia*) e pela Organização Mundial de Saúde com o propósito de introduzir ensaios de qualidade capazes de detectar o sulfato de condroitina supersulfatado, testes anteriormente não contemplados[7,8]. No Brasil, a carência de heparina estimulou uma reestruturação do mercado com introdução de novos fornecedores e, consequentemente, de novas fontes de matéria-prima do produto[4,9,10]. Todas essas alterações impactam a farmacovigilância da heparina, introduzindo novas questões de segurança com relação à vigilância da terapia de anticoagulação com HNF. Diante dessas questões, o objetivo desse estudo foi investigar a origem das preparações de heparina na forma farmacêutica injetável disponíveis no mercado brasileiro, discutindo o impacto do perfil dos produtos comercializados e das recentes alterações na monografia da heparina na farmacovigilância do fármaco.

Métodos

Para determinar a origem biológica das preparações injetáveis de HNF comercializadas no Brasil, pesquisou-se o banco de dados de Produtos Registrados das Empresas de Medicamentos da Agência Nacional de Vigilância Sanitária (Anvisa)[11] para identificação das indústrias farmacêuticas com autorização para o comércio do fármaco no país. Adicionalmente, o Dicionário de Especialidades Farmacêuticas (DEF 2008/2009) também foi consultado. As indústrias identificadas com autorização ativa para o comércio do fármaco foram contatadas inicialmente por telefone através do Serviço de Atendimento ao Consumidor (SAC) e, posteriormente, via e-mail. O contato com as indústrias seguiu um formulário padronizado objetivando responder as seguintes questões: se a empresa produzia algum tipo de heparina atualmente; o nome comercial e a apresentação farmacêutica do produto produzido; o principal destino comercial do medicamento (hospital ou drogarias); e a origem biológica da heparina.

A pesquisa foi realizada entre 19 de agosto a 28 de setembro do ano de 2010 pela equipe do Centro de Estudos do Medicamento da Faculdade de Farmácia da Universidade Federal de Minas Gerais (Cemed – UFMG).

Resultados

Foram identificadas nove marcas registradas junto à Anvisa, referentes a oito indústrias farmacêuticas fabricantes de heparina, na forma farmacêutica injetável. Uma das indústrias não pôde ser localizada pelas referências disponíveis e foi excluída do inquérito. Das oito marcas registradas e que a indústria produtora pôde ser contatada, uma (Heparin®) não é mais produzida, tendo sido substituída por outro produto (Hemofol®) do mesmo laboratório produtor. Das marcas registradas restantes, duas possuem registro expirado, duas apresentam registro junto à Anvisa em processo de renovação (informação do fabricante) e três indústrias possuem autorização ativa para o comércio de heparina no país.

Dos produtos com licença ativa para comércio ou em renovação, três consistem de formulação injetável para aplicação subcutânea (3/5), um consiste de formulação

para aplicação intravenosa (1/5) e um de formulação destinada à aplicação subcutânea ou intravenosa (1/5).

A respeito da origem biológica dos fármacos, três (60%) são de origem suína e dois (40%) de origem bovina, sendo que apenas um possui essa informação explicitada na bula. Das cinco indústrias farmacêuticas produtoras de heparina no Brasil, quatro comercializam seus produtos apenas para hospitais, e uma para hospitais e drogarias.

O nome comercial dos fármacos identificados, bem como a origem biológica da matéria-prima, as formas farmacêuticas disponíveis e o principal destino comercial dos produtos estão resumidos na Tabela 1.

Discussão

A farmacovigilância, Fase IV da pesquisa clínica dos fármacos, compreende as atividades relacionadas a detecção, avaliação, compreensão e prevenção dos eventos adversos ou quaisquer problemas relacionados a medicamentos com objetivo de identificar riscos e prevenir danos aos pacientes[12]. À farmacovigilância interessa diversos aspectos do período de pós-comercialização dos fármacos, incluindo a revisão contínua da eficácia, as reações adversas, os eventos adversos por desvios da qualidade, o uso de medicamentos para indicações não aprovadas, as interações medicamentosas, os eventos de inefetividade terapêutica, intoxicações relacionadas a medicamentos e também os erros de medicação potenciais ou reais[13,14].

Apesar de a HNF ter sido o principal instrumento no manejo de alterações tromboembólicas por mais de meio século[15], o fármaco vem despertando grande interesse para o sistema de farmacovigilância em função de recentes circunstâncias comerciais e clínicas, demonstrando um necessário alerta referente à sua utilização.

A origem biológica da HNF é um fator pouco explorado nas diretrizes de dose e posologia do fármaco. No entanto, a fonte animal do medicamento altera o seu perfil de efetividade e segurança[16,17]. De fato, a heparina bovina e a heparina suína não são fármacos equivalentes[16]. A heparina bovina possui maior grau de sulfatação de seus compostos, e isso determina efeitos distintos na coagulação, trombose e sangramento da heparina de origem suína. A heparina bovina também difere em sua afinidade pela protamina, substância utilizada na inibição do efeito anticoagulante do fármaco. Todas essas questões podem evidenciar a ausência de intercambialidade de doses entre as heparinas de origem bovina e suína, reforçando a necessidade de monitoramento do tratamento. A origem biológica da HNF pode, então, afetar aspectos de efetividade e segurança do tratamento com o fármaco. A questão ganha especial relevância ao observamos que essas informações estão comumente ausentes nas bulas dos medicamentos, e que em nosso país fármacos de diferentes origens biológicas são comercializados simultaneamente e de forma intercambiável.

As HBPM, por sua vez, não são afetadas por aspectos da origem biológica do fármaco, porque a heparina bovina não é utilizada na produção desses produtos devido a possíveis contaminantes virais, como o da encefalite espongiforme bovina[15].

A crise recente no mercado da heparina originou outra questão de impacto mundial, reforçando o alerta com relação à utilização da HNF: as modificações na monografia do fármaco. Todos os produtos comercializados no Brasil e no mundo devem ser submetidos, desde outubro de 2009, às novas recomendações instituídas na monografia da HNF pela Organização Mundial de Saúde e pela Farmacopeia dos Estados Unidos com o objetivo de assegurar a segurança e a qualidade dos ingredientes farmacêuticos ativos[7,8,18]. As alterações preveem a introdução de testes adicionais que devem ser utilizados pelos produtores para a identificação de contaminantes e a implementação de um novo ensaio de potência. O novo teste de potência recomendado, o teste cromogênico antifator IIa, oferece maior especificidade e segurança adicional contra potenciais adulterantes que mimetizem a atividade da heparina. Em paralelo à introdução do novo teste, novo padrão de referência de potência também foi definido[8]. Adicionalmente, a unidade de potência da heparina utilizada pela farmacopeia americana foi harmonizada com a Unidade Internacional (UI) utilizada pela Organização Mundial de Saúde.

Tabela 1 – Heparinas não fracionadas comercializadas no Brasil segundo nome comercial, origem biológica da matéria-prima, formas farmacêuticas e principal destino comercial dos produtos

Nome comercial	Origem biológica	Formas farmacêuticas	Principal destino comercial
Actparin	Bovina	5.000 UI/mL (IV) 5.000 UI/0,25 mL (SC)	Hospital (IV e SC) Drogarias (IV)
Hemofol	Suína	5.000 UI/0,25 mL (SC) 5.000 UI/mL (IV)	Hospital
Hepamax-s	Suína	5.000 UI/mL (IV e SC)	Hospital
Heptar	Bovina	5.000 UI/mL (IV)	Hospital
Parinex	Suína	5.000 UI/mL (IV) 5.000 UI/0,25 mL (SC)	Hospital

IV, injeção intravenosa; SC, subcutânea

Apesar de as alterações na monografia da heparina contribuírem para um produto mais seguro e de qualidade, a questão remanescente trata da significância clínica da mudança de potência do fármaco. Estudos realizados pela agência americana que regulamenta o setor de medicamentos, a *Food and Drug Administration* (FDA), demonstrou que a heparina produzida, segundo às novas especificações da monografia da USP, tem aproximadamente 10% menos atividade anticoagulante que a heparina anteriormente produzida, e reforça que a alteração na potência pode ter implicações clínicas em determinadas situações, como na administração intravenosa *in bolus*[19]. Já a USP, responsável pela nova monografia, não antecipa que a alteração na potência demonstre importância clínica[20].

Independentemente das divergentes opiniões sobre o impacto clínico da nova potência da heparina, é consenso a necessidade de um cuidadoso acompanhamento clínico do efeito do fármaco e da resposta dos pacientes ao "novo" medicamento[7,8,19]. Qualquer suspeita de resposta alterada deve ser avaliada e disseminada para a comunidade através de notificação ao setor responsável[19,20].

Dos eventos adversos de interesse da farmacovigilância, destacam-se as reações adversas a medicamentos, por estarem na base das grandes tragédias relacionadas à utilização de medicamentos em populações e pelo conhecimento de que cerca de metade dos fármacos induz reações adversas detectadas somente na fase pós-comercialização (farmacovigilância)[13]. As reações adversas induzidas pela heparina incluem hemorragia, reação anafilática, elevação de enzimas hepáticas, osteoporose (com longo período de utilização) e trombocitopenia induzida por heparina (conhecida pela sigla HIT, originária da língua inglesa)[21]. Destacam-se, pela frequência e gravidade, a hemorragia e a trombocitopenia induzida por heparina.

Os sangramentos associados à utilização do fármaco podem ocorrer em qualquer local e incidem em uma frequência entre 5% e 10%[21]. De fato, a hemorragia é um risco conhecido das heparinas e é uma extensão da ação terapêutica do fármaco. A utilização da heparina bovina aumenta esse risco de hemorragia porque as doses requeridas para a indução de sangramento parecem ser menores que as de heparina suína[16]. Reações adversas hemorrágicas induzidas por heparina devem, portanto, ser notificadas ao sistema de farmacovigilância com o cuidado de inclusão no relato do evento da origem biológica do fármaco. Essa informação permite o adequado desenvolvimento do conhecimento sobre essa questão e o estabelecimento do perfil epidemiológico dos riscos das heparinas comercializadas em nosso país.

A trombocitopenia induzida por heparina é uma reação adversa imunodependente, frequente e potencialmente fatal[22-24]. Sua consequência mais importante é o aumento paradoxal do risco de complicações tromboembólicas. O conhecimento sobre essa reação está ainda em aprimoramento. Sabe-se que sua incidência varia de acordo com o tipo de heparina utilizada, se HNF ou HBPM, bem como com a população de paciente exposta[17]. O subgrupo em maior risco inclui os pacientes pós-cirúrgicos em tratamento com HNF (1% a 5%). A incidência da reação varia, ainda, com a origem biológica da HNF, sendo a bovina mais imunogênica que a suína[17].

No Brasil, a incidência da trombocitopenia induzida por heparina permanece desconhecida, bem como a gravidade de sua implicação clínica. Isso é preocupante considerando-se as diferenças na HNF comercializada em nosso país: enquanto a maioria dos países não produz a heparina bovina, 40% dos produtos ofertados em nosso mercado é esse tipo de heparina. Assim, o conhecimento baseado em dados sobre essa reação, produzidos em mercados e populações estrangeiras, podem não representar a nossa realidade. Dada a relevância do problema, demonstrada por diversos estudos em outros países, notadamente em países do hemisfério norte, bem como a frequência e a potencialidade para o desenvolvimento de importantes eventos clínicos consequentes da trombocitopenia induzida por heparina, esta é uma lacuna preocupante em nosso sistema de farmacovigilância.

O desconhecimento do perfil epidemiológico da HIT em nosso país pode ainda estar associado a um importante impacto econômico. Cada 15 novos casos reconhecidos por ano custam entre 700.000 e 1,8 milhões de dólares para a instituição[25]. Por outro lado, o não reconhecimento clínico da reação poderá resultar em tratamentos incorretos, aumento do risco de vida ou de amputações e aumento ainda maior de custos financeiros e de vidas.

No Brasil, a Anvisa vem ampliando suas atividades na área de farmacovigilância e o setor conta, atualmente, com um sistema de notificação on-line aprimorado e eficiente[26]. Profissionais de saúde, usuários e indústria são estimulados a alimentar o sistema e colaborar para a utilização segura e efetiva dos fármacos comercializados no país.

Conclusão

Evidências acerca da ausência de intercambialidade de doses entre as heparinas de origem bovina e suína, o diferenciado perfil de segurança entre esses fármacos e a permanência de heparinas produzidas a partir das diferentes fontes animais no mercado brasileiro indicam necessidade de acompanhamento do tratamento e da resposta dos pacientes. A efetividade e a segurança da heparina estudadas em populações estrangeiras podem não representar a nossa realidade, já que a maioria dos países não produz a heparina bovina e a heparina atualmente comercializada tem ainda aproximadamente 10% menos atividade anticoagulante que a anteriormente produzida, e essa alteração pode ter implicações clínicas. Destaca-se também a necessidade de realização de estudos clínicos no Brasil para se testar a segurança e a eficácia das formulações de heparina

utilizadas no país. Eventos que ameacem a segurança do paciente devem ser comunicados ao sistema de farmacovigilância do país.

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4.2 Revisão sistemática de reações adversas a medicamentos e a trombocitopenia induzida por heparina

Esse artigo determinou o risco (incidência) de trombocitopenia induzida por heparina em pacientes pós-cirúrgicos utilizando o método de revisão sistemática de ensaios clínicos adaptado para a investigação com foco numa reação adversa medicamento, a trombocitopenia induzida por heparina. As análises confirmaram uma redução do risco de trombocitopenia induzida por heparina na utilização de heparina de baixo peso molecular e indicaram que a frequência da reação em pacientes submetidos a cirurgias de alta complexidade tende a ser superior a 1% caracterizando a reação como comum segundo a classificação de frequência das reações adversas da Organização Mundial de Saúde (*The Upsalla Monitoring Centre* - http://www.who-umc.org). Os dados necessitam de suporte devido ao limitado número de estudos incluídos, mas apresentam o potencial de impactar os protocolos de monitoramento dos pacientes em utilização de heparinas. Destaca-se que esse estudo foi importante para demonstrar a escassez de ensaios clínicos que abordam o tema da segurança das heparinas de forma apropriada e em paralelo ao desfecho de eficácia, limitação grave que deve ser considerada pela comunidade científica, as agências reguladoras e os profissionais de saúde.

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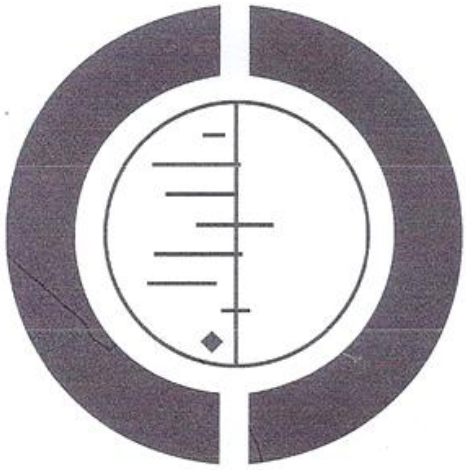
Unfractionated heparin versus low molecular weight heparin for avoiding heparin-induced thrombocytopenia in postoperative patients (Review)

Junqueira DRG, Perini E, Penholati RRM, Carvalho MG



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[Intervention Review]

Unfractionated heparin versus low molecular weight heparin for avoiding heparin-induced thrombocytopenia in postoperative patients

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ABSTRACT

Background

Heparin-induced thrombocytopenia (HIT) is an adverse drug reaction presenting as a prothrombotic disorder related to antibody-mediated platelet activation. It is a poorly understood paradoxical immune reaction resulting in thrombin generation in vivo, which leads to a hypercoagulable state and the potential to initiate venous or arterial thrombosis. A number of factors are thought to influence the incidence of HIT including the type and preparation of heparin (unfractionated heparin (UFH) or low molecular weight heparin (LMWH)) and the heparin-exposed patient population, with the postoperative patient population presenting a higher risk.

Although LMWH has largely replaced UFH as a front-line therapy, there is evidence supporting a lack of superiority of LMWH compared with UFH regarding prevention of deep vein thrombosis and pulmonary embolism following surgery, and similar frequencies of bleeding have been described with LMWH and UFH. The decision as to which of these two preparations of heparin to use may thus be influenced by adverse reactions such as HIT. We therefore sought to determine the relative impact of UFH and LMWH specifically on HIT in postoperative patients receiving thromboembolism prophylaxis.

Objectives

The objective of this review was to compare the incidence of HIT and HIT complicated by thrombosis in patients exposed to UFH versus LMWH in randomised controlled trials (RCTs) of postoperative heparin therapy.

Search methods

The Cochrane Peripheral Vascular Diseases Group searched their Specialised Register (March 2012) and CENTRAL (2012, Issue 2). In addition, the authors searched LILACS (March 2012) and additional trials were sought from reference lists of relevant publications.

Selection criteria

We were interested in comparing the incidence of HIT occurring during exposure to UFH or LMWH after any surgical intervention. Therefore, we studied RCTs in which participants were postoperative patients allocated to receive UFH or LMWH, in a blinded or unblinded fashion. Eligible studies were required to have as an outcome clinically diagnosed HIT, defined as a relative reduction in the

platelet count of 50% or greater from the postoperative peak (even if the platelet count at its lowest remained > 150 x 10^9/L) occurring within five to 14 days after the surgery, with or without a thrombotic event occurring in this timeframe. Additionally, circulating antibodies associated with the syndrome were required to have been investigated through laboratory assays.

Data collection and analysis

Two review authors independently extracted data and assessed the risk of bias. Disagreements were resolved by consensus with participation of a third author.

Main results

In total two studies involving 923 participants met all the inclusion criteria and were included in the review. Pooled analysis showed a statistically significant reduction in the risk of HIT with LMWH compared with UFH (risk ratio (RR) 0.24, 95% confidence interval (CI) 0.07 to 0.82; P = 0.02). This result suggests that patients treated with LMWH would have a relative risk reduction (RRR) of 76% in the probability of developing HIT compared with patients treated with UFH.

Venous thromboembolism (VTE) complicating HIT occurred in 12 of 17 patients who developed HIT. Pooled analysis showed a statistically significant reduction in HIT complicated by VTE with LMWH compared with UFH (RR 0.20, 95% CI 0.04 to 0.90; P = 0.04). This result indicates that patients using LMWH would have a RRR of 80% for developing HIT complicated by VTE compared with patients using UFH. Arterial thrombosis occurred in only one patient who received UFH and there were no amputations or deaths documented.

Authors' conclusions

There was a lower incidence of HIT and HIT complicated by VTE in postoperative patients undergoing thromboprophylaxis with LMWH compared with UFH. This is consistent with the current clinical use of LMWH over UFH as front-line heparin therapy. However, conclusions are limited by a scarcity of high quality evidence. We did not expect the paucity of RCTs including HIT as an outcome as heparin is one of the most commonly used drugs worldwide and HIT is a life-threatening adverse drug reaction. To address the scarcity of clinically-relevant information on the topic of HIT as a whole, HIT should be included as an outcome in future RCTs of heparin, and HIT as an adverse drug reaction should be considered in clinical recommendations regarding monitoring of the platelet count for HIT.

PLAIN LANGUAGE SUMMARY

Frequency of heparin-induced thrombocytopenia in postoperative patients according to type of heparin

Heparin is a natural agent with antithrombotic action. Two types of heparins are widely used, unfractionated heparin (UFH) and low molecular weight heparin (LMWH). Heparin-induced thrombocytopenia (HIT) is an adverse reaction that can occur during treatment with heparin. It is common in practice and its most important consequence is a paradoxical increase in the risk of thromboembolic complications. The frequency of HIT is still poorly understood. A number of factors are thought to influence its frequency, including the type of heparin and the type of patient exposed; postoperative patients are at higher risk. This review aimed to compare the risk of HIT in postoperative patients exposed to UFH or LMWH. A better understanding of this problem should contribute to safer management of postoperative patients who need thromboprophylaxis with heparin.

High quality evidence from randomised controlled trials is sparse about HIT. Only two randomised controlled trials were good enough to be included in this review. Patients given LMWH had a lower risk of HIT than those given UFH (risk ratio 0.24, 95% confidence interval 0.07 to 0.82). Though little evidence is available, it appears that HIT induced by both types of heparins can be considered common (incidence > 1% and < 10%). This should be considered in clinical recommendations on monitoring of the platelet count for HIT. The research community and pharmaceutical industry should be aware of the need to include appropriate monitoring and testing for HIT in trials of anticoagulant therapy with heparin

BACKGROUND

Description of the condition

Heparin is a commonly used medication worldwide since it is essential in the treatment and prophylaxis of thromboembolic disorders. There are two types of heparin drugs comprising unfractionated heparin (UFH), also known as standard heparin, and low molecular weight heparin (LMWH). LMWH is constituted by a group of several drugs (for example enoxaparin, dalteparin, nadroparin, tinzaparin, certoparin) (Hirsh 2004; Micromedex® 2.0). LMWH has been largely replacing UFH as front-line therapy because it is judged to be at least as efficacious in preventing thromboembolic complications and to cause fewer bleeding adverse outcomes. However, similar efficacy and risks have been described (Handoll 2002; Kakkar 2000; Levine 2004; Mismetti 2001; Wille-Jørgensen 2003).

Although haemorrhagic events are the main recognised risk of heparin use, heparin-induced thrombocytopenia (HIT) is a potential severe, morbid complication of heparin therapy. HIT is defined as a relative reduction in platelet count of about 50% (even if the platelet count at its lowest remains > 150 x 10^9/L) occurring within five to 14 days after the start of heparin therapy (Warkentin 2003a; Warkentin 2003; Warkentin 2006). Patients re-exposed after a recent treatment may develop a rapid onset of HIT within 24 hours of heparin administration (Warkentin 2009). Also, a less frequent delayed onset of HIT, when it occurs after discontinuation of heparin, has been described (Smythe 2005; Warkentin 2001). Formerly designated as white clot syndrome or HIT type II, it is considered an acquired hypercoagulability syndrome caused by an immune-mediated reaction which is commonly followed by venous or arterial thrombosis (Greinacher 1995; Hong 2003; Walenga 2000; Warkentin 1995).

Description of the intervention

According to the elucidated mechanism, platelet activation and aggregation occur due to the interaction with an immunocomplex formed by platelet factor 4 (PF4)/heparin/IgG (HIT antibodies) (Amiral 1992; Januzzi 2000; Kelton 1994; Warkentin 1994a). In a few HIT cases (< 10%) only IgA or IgM antibodies against PF4/heparin complexes are detectable (Amiral 1996), but their clinical importance remains uncertain (Bircher 2006; Warkentin 2009). Considering that patients requiring antithrombotic therapy with heparin may be bedridden, at least to some extent, the procoagulant state together with vascular injury and stasis may be a central mechanism of the venous and arterial thrombosis associated with HIT.

The diagnosis of HIT requires the combination of clinical likelihood and laboratory tests to detect platelet activation induced by the HIT antibodies (Keeling 2006). The functional assays, ^{14}C-serotonin released assay (SRA) and heparin-induced platelet activation assay (HIPA), present the most favourable sensitivity and specificity trade-off (Warkentin 2008) as they demonstrate the presence of clinically relevant antibodies (Otis 2010). The platelet aggregation assay is not generally recommended (Leo 2003). Also, a number of commercial enzyme-linked immunoassays (ELISA) are available to diagnose HIT. These immunoassays represent an ideal test to rule out HIT but they may be combined with functional assays to confirm a diagnosis since they detect both pathogenic and non-pathogenic antibodies (Otis 2010).

How the intervention might work

HIT can occur following any mode of heparin administration (Januzzi 2000; Warkentin 2008), including parenteral infusions (Smythe 2005), subcutaneous therapy (Girolami 2003), and even with low-grade exposures such as heparin line flushes or following the insertion of heparin-bonded pulmonary artery catheters (Mureebe 2004). All sources of heparin must be suspended when the reaction occurs and the rationale for the treatment is the use of direct thrombin inhibitors and anti-factor Xa agents (Warkentin 2008).

The precise incidence of HIT varies due to different definitions of thrombocytopenia in HIT and due to distinctive methods to demonstrate the HIT antibodies (Warkentin 2008). Moreover, the development of HIT is associated with the type of heparin used (UFH or LMWH) and the type of heparin-exposed patient population (Warkentin 2008). The incidence of HIT seems to be higher with the use of bovine heparin when compared with porcine heparin (Ahmad 2007; Francis 2003).

Overall, an absolute risk of HIT induced by UFH or LMWH is estimated to be approximately 2% to 3% and 0.2% to 0.6%, respectively (Martel 2005; Warkentin 2003). This association of HIT with the type of heparin may be justified by a different immunogenicity attributed to UFH as it has a higher molecular weight and degree of sulphation. The high-risk subgroup is constituted of postoperative patients receiving UFH (incidence estimated between 1% to 5%) (Warkentin 2008). Postoperative patients receiving LMWH show a lower risk of HIT (incidence estimated between 0.1% to 1%) as do medical and obstetrical patients exposed to UFH (Girolami 2003). Specific characteristics of the patients and of certain surgery types have been shown to influence the risk profile of HIT (Lubenow 2010; Warkentin 2000), but most studies consist of patients after orthopaedic surgery.

Why it is important to do this review

Heparin-induced thrombocytopenia is an important adverse drug reaction and delayed recognition contributes to morbidity and mortality of patients (Rice 2005). However, there is a lack of robust evidence supporting knowledge on the frequency and the diagnosis

of HIT, which weaken the decision-making therapeutical process. Therefore, we aimed to compare the risk of HIT in postoperative patients exposed to UFH or LMWH.

OBJECTIVES

The objective of this review was to compare the incidence of heparin-induced thrombocytopenia (HIT) in postoperative patients exposed to unfractionated heparin (UFH) or low molecular weight heparin (LMWH).

METHODS

Criteria for considering studies for this review

Types of studies

This review included randomised controlled trials (RCTs) in which participants were postoperative patients allocated to receive UFH or LMWH, in a blinded or unblinded fashion.

Types of participants

Patients undergoing surgical procedures and treated with UFH or LMWH for prophylaxis of thrombotic events during at least five days.

Types of interventions

We were interested in the incidence of HIT occurring during prophylaxis treatment with UFH or LMWH after any surgical intervention. In order to achieve this objective, we studied RCTs in which participants were postoperative patients allocated to receive UFH or LMWH in a blinded or unblinded fashion.

Types of outcome measures

Primary outcomes

The main outcome of interest was the occurrence of HIT. The accepted definition of HIT was relative reduction in the platelet count of 50% or greater from the postoperative peak (even if the platelet count at its lowest remains >150 x 10^9/L) occurring within five to 14 days after the surgery. The onset of a thromboembolic event in the time frame defined above could also prompt suspicion of HIT. All clinically suspected cases of HIT needed to have the diagnosis confirmed through the demonstration of HIT antibodies by functional or immunological laboratory tests.

Cases of early-onset or delayed-onset HIT would also be considered as long as they had properly performed laboratory tests for HIT. These outcomes are defined as follows:

- early-onset is when HIT develops within 24 hours of heparin administration;
- delayed-onset is when HIT is diagnosed after the discontinuation of heparin.

Secondary outcomes

The secondary outcomes investigated were HIT complicated by the following events:

1. venous thromboembolism (presenting clinically as deep vein thrombosis, pulmonary embolism, or both);
2. arterial thrombosis (presenting clinically as myocardial infarction, stroke, or other artery thrombosis);
3. amputation;
4. death.

All secondary outcomes should be confirmed by an objective method (Büller 2003; Emea 1998; Emea 2008; Prandoni 2005), depending on the specific situation, as follows.

Arterial thrombosis:

- arteriography for an arterial thrombosis investigation;
- electrocardiography with enzymatic support in the case of suspected myocardial infarction;
- cerebral computed tomography (CT) scan or magnetic resonance imaging (MRI) in the case of suspected stroke.

Venous thromboembolism should be confirmed by at least one objective test:

- ascending contrast venography;
- duplex venous ultrasonography, MRI or venography in the case of suspected deep vein thrombosis (DVT);
- ventilation/perfusion lung scan, pulmonary angiogram or spiral CT lung scan for clinical diagnosis of pulmonary embolism.

Recurrent venous thrombosis would also be considered as a secondary outcome and the criteria for its diagnosis include abnormal venous ultrasonography where compression had been normal or there was a substantial increase (4 mm or more) in the diameter of the thrombus during full compression.

Search methods for identification of studies

Electronic and manual searches were performed with no restriction on language.

Electronic searches

The Cochrane Peripheral Vascular Diseases (PVD) Group Trials Search Co-ordinator (TSC) searched the Specialised Register (last

searched March 2012) and the Cochrane Central Register of Controlled Trials (CENTRAL) (2012, Issue 2) on *The Cochrane Library* (www.thecochranelibrary.com). See Appendix 1 for details of the search strategy used to search CENTRAL. The Specialised Register is maintained by the TSC and is constructed from weekly electronic searches of MEDLINE, EMBASE, CINAHL, AMED, and through handsearching relevant journals. The full list of the databases, journals and conference proceedings which have been searched, as well as the search strategies used, are described in the Specialised Register section of the Cochrane Peripheral Vascular Diseases Group module in *The Cochrane Library*.

The following trials databases were searched by the TSC in March 2012 using the terms thrombocytopenia and heparin for details of ongoing and unpublished studies:

World Health Organization International Clinical Trials Registry (http://apps.who.int/trialsearch/);

ClinicalTrials.gov (http://clinicaltrials.gov/);

Current Controlled Trials (http://www.controlled-trials.com/).

In addition, the authors searched LILACS with two search strategies, one of them using a high sensitive filter (Manríquez 2008). The search strategies are described (Appendix 2; Appendix 3). The searches in LILACS were last updated in March 2012.

Searching other resources

Additional trials were sought through manual screening of reference lists of eligible studies. The OpenSIGLE (System for Information on Grey Literature in Europe) was also searched by the authors in order to identify reports not published in indexed journals. The search strategy for this database included the terms heparin-induced thrombocytopenia, heparin induced thrombocytopenia, thrombocytopenia, heparin and adverse drug reaction.

Data collection and analysis

Selection of studies

The potentially relevant reports retrieved from the electronic searches were first assessed through analysis of titles and abstracts. The full report of the studies was accessed whenever necessary in order to conclude the selection process. This process was carried out independently by the authors Daniela RG Junqueira (DRGJ) and Raphael RM Penholati (RRMP). Disagreements were resolved by consensus with the participation of a third author, Edson Perini (EP).

In instances of lack of data, a request for further information was sent to trialists. Contact with trialists was also sought in order to clarify the definition of HIT used or to obtain detailed data needed to perform analyses.

Data extraction and management

Data collection on each trial was performed using a standard form that addressed characteristics of the trials and participants:

- details of trial design;
- setting where trial was conducted;
- eligibility criteria and trial exclusion criteria;
- number of participants randomised for each intervention group;
- mean age of participants;
- losses to follow-up;
- randomisation and concealment allocation method;
- type of heparin used (dose, commencement of therapy relative to surgery, duration of therapy);
- definition of HIT;
- time points when clinical and laboratory measurements were made to diagnose HIT during the study;
- type of laboratory assay performed to confirm HIT;
- number of primary and secondary outcomes (as mentioned in the section 'Criteria for considering studies for this review').

The data extraction tool was developed in accordance with the PVD Group methods (PVD Review Group). Data extraction was independently carried out by DRGJ and RRMP and the results were checked for accuracy. Disagreements or unclear data were discussed with a third author (EP or Maria das Graças Carvalho (MGC)). Additional information was sought when needed.

Assessment of risk of bias in included studies

Data regarding the sources related to the risk of bias were independently assessed by DRGJ and RRMP using forms designed according to Cochrane and PVD Group guidelines (Higgins 2009; PVD Review Group). Additional information was sought when necessary.

Measures of treatment effect

Dichotomous data were extracted and the absolute risk (incidence) of HIT was calculated with corresponding 95% confidence interval (CI). Outcome frequencies were transformed into risk ratio (RR) with 95% CI in the meta-analysis. A fixed-effect model was used for pooling data. In the event of a statistical difference, we calculated the relative risk reduction (RRR) for the outcomes.

Dealing with missing data

The authors of trials presenting a lack of data were always contacted. Clarifications were also sought whenever facing unclear data.

Assessment of heterogeneity

Statistical heterogeneity was tested using the Chi^2 test and I^2 statistic (Higgins 2009). The Chi^2 test was used to assess whether observed differences in results were compatible with chance alone. The I^2 statistic was applied to quantify inconsistency across studies.

Assessment of reporting biases

Data were obtained from full papers and unpublished data were sought. However, the insufficient number of studies that were included would not provide appropriate visual inspection for asymmetry on the scatter plot and therefore the funnel plot was not used to look for publication bias.

Data synthesis

The outcomes of interest in this review were dichotomous and we recorded the number of participants who developed the outcomes according to the allocated group of heparin type. The risk of HIT was calculated according to the type of heparin used.

Subgroup analysis and investigation of heterogeneity

Because of a lack of trials, we could not carry out a subgroup analysis exploring the effect of different laboratory methods for the diagnosis of HIT or the different types of heparins. One subgroup analysis, not planned in advance, was nonetheless possible. Thus, we performed an analysis exploring the risk of HIT with LMWH versus UFH in patients undergoing major surgical procedures (as opposed to patients undergoing any, that is major or minor, surgical procedure).

Sensitivity analysis

There were not enough studies to perform a sensitivity analysis exploring heterogeneity according to factors previously stated (methodological quality, blinding method performed, unpublished studies, study sample size, age of patients, gender of patients, drug posology).

RESULTS

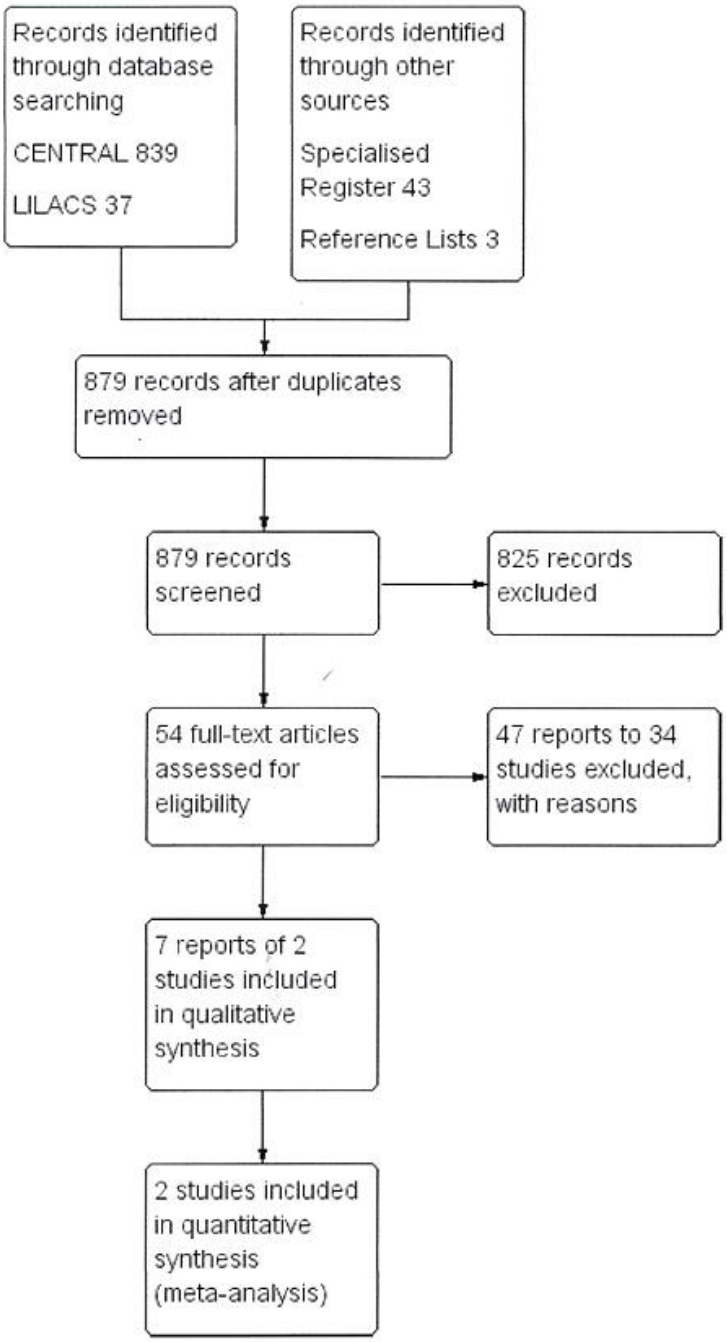
Description of studies

See: Characteristics of included studies; Characteristics of excluded studies.

Results of the search

See the study flow diagram (Figure 1).

Figure 1. Study flow diagram.



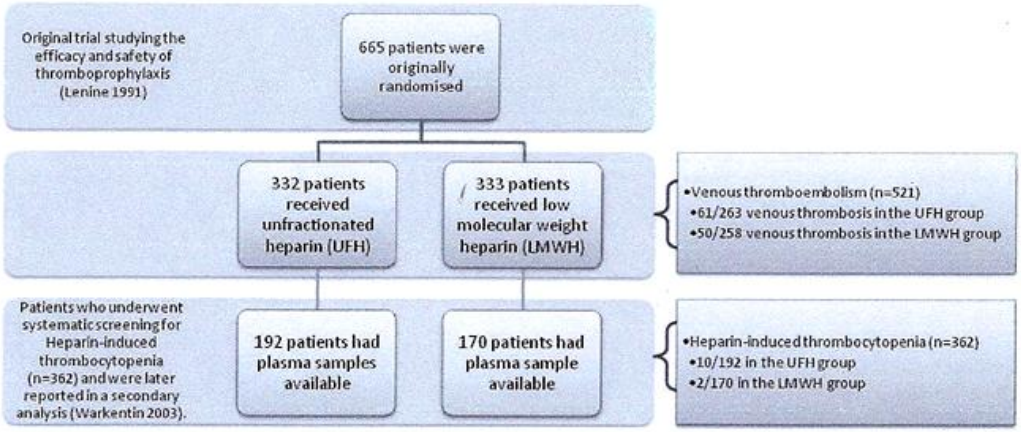
Details are listed below and in the tables Characteristics of included studies and Characteristics of excluded studies.

Included studies

Two studies were included in the review with data available for 923 postoperative patients recruited in hospitals located in Canada and Germany.

One of them, performed by Warkentin and colleagues (Warkentin 2003), compared the incidence of HIT in orthopaedic postoperative patients allocated to one of two groups treated with standard heparin or enoxaparin, a type of low molecular weight heparin. This study represented a secondary analysis using patients enrolled in a major clinical trial. The participants were originally recruited to study the efficacy and safety of enoxaparin compared with standard calcium heparin for the prevention of postoperative DVT in patients undergoing elective hip surgery. A total of 665 patients were randomised in the original trial (Levine 1991) and 362 underwent systematic monitoring for HIT and had the presence of HIT antibodies determined. A flowchart illustrating the sequence of participants enrolled in these two studies is provided (Figure 2).

Figure 2. Flowchart illustrating the recruitment of the patients included in this systematic review and extracted from the study reported by Warkentin 2003.



Participants enrolled in this trial received treatment until postoperative day 14 or until discharge. Preoperative and daily postoperative platelet counts were measured for all patients and the peak postoperative platelet count represented the platelet count baseline. Plasma samples used to investigate HIT were collected around postoperative day 7 or later (Warkentin 2003). The study confirmed the presence of functional antibodies of HIT through the SRA. The result of the SRA was considered positive if the sample caused greater than 20% serotonin release at 0.1 U/mL heparin, less than 20% serotonin release at 100 U/mL heparin, and less than 20% serotonin release at 0.1 U/mL heparin in the presence of Fc receptor blocking monoclonal antibody. Samples tested positive in the SRA were screened through enzyme immunoassay to confirm the presence of antibodies of the IgG class in the sample.

The second included study (Lubenow 2010a) compared the incidence of HIT in patients admitted to a trauma surgery department. Although 8.6% of the participants enrolled were not submitted to a surgery procedure, data could be extracted separately for patients who underwent major or minor surgeries. Patients in this study received UFH or certoparin, a type of LMWH, until median postoperative day 10. Daily platelet counts were measured and HIT was clinically defined when a patient scored four or more

points on the 4T's score system. The 4T's score system is a risk assessment tool that classifies patients according to their probability for having HIT based on the sum of points attributed to four clinical features of HIT (magnitude of thrombocytopenia, timing of thrombocytopenia regarding heparin exposure, occurrence of thrombosis or other sequelae, and the absence of other explanations for the thrombocytopenia) (Lo 2006; Warkentin 2003a). Considering the controlled design of the trial, any patient who would have a relative reduction in the platelet count of 50% or greater from the postoperative peak, occurring within days five to 14 after the surgery, or have a thromboembolic event in this time frame would score four points in the score system and therefore fall within the definition of HIT specified for the inclusion in this systematic review. The study confirmed the presence of functional antibodies of HIT through the HIPA test and an in-house enzyme immunoassay for IgG, IgM and IgA (cut off 0.5 optical density units).

Details of the study design are shown in the table Characteristics of included studies and in Table 1.

Excluded studies

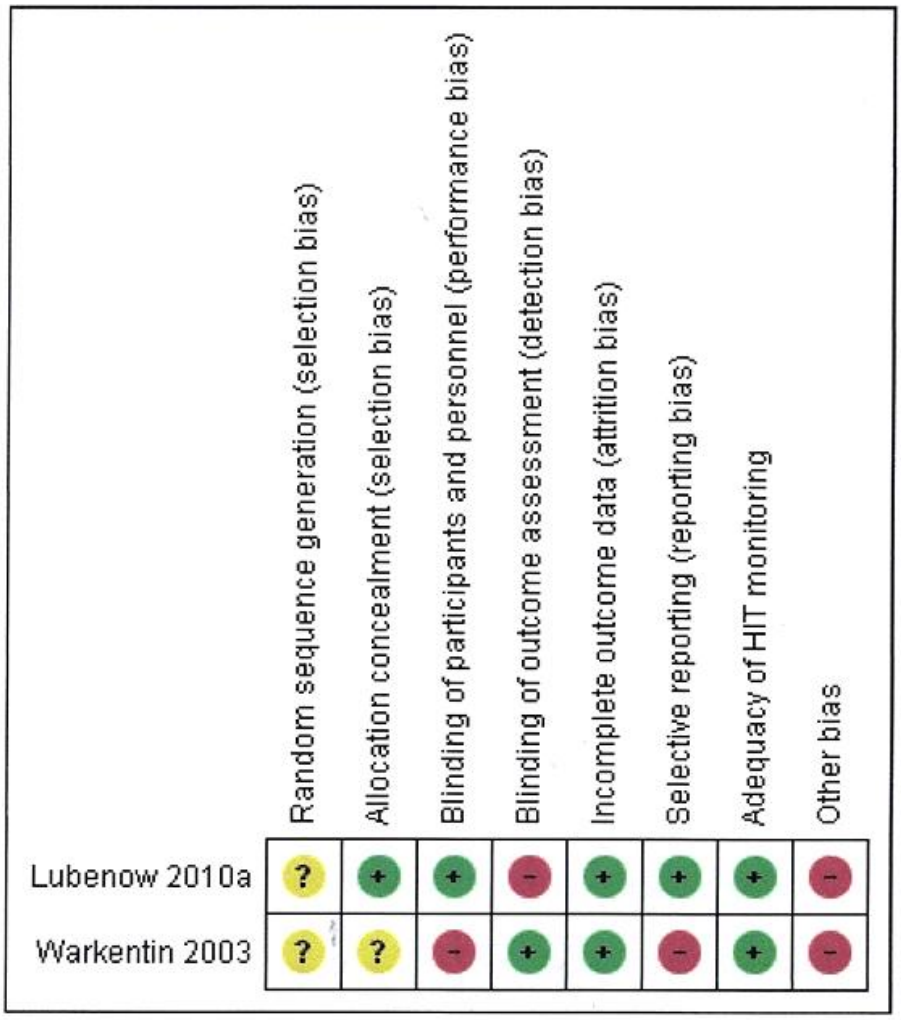
A total of 34 trials were excluded due to one or more of the following reasons: nonoperative patients (Ansell 1980; Bailey 1986; Berkowitz 2001; Chen 2005; CORTES Study; Daskalopoulos 2005; Fier 2011; Harenberg 1996; Lage 2007; Mitic 2010; PROTECT 2011; Reeves 1999; Wang 2006); no randomisation (Eika 1980; Funk 2000; Huhle 2000; Mahfeld 2002; Oliveira 2008; Savi 2005; Stenske 1998); irrelevant intervention (studies of drugs used to treat HIT) or combined intervention (heparin plus another drug or heparin compared with a different anticoagulant drug) (Assadian 2008; Chong 2001; Daskalopoulos 2005; Francis 2003; Mohiuddin 1992; Sarduy 2004; Savi 2005; Warkentin 2005); length of treatment with heparin (Francis 2003; Mohiuddin 1992); definition or monitoring of HIT (Ahmad 2003; Bailey 1986; Bell 1980; Bergqvist 1997; Kanan 2008; Konkle 2001; Leyvraz 1991; Powers 1984; Yeh 2007) not in accordance with the inclusion criteria of this review.

Risk of bias in included studies

Overall, the included trials presented a somewhat important risk of bias. The randomisation process was unclear in the included studies and bias due to allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias) and selective reporting (reporting bias) were considered possible for one or both trials. Incomplete outcome data (attrition bias) were judged as adequately addressed but other bias comprising 'funding' was judged to be high risk.

Details are described in Characteristics of included studies. Visual information on the risk of bias is provided (Figure 3).

Figure 3. Methodological quality summary: review authors' judgements about each methodological quality item for each included study.



Allocation

The selection bias in the included studies was judged to have an unclear risk because the randomisation process was mentioned but none of the trials specified the method used.

Blinding

Performance bias was judged to be high risk in one study (Warkentin 2003) and detection bias was judged high risk in the other trial (Lubenow 2010a). This high level of bias could have introduced systematic differences between groups and how outcomes were determined.

Incomplete outcome data

The trial reports appeared to include all expected outcomes. Therefore, they were probably free of attrition bias. Complementary data were sought from the authors and successfully used in the analysis.

Selective reporting

Reporting bias was considered high risk in one study (Warkentin 2003). This study represents a secondary analysis using patients enrolled in a major clinical trial. A total of 665 patients were ran-

domised in the original trial (Levine 1991) and Warkentin 2003 determined the presence of HIT antibodies with the ability to activate platelets in a subgroup consisting of 362 patients in whom serial plasma samples were available. Losses to follow-up in the original trial were minor and adequately reported. However, the selection process for the subgroup of patients used in the secondary analysis regarding HIT was not described and may have been conducted according to the researcher's convenience. Therefore, one cannot be assured of the randomisation process.

Other potential sources of bias

Monitoring for HIT

Special attention was paid to the appropriateness of HIT monitoring and of the diagnosis process according to the clinical and serological profile of the syndrome (see Table 1 and Characteristics of included studies). Addressing this issue is essential to avoid underrecognition of the condition. Both of the included studies performed systematic assays to determine the presence of functional antibodies related to HIT.

Conflict of interest

Funding for both included trials was provided by pharmaceutical industries. This financial support was judged as high risk of bias.

Effects of interventions

Primary outcomes

Participants originally enrolled in a major clinical trial (Levine 1991) were assessed for thrombosis through venograms (efficacy outcome) and haemorrhagic complications (safety outcome). In this study, no statistically significant difference was detected in the frequency of thrombosis between patients receiving UFH or LMWH (23.2% versus 19.4%, respectively; P > 0.2). Haemorrhagic complications were statistically significantly different comparing the UFH and the LMWH groups (9.3% versus 5.1%, respectively; P = 0.035). The number needed to treat to avoid an additional harm outcome (NNTH) would be 24, meaning an interesting safety improvement in terms of haemorrhagic outcomes for the group of patients receiving LMWH despite similar levels of efficacy regarding thrombosis prevention. No deaths occurred.
Later these participants became the source of a secondary analysis (Warkentin 2003) for assessing the HIT syndrome. This study reported 12 patients who developed HIT (12/362): 2/170 in the LMWH group (1.2%, 95% CI 0.2 to 3.8) and 10/192 in the UFH group (5.2%, 95% CI 2.7 to 9.1).
In the study performed by Lubenow 2010a and colleagues, HIT was reported in 1/272 patients in the LMWH group (0.4%, 95% CI 0.1 to 2.4) and in 4/289 in the UFH arm (1.4%, 95% CI 0.4 to 3.6).
Considering the studies individually, the difference in the incidence of HIT between groups was not statistically significant. However, a pooled analysis (the scores of 923 people) indicated a significant reduction in the risk of HIT with LMWH compared with UFH (RR 0.24, 95% CI 0.07 to 0.82; P = 0.02). There was no evidence of statistical heterogeneity (P = 0.90; I^2 = 0.0%). This result suggests that patients treated with LMWH would have a RRR of 76% in the probability of developing HIT compared with patients receiving UFH.
Combining the subgroups of patients undergoing major surgical procedures was possible since one study included only this type of participants (Warkentin 2003) and the other presented extractable data according to type of surgical procedure (Lubenow 2010a). The meta-analysis (the scores of 586 patients undergoing major surgeries) demonstrated a significant reduction in the risk of HIT with LMWH compared with UFH (RR 0.22, 95% CI 0.06 to 0.75; P = 0.02). There was no evidence of statistical heterogeneity (P = 0.93; I^2 = 0.0%).
See Analysis 1.1, Analysis 1.2, Figure 4 and Figure 5.

Figure 4. Forest plot of comparison: 1 Risk of heparin-induced thrombocytopenia (HIT) following LMWH or UFH exposure, outcome: 1.1 Heparin-induced thrombocytopenia.

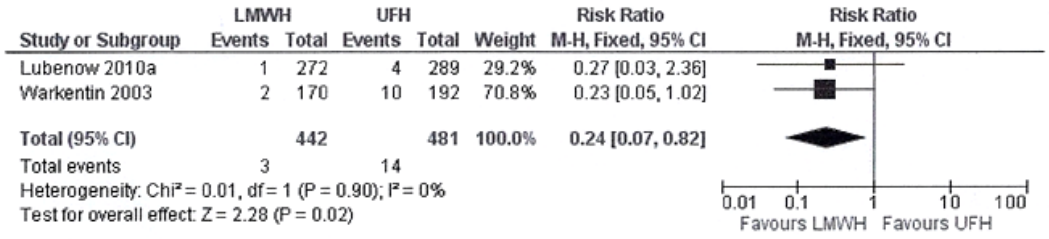
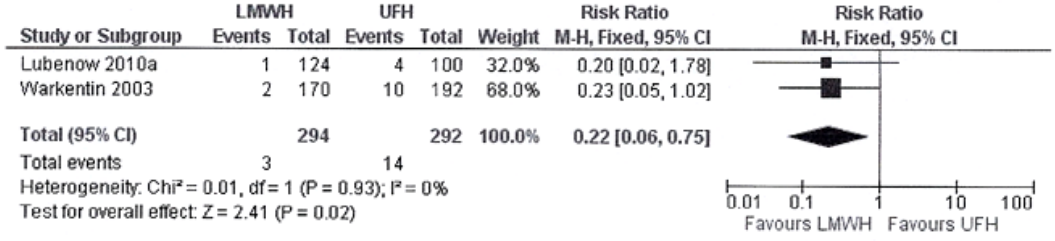


Figure 5. Forest plot of comparison: 1 Risk of heparin-induced thrombocytopenia (HIT) following LMWH or UFH exposure, outcome: 1.2 HIT in patients undergoing major surgical procedures.



Secondary outcomes

Venous thromboembolism (VTE) occurred in a total of 57 of the 362 patients enrolled in one study (Warkentin 2003) but the trial report did not present data on VTE events according to treatment group assignment. Considering just patients who developed HIT, venous thromboembolism occurred in a total of seven patients: 1/170 in the LMWH group (0.59%, 95% CI 0.0 to 3.59) and 6/192 in the UFH arm (3.14%, 95% CI 1.29 to 6.83). This means that VTE occurred in one out of two HIT cases detected in the LMWH group (50.0%) and in six out of 10 cases of HIT in the UFH arm (60%).

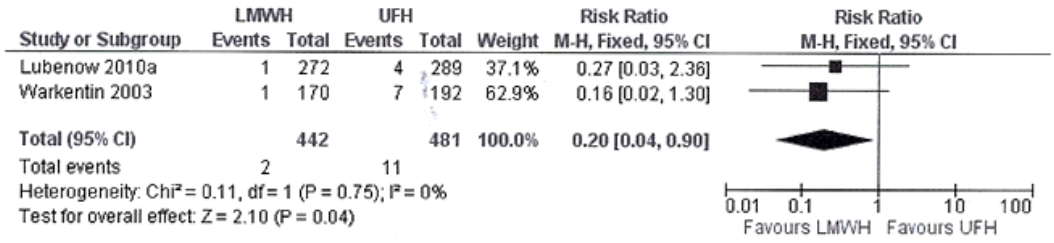
The VTE outcomes reported by Lubenow 2010a comprised a total of five events (5/561). Considering just patients who developed HIT, one event was reported in the LMWH group (0.37%, 95% CI 0.05 to 2.36) and 4/289 events occurred in the UFH arm (1.38%, 95% CI 0.41 to 3.63). Of note, all patients who developed HIT had a thromboembolic event.

Pooled analysis (the scores of 923 people) showed a significant reduction in HIT complicated by VTE with LMWH compared with UFH (RR 0.20, 95% CI 0.04 to 0.90; P 0.04), with no evidence of statistical heterogeneity (P = 0.75; I^2 = 0.0%). This result indicates that patients using LMWH would have a RRR of 80.0% for developing HIT complicated by VTE.

Arterial thrombosis occurred in one patient who received UFH (Warkentin 2003). There were no amputations, deaths or recurrent venous thromboses documented.

See Analysis 2.1 and Figure 6.

Figure 6. Forest plot of comparison: 2 Risk of HIT complicated by venous thromboembolism, outcome: 2.1 HIT complicated by venous thromboembolism.



DISCUSSION

Summary of main results

An adverse drug reaction is a response to a drug which is noxious and unintended, and which occurs at doses normally used in humans for the prophylaxis, diagnosis, or therapy of a disease, or for the modification of a physiological function (ASHP 1995; Emea 2006; WHO 1972). The adverse drug reactions are the most clinically significant medication-related problems and evidence con-

tinues to mount that adverse reactions to medicines are a common cause of illness, disability and even death (Handler 2007; WHO 1994).

Heparin-induced thrombocytopenia (HIT) is an adverse drug reaction induced by exposure to heparin. It is a potentially morbid syndrome since its main consequence is a hypercoagulate state associated with an increased risk of thrombotic events. However, despite its clinical importance and life-threatening presentation, HIT continues to be unrecognised and most physicians think that they have never seen a case (Levine 2005). Heparins are one of the most widely used drugs in hospitals worldwide and heparin is an essential drug in many clinical settings, from cardiovascular and surgery interventions to haemodialysis and pregnancy management. Postoperative patients stand out as a subgroup with an important risk of developing thromboembolic complications. The surgery procedure itself and the associated mobility restrictions contribute to this exacerbated occurrence of thromboembolic events. Moreover, it has been shown that this subgroup of patients is at higher risk of developing HIT (Warkentin 2008). Therefore, increasing the knowledge of the incidence of HIT in postoperative patients should improve the clinician's decision-making processes.

We carried out a comprehensive search of the medical literature and 879 articles were screened. Two randomised controlled trials were identified and included in this systematic review, both with some relatively important risk of bias. Pooling data of the trials in a meta-analysis showed a significant difference in the risk of HIT between LMWH and UFH. Regarding secondary outcomes, meta-analysis also showed a significant difference in rates of HIT complicated by VTE between heparin types. The relative risk reduction calculated for these outcomes demonstrated a substantial reduction in the risk of HIT and in the risk of HIT complicated by VTE with the use of LMWH. Interestingly, a subgroup analysis exploring the risk of HIT exclusively in patients who had undergone major surgeries showed a similar risk of HIT developing for this subgroup when compared with all patients, except for a slightly narrower confidence interval. Of note, the observed incidence of HIT for this group of patients undergoing major surgical procedures and treated with LMWH was higher than previously described in the scientific literature. This finding could indicate that, at least in patients submitted to major surgeries, HIT is a frequent occurrence with UFH and LMWH exposure.

There are some limitations in these results. A small number of clinical trials could be identified that were in accordance with the question addressed by the systematic review. Both trials included in the analysis were underpowered to detect HIT. From the frequencies demonstrated by the studies individually, the power to detect HIT was equal to 56.0% (Warkentin 2003) and to 24.6% (Lubenow 2010a) (Results from OpenEpi, Version 2, open source calculator--powerRCT, two-sided test at a significant level of 95%). The pooled analyses and the subgroup analyses also had small sample sizes. Regarding heterogeneity, although there was no evidence of statistical heterogeneity, the studies present some level of clinical and methodological diversity. For instance, doses of heparin and duration of treatment were different between studies. Moreover, from a methodological view point, the follow-up strategy for HIT assessment differed between the studies. It is difficult to determine exactly if and how these characteristics could impact the results. Therefore, despite the reduced incidence of HIT with LMWH compared with UFH, the limitations weaken the conclusions of this systematic review and meta-analysis. Considering the available evidence highlighted by this systematic review, we are unable to support a definitive conclusion regarding HIT as a more preventable adverse drug reaction by using LMWH compared with the use of UFH.

Overall completeness and applicability of evidence

The pathophysiology of HIT is in accordance with the existing consensus that UFH would induce HIT to a higher degree than LMWH, and anecdotal data have also shown this tendency (Martel 2005; Warkentin 1995), thus supporting existing guidelines for HIT monitoring. In this systematic review, the meta-analysis showed a significant difference in terms of the incidence of HIT induced by the two types of heparin, UFH and LMWH, with the LMWH presenting a lower risk for inducing HIT. However, this systematic review also highlighted the paucity of evidence regarding HIT, a life-threatening adverse drug reaction caused by an important drug that is used worldwide. In conclusion, the research community should note this problem for future studies related to heparin drugs. Moreover, considering that there are data regarding prevention of thromboembolic complications showing similar efficacy of LMWH and UFH (Handoll 2002; Mismetti 2001; Wille-Jørgensen 2003), and that drug costs remain a barrier to widespread use of LMWH, the actual cost-benefit balance of both heparins has not been based on accurate, high quality evidence. Additionally, complementary data also suggest that the rate of bleeding with heparin treatment may vary according to dose regimens and medical conditions but similar frequencies of bleeding have recently been described with LMWH and UFH (Kakkar 2000; Levine 2004).

Another question which may be taken into account is the existence of the different types of standard heparin which are commercialised in different countries. Whereas in the United States and in Europe the bovine UFH is no longer produced, because of the bovine spongiform encephalopathy epidemic (Blossom 2008; Brown 2001), in developing countries the delivery of heparins extracted from both bovine and porcine animal sources remains a reality (Junqueira 2011).

Quality of the evidence

The evidence included in this review was extracted from two RCTs involving 923 participants, of which 442 received LMWH (enoxa-

parin or certoparin) and 481 received UFH. Methodological limitations of the studies can be summarised as follows: an unclear risk of bias for one or more key domains (random sequence generation and allocation concealment); high risk of bias for one or more key domains (blinding of participants and personnel, blinding of outcome assessment, selective reporting and other bias); and low risk of bias for one or more key domains (incomplete outcome data and adequacy of HIT monitoring).

Overall, the included trials presented a somewhat important degree of risk of bias and the quality of the evidence can be considered as moderate.

Potential biases in the review process

Randomised controlled trials are largely used to study therapeutic interventions. However, the emphasis on treatment benefit, together with omission of information on harmful effects, could misinform anyone trying to make balanced decisions. Therefore, this review has drawn on the knowledge that harmful effects of any intervention may be reviewed with similar rigour as treatment benefits (Loke 2007). We followed a well recognised methodology which assures the quality of the Cochrane reviews for interventions (Higgins 2009). Publication bias was avoided by searching numerous databases and performing manual citation tracking. However, it was not evaluated by objective methods because of the small numbers of studies included. Despite our efforts, we identified only two clinical trials in accordance with the inclusion criteria.

The resulting low number of studies identified to answer the clinical question of this review may illustrate a limitation of RCTs to accurately evaluate adverse events induced by drug use, since this design is typically applied to study positive outcomes, particularly those with a higher frequency. However, considering the available body of knowledge related to HIT, and the fact that HIT is not a rare event, any clinical trial studying heparins must be designed to consider monitoring of HIT. It is an important limitation, and even unethical, when clinical trials are conducted focusing on just the efficacy or when they do not perform screening for a well-known and potentially dangerous adverse reaction to the drug under study.

It is now becoming clear that the systematic evaluation of adverse effects of drugs may require other study designs, mainly cohort and case-control studies. The methodology for inclusion of observational studies is still in need of further improvement. We believe that the continuous search for high quality studies focusing on HIT and the ongoing development of the methodology for systematic reviews of adverse effects according to the quality standards of the Cochrane Collaboration (Cochrane 2010; Loke 2007) may improve the evidence on the risk of HIT highlighted in this systematic review. Of relevance, pharmaceutical industries and researchers involved in clinical trials regarding heparins have the responsibility to use high quality methods to assess HIT.

Agreements and disagreements with other studies or reviews

The incidence of HIT among patients exposed to heparin is highly variable and is influenced by the type of heparin used (UFH or LMWH) and the type of heparin-exposed patient population. One important evidence-based clinical practice guideline (Warkentin 2008) summarises the various risk factors for HIT and classifies them into three categories: high risk (incidence > 1.0%), intermediate risk (incidence ranging from 0.1 to 1.0%), and low risk (incidence < 0.1%). Patient groups with a risk estimated to be higher than 1.0% are postoperative patients receiving prophylactic or therapeutic doses of UFH. Medical and obstetric patients receiving a prophylactic or therapeutic dose of UFH, postsurgery patients receiving LMWH, postsurgery patients receiving UFH 'flushes', and medical and obstetric patients receiving LMWH after first receiving UFH are groups with a risk for HIT that is estimated to be intermediate. Medical and obstetric patients receiving LMWH and medical and obstetric patients receiving only heparin 'flushes' are groups at lower risk. The methodological quality supporting this guideline is based on evidence from observational studies, case series, RCTs with serious flaws, or indirect evidence. This means that higher-quality research is likely to have an important impact on the confidence in the estimates of the effect and may well change them (Guyatt 2008).

Our systematic review corroborates the evidence that LMWH induces HIT to a lower degree than UFH (Martel 2005; Prandoni 2005; Warkentin 2000; Warkentin 2003). Also, the analyses demonstrated a high risk of HIT complicated by venous thromboembolic events when patients are exposed to UFH. Because of the small sample sizes used in the analyses, this finding may be closely related to the frequency of HIT induced by each type of heparin and it may exclusively be a consequence of the small number of HIT cases among patients who received LMWH. Nevertheless, and despite the sparse data on this outcome, the number of venous thromboembolic events linked to HIT was striking (Greinacher 1995; Hong 2003; Walenga 2000; Warkentin 1995). In disagreement with other studies, the results demonstrated a higher than expected incidence of HIT (absolute risk) with LMWH exposure in those patients submitted to major surgeries.

A recently published study demonstrated that patients who have had major surgical procedures have a much greater risk for developing the immune response to platelet factor 4/heparin than patients undergoing minor surgical procedures, irrespective of the type of heparin received (Lubenow 2010a). In line with this, we showed a tendency for a slightly more confident estimate of the relative risk of HIT associated with major surgeries. Together, these findings may support the existence of a non-drug factor acting as a marker for the immune response which leads to this adverse drug reaction, as discussed in the paper of Lubenow 2010a.

An interesting systematic review with meta-analysis that was recently published (Morris 2007) evaluated the incidence of throm-

bocytopenia and heparin-induced thrombocytopenia in patients treated with either LMWH or UFH for venous thromboembolism. The results showed no statistically significant difference between UFH and LMWH with regards to the incidence of thrombocytopenia alone. The incidence of HIT highlighted by Morris 2007 was too low to permit any firm conclusion. However, the definition of HIT used in this study was significantly less rigorous than the definition considered in the present review thus weakening any comparison.

Another systematic review with meta-analysis screened 368 articles with the aim of evaluating HIT incidence in patients receiving thromboprophylaxis with both types of heparin (Martel 2005). Two RCT addressing HIT following orthopaedic surgery (Leyvraz 1991; Warkentin 1995) and three non-RCT prospective studies (two addressing HIT following orthopaedic surgery and one addressing HIT following cardiac surgery) were considered eligible for the review by Martel 2005. Other studies addressing only thrombocytopenia (without confirmatory tests for HIT) were also analysed. Both RCTs included in the systematic review by Martel 2005 were retrieved by our electronic searches. The trial performed by Leyvraz 1991 was excluded from our review because it used an obsolete laboratory test to diagnose HIT, which would underestimate cases of the outcome. The study performed by Warkentin 1995 was considered to be a previous report of the study reported by Warkentin 2003. The latter was included in the present systematic review.

Warkentin and colleagues (Warkentin 1995) published a report investigating HIT in a total of 665 patients who had been randomised in a larger trial (Levine 1991) to receive UFH or LMWH. The HIT was defined as a decrease in platelet count below 150 x 10^9/L beginning five or more days after initiation of heparin therapy together with a positive test for PF4/heparin IgG antibodies. Plasma samples were not available from all the 665 patients and the article analysed data describing the study design and results for the 387 patients tested. Later, in the year of 2003, the same group of researchers published a secondary report (Warkentin 2003) discussing a more accurate definition for thrombocytopenia in HIT (the currently accepted definition) and re-analysing the same data from the previous article of 1995. A detailed analysis of the reports shows that Warkentin 2003 determined the presence of HIT antibodies in a subgroup consisting of 362 patients who underwent systematic monitoring for HIT and had serial plasma samples available. Therefore, the study actually investigated 362 patients according to our definition of HIT resulting in a description of 12 HIT cases: 2/170 in the LMWH group and 10/192 in the UFH group. There were another three patients in the UFH group with serologically-proven HIT (positive SRA in all three, positive immunoassay IgG specific in 2/2 tested) and with a large magnitude drop in platelet count (> 50% fall and timing consistent with HIT) who were not in the 362 patient subgroup but who underwent serological testing for HIT antibodies as ordered by the treating physicians. The author included these three patients in his analysis and also extended the analysis approaching the problem based on the total sample initially randomised (665 patients). The resulting analysis was 13 cases of proven HIT among 332 patients exposed to UFH versus two HIT cases among 333 patients exposed to LMWH. These detailed data were available thanks to the information provided by the trialists through electronic correspondence.

When extracting data from Warkentin 2003 for this review we considered the cohort of patients who actually followed the same methodological approach, which is the 362 patients who had platelets monitored and serological tests performed for HIT according to standard protocols predefined in the trial. The meta-analysis performed by Martel 2005 considered eight cases of HIT in the UFH arm (8/332) and no cases of HIT in the LMWH arm (0/333). The resulting Mantzel-Haensel odds ratio, using a random-effects model, was 0.06 (95% CI 0.00 to 1.00). The total of HIT cases extracted in the meta-analysis by Martel 2005 is not in accordance with ours but the resulting risk rate for this individual study is similarly imprecise.

AUTHORS' CONCLUSIONS

Implications for practice

Our results from two trials estimated a higher absolute risk (incidence) of HIT induced by UFH compared with LMWH, which is consistent with the scientific literature and the current clinical use of LMWH over UFH as front-line therapy. Conversely, the observed risk of HIT induced by LMWH was higher than previously shown in patients submitted to major surgery. According to WHO standard categories for adverse drug reaction frequency, and considering our results, HIT induced by both types of heparins may be considered as a common adverse drug reaction (incidence > 1% and < 10%) and this may impact clinical recommendations regarding platelet count monitoring for HIT. Of note, a significantly lower risk for venous thromboembolism was seen in patients who developed HIT when exposed to LMWH compared with UFH.

Implications for research

The pathophysiology of HIT together with observational evidence seem to be in accordance with a higher risk of HIT developing when patients are exposed to UFH. However, high quality evidence from randomised controlled trials is sparse. Indeed, it is unexpected that most clinical trials did not include HIT as an outcome considering that it is a life-threatening adverse drug reaction and that heparin is an important drug that is used worldwide.

Randomised clinical trials that include appropriate screening for HIT are needed and the research community should note this

problem for future clinical studies related to heparin drugs. Pharmaceutical industries and researchers involved in clinical trials regarding anticoagulant therapy with heparin have the responsibility to use high quality methods to appraise HIT.

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* *Indicates the major publication for the study*

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Lubenow 2010a

Methods	A randomised and double-blind clinical trial.
Participants	Participants were patients admitted to the Trauma Surgery Department at the University Hospital Grifswald, Germany. A total of 696 participants were randomised and 614 patients received the intended treatment (13.36% loss to follow-up). Mean age of the group of participants was 49.0 years (range: 18 - 98) for UFH arm and 50.0 years (range: 18 - 94) for the LMHW arm (P = 0.99) From the total of participants who received the intended treatment, 561 were postoperative patients (39.9% major surgeries; 60.1% minor surgeries). All participants had daily platelet counts measured in capillary blood (SE 9000; Sysmex). The heparin-induced platelet activation (HIPA) test was used to demonstrate platelet-activating antibodies and an in-house platelet factor 4/heparin enzyme immunoassay for IgG, IgM and IgA was used to screen patients for HIT antibody seroconversion defined as negative HIPA test and immunoassay on admission and positive tests from day five onwards of heparin The HIT antibody testing were planned to be performed on admission, at discharge (if before day 10) and between day 10 and 14. Participants undergoing major surgeries had blood samples for HIT testing obtained on day 11.0 (± 3.3 days). Patients undergoing minor surgeries had blood samples for HIT testing obtained on day 10.6 (± 3.3 days); P = 0.18. Patient characteristics did not differ between the heparin groups
Interventions	A group of 316 patients received UFH (B, Braun) and 298 received the LMWH Certoparin (Certoparin, Monoembolex, Novartis). The subgroup of postoperative patients comprised a number of 289 patients who received UFH and 272 patients who received LMWH Patients were followed until end point (HIT or new thrombosis) or until discharge. The study drugs were given for a median of ten days (range: 5 - 20 days) in patients undergoing major surgeries and for a median of seven days (range: 5 - 19 days) in patients undergoing minor procedures. Of note, the report states in its 'Methods Section' that all patients received LMWH after day 10. However, results presented are not in accordance with this statement
Outcomes	The primary outcome assessed was HIT defined as a patient with a 4Ts score of 4 or more points as agreed by two independent investigators, and tested positive for anti-platelet factor 4/heparin immunoglobulin IgG antibodies, and showing platelet-activating antibodies in the HIPA test Secondary outcomes were venous thromboembolism diagnosed through compression ultrasonography performed as screening at discharge, or in case of clinical suspicion of deep vein thrombosis
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement

Lubenow 2010a *(Continued)*

Random sequence generation (selection bias)	Unclear risk	Investigators did not state the method of randomisation neither in the protocol of the study (NCT00196417) nor in this trial report
Allocation concealment (selection bias)	Low risk	Investigators used sealed envelopes to conceal allocation of treatment groups. They did not describe the method to preserve concealment during the randomisation process
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Blinding of participants and personnel to the assigned treatment group was assured by a special coding of the medications and by the use of placebo injections when necessary
Blinding of outcome assessment (detection bias) All outcomes	High risk	Blinding of investigators who assessed the primary outcome (HIT) was probably not done since participants were assessed by the investigators by the use of the 4T's score after they were known to be positive in at least one of the HIT tests used In the evaluation of venous thrombosis, although the study states that abnormal findings were adjudicated by an investigator blinded to treatment assignment to the patient, it does not tell anything about the personnel who first assessed patients
Incomplete outcome data (attrition bias) HIT	Low risk	Numbers of exclusions and reasons for exclusions were adequately described
Selective reporting (reporting bias)	Low risk	The study protocol is available and all of the study's prespecified (primary and secondary) outcomes that are of interest for this review have been reported in the prespecified way
Adequacy of HIT monitoring	Low risk	Assessment of HIT antibodies occurred independently of clinical suspicion of HIT
Other bias	High risk	Although investigators state that the sponsor Novartis had no role in the study design, collection analysis and interpretation of data, the study was supported by an unrestricted grant from Novartis (Nürnberg, Germany)

Warkentin 2003

Methods	A multicentre, randomised clinical trial.
Participants	The LMWH, enoxaparin (Lovenox, Rhône-Poulenc Rorer, Montreal), was compared with a standard heparin (Calciparine, Laboratoires Anglo-French, Dorval, Quebec, Canada; prepared from porcine intestinal mucosa) for the prevention of thrombosis after elective hip surgery. A total of 665 patients were randomised in the original trial (Levine 1991) and Warkentin 2003 intended to determine the presence of platelet-activating HIT-IgG antibodies in a subgroup consisting of 362 patients in whom serial plasma samples were available, with at least one sample that was obtained on postoperative day seven or later. These patients were tested for HIT by the serotonin release assay (SRA) and by an enzyme immunoassay to confirm the presence of antibodies of IgG class in the samples which tested positive for HIT in the SRA Mean age of patients was equal to 66.8 years (UFH group) and 66.2 years (LMWH group). Patient characteristics did not differ between the heparin groups
Interventions	333 patients were randomised to receive LMWH and 332 patients to UFH The subgroup analysis consisted of 192 patients receiving UFH and 170 patients receiving LMWH The study drugs were given for a mean (± SD) period of 10 ± 3 days (maximum: 14)
Outcomes	The primary outcome assessed was HIT defined as a 50% or greater fall in the platelet count from the postoperative peak (up to postoperative day 14). The HIT was assured by demonstration of functional IgG antibodies through positive SRA and an immunoassay specific to IgG immunoglobulin class Secondary outcomes reported in patients whom developed HIT were thrombosis and thromboembolism
Notes	This study represents a secondary analysis using patients enrolled in a major clinical trial (Levine 1991).

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	The trial report stated the accomplishment of a randomisation process but no information regarding the method used was available
Allocation concealment (selection bias)	Unclear risk	Authors did not report any allocation concealment approach.
Blinding of participants and personnel (performance bias) All outcomes	High risk	Authors did not report the method assuring blinding of participants. Indeed, the authors describe the study merely as a randomised trial
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Although the assessment of thrombocytopenia appears to have been done in an unblinded manner, tests for heparin-dependent

		IgG antibodies were performed by an investigator unaware of the study group received by the patient For the diagnosis of venous thromboembolism, a central committee blinded to the patient assigned treatment interpreted results of venograms
Incomplete outcome data (attrition bias) HIT	Low risk	Trial report appears to include all expected outcomes and it is probably free of any suggestion of selective reporting
Selective reporting (reporting bias)	High risk	Losses of follow-up of the original trial were minor and adequately reported. However, the selection process for the subgroup of patients used in the secondary analysis regarding HIT was conducted according to researchers convenience. Therefore, one cannot assure that comparativeness allowed by the randomisation process was not missed
Adequacy of HIT monitoring	Low risk	Assessment of HIT antibodies occurred independently of clinical suspicion of HIT
Other bias	High risk	Funding for the clinical trial was provided by Aventis Pharma, Laval, Quebec

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Ahmad 2003	This trial includes nonoperative patients, therefore not meeting our type of participant inclusion criteria. Moreover, the definition of thrombocytopenia is obscure
Ansell 1980	This study was excluded because it recruited nonoperative patients
Assadian 2008	Patients recruited for this trial were randomised to receive unfractionated heparin or enoxaparin at the surgical procedure. However, all patients received enoxaparin plus aspirin during three days after surgery. The combined intervention (enoxaparin plus aspirin) and the time of prophylaxis for thrombotic events are not in accordance with our predefined inclusion criteria
Bailey 1986	This trial includes patients treated with heparin for deep venous thrombosis, pulmonary embolism, peripheral arterial embolism and cerebrovascular thromboembolism. It does not meet our entry criteria for type of participants. Additionally, the study did not confirm the diagnosis of heparin-induced thrombocytopenia through the demonstration of HIT antibodies by functional or enzyme immunoassays

(*Continued*)

Bell 1980	This study did not confirm the diagnosis of heparin-induced thrombocytopenia through the demonstration of HIT antibodies by functional or enzyme immunoassays
Bergqvist 1997	The study did not investigate heparin-induced thrombocytopenia
Berkowitz 2001	This trial includes nonoperative patients, not meeting our type of participant inclusion criteria
Chen 2005	Eligible participants in this trial were those requiring treatment for acute pulmonary thromboembolism thus it did not evaluate postoperative patients
Chong 2001	This study compares clinical outcomes of two treatments for heparin-induced thrombocytopenia: danaparoid versus dextran 70
CORTES Study	The CORTES Study investigated the incidence and clinical relevance of platelet factor 4/heparin antibodies in patients who had acute deep vein thrombosis of the leg and excluded all patients submitted to surgical procedures
Daskalopoulos 2005	This trial includes patients not submitted to a surgical procedure, not meeting our type of participant inclusion criteria. Additionally, unfractionated heparin in this trial was administered followed by acenocoumarol
Eika 1980	This study is a cohort study and enrolled nonoperated on patients
Fier 2011	This is a phase II trial so it studied only healthy volunteers
Francis 2003	This study was excluded because it focused on the effect of heparin exposure during coronary surgery and only 18.8% of the participants enrolled used heparin after surgery
Funk 2000	This is cohort study and therefore it did not use a randomisation process
Harenberg 1996	This trial includes nonoperated on patients, therefore not meeting our type of participant inclusion criteria
Huhle 2000	This study is a controlled clinical trial, not a randomised controlled trial
Kanan 2008	The study did not investigate heparin-induced thrombocytopenia
Konkle 2001	The main primary outcome in this trial was antibody formation to heparin/platelet factor 4 complexes studied using the serotonin release assay and a heparin/platelet factor 4 enzyme linked immunosorbent assay. Platelet counts were not monitored in accordance with the defined inclusion criteria for this systematic review
Lage 2007	This trial excluded any patient which could be submitted to any invasive procedure, therefore it excluded surgical patients
Leyvraz 1991	This trial defined thrombocytopenia as a platelet count drop of more than 40% and an absolute count decrease below 100 x 10^9/L on two consecutive measurements with laboratory confirmation by in vitro aggregation tests. It was excluded because the definition of heparin-induced thrombocytopenia is not in accordance with our previously defined criteria. Moreover, using such a definition for thrombocytopenia in HIT may underestimate cases of the outcome

(*Continued*)

Mahfeld 2002	This study is a controlled clinical trial, not a randomised controlled trial
Mitic 2010	This trial studied patients receiving treatment for venous thromboembolism during pregnancy and puerperium therefore not meeting our type of participant inclusion criteria
Mohiuddin 1992	This trial studied efficacy and safety of early initiation of warfarin during heparin therapy in acute thromboembolism. The intervention was heparin (UFH or LMHW) followed by warfarin starting within 48 hours or 96 hours. It does not meet our entry criteria for type of intervention
Oliveira 2008	This was a cohort, not a randomised controlled trial.
Powers 1984	This study was excluded because the definition of HIT was not in accordance with our inclusion criteria
PROTECT 2011	This trial excluded patients submitted to major trauma, neurosurgery or orthopaedic surgery, therefore it excluded the population of interest in this systematic review
Reeves 1999	Eligible participants in this trial were patients requiring haemodialysis for acute renal failure or as adjunctive therapy in systematic inflammatory response syndrome therefore not meeting our type of participant inclusion criteria
Sarduy 2004	This trial compares treatment with unfractionated heparin and warfarin, therefore it is not in accordance with our inclusion criteria
Savi 2005	This trial is not a randomised controlled trial. It studied the cross-reactivity of HIT sera with fondaparinux in 39 HIT confirmed patients. It does not meet our inclusion criteria
Stenske 1998	This study is a controlled clinical trial, not a randomised controlled trial
Wang 2006	This trial includes non-operative patients, not meeting our type of participant inclusion criteria
Warkentin 2005	This trial tested patient sera from two randomised, double-blind clinical trials that compared the LMWH (enoxaparin) with another anticoagulant drug, fondaparinux
Yeh 2007	This trial investigated the incidence of thrombocytopenia in a randomised fashion and HIT was not confirmed by laboratory assays

DATA AND ANALYSES

Comparison 1. Risk of heparin-induced thrombocytopenia (HIT) following LMWH or UFH exposure

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Heparin-induced thrombocytopenia	2	923	Risk Ratio (M-H, Fixed, 95% CI)	0.24 [0.07, 0.82]
2 HIT in patients undergoing major surgeries procedures	2	586	Risk Ratio (M-H, Fixed, 95% CI)	0.22 [0.06, 0.75]

Comparison 2. Risk of venous thromboembolism in patients whom developed heparin-induced thrombocytopenia

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Venous thromboembolism	2	923	Risk Ratio (M-H, Fixed, 95% CI)	0.20 [0.04, 0.90]

Analysis 1.1. Comparison 1 Risk of heparin-induced thrombocytopenia (HIT) following LMWH or UFH exposure, Outcome 1 Heparin-induced thrombocytopenia.

Review: Unfractionated heparin versus low molecular weight heparin for avoiding heparin-induced thrombocytopenia in postoperative patients

Comparison: 1 Risk of heparin-induced thrombocytopenia (HIT) following LMWH or UFH exposure

Outcome: 1 Heparin-induced thrombocytopenia

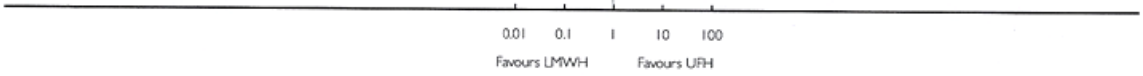
Study or subgroup	LMWH n/N	UFH n/N	Risk Ratio M-H,Fixed,95% CI	Weight	Risk Ratio M-H,Fixed,95% CI
Lubenow 2010a	1/272	4/289		29.2 %	0.27 [0.03, 2.36]
Warkentin 2003	2/170	10/192		70.8 %	0.23 [0.05, 1.02]
Total (95% CI)	**442**	**481**		**100.0 %**	**0.24 [0.07, 0.82]**

Total events: 3 (LMWH), 14 (UFH)

Heterogeneity: Chi?? = 0.01, df = 1 (P = 0.90); I?? =0.0%

Test for overall effect: Z = 2.28 (P = 0.023)

Test for subgroup differences: Not applicable

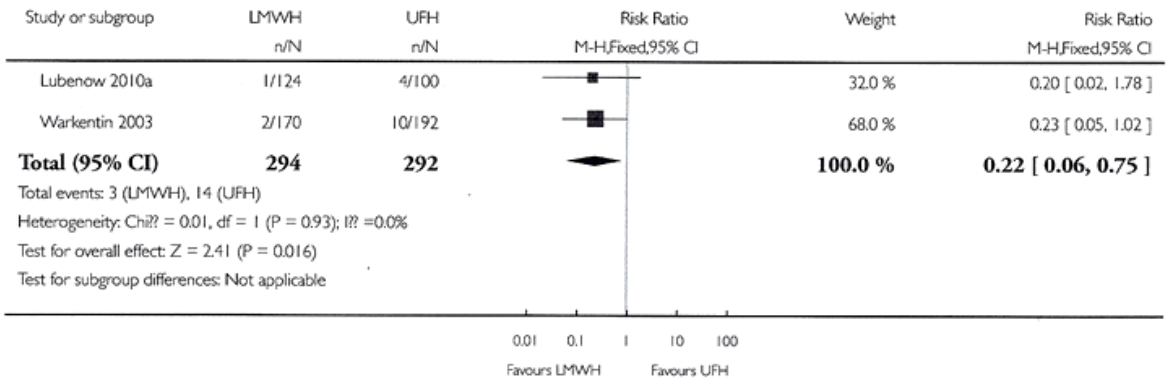


Analysis 1.2. Comparison 1 Risk of heparin-induced thrombocytopenia (HIT) following LMWH or UFH exposure, Outcome 2 HIT in patients undergoing major surgeries procedures.

Review: Unfractionated heparin versus low molecular weight heparin for avoiding heparin-induced thrombocytopenia in postoperative patients

Comparison: 1 Risk of heparin-induced thrombocytopenia (HIT) following LMWH or UFH exposure

Outcome: 2 HIT in patients undergoing major surgeries procedures

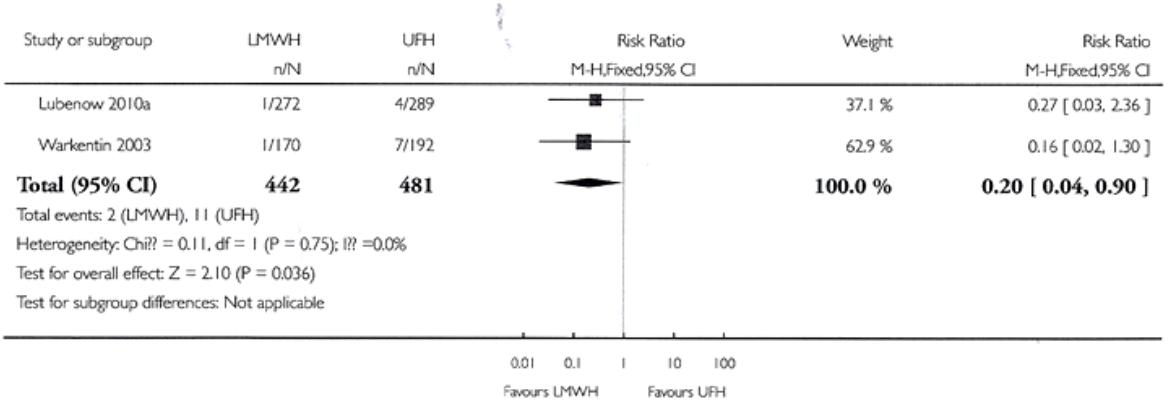


Analysis 2.1. Comparison 2 Risk of venous thromboembolism in patients whom developed heparin-induced thrombocytopenia, Outcome 1 Venous thromboembolism.

Review: Unfractionated heparin versus low molecular weight heparin for avoiding heparin-induced thrombocytopenia in postoperative patients

Comparison: 2 Risk of venous thromboembolism in patients whom developed heparin-induced thrombocytopenia

Outcome: 1 Venous thromboembolism



ADDITIONAL TABLES

Table 1. Details of the dose, type of medication used and length of follow-up

Study ID	UFH	Number of participants	Dose	LMWH	Number of participants	Dose	Treatment duration	Time point when plasma sample were obtained for HIT-IgG antibodies test	Laboratory test for HIT
Warkentin 2003	Standard calcium heparin	192	7500 units subcutaneously twice daily	Enoxaparin	170	30 mg subcutaneously twice daily	Started 12 to 24 hours after surgery and continued for 14 days or until discharge if it occurred sooner	At least 1 plasma sample obtained on postoperative day 7 or later	Serotonin release assay, with confirmatory investigation for the presence of functional antibodies of IgG class
Lubenow 2010a	Standard UFH	289	5000 units subcutaneously three times daily	Certoparin	272	3000 anti-factor XaU subcutaneously once daily	Started immediately after admission of patient and continued until day 10 or until discharge. After day 10 all patients received LMWH	Obtained on admission, at discharge (if before day 10) and between day 10 and 14.	Anti-platelet factor 4/heparin for immunoglobulin IgG class and platelet-activating antibodies in the heparin-induced platelet activation (HIPA) test

HIT: heparin-induced thrombocytopenia
LMWH: low molecular weight heparin
UFH: unfractionated heparin

APPENDICES

Appendix 1. Search strategy used for CENTRAL

#1	MeSH descriptor Thrombocytopenia explode all trees	809
#2	(HIT):ti,ab,kw	429
#3	(thrombocytopen*):ti,ab,kw	2500
#4	MeSH descriptor Autoantibodies explode all trees	800
#5	MeSH descriptor Autoimmunity, this term only	51
#6	autoantibod* OR auto-antibod* or antibod* OR (heparin near3 induced)	16235
#7	(#1 OR #2 OR #3 OR #4 OR #5 OR #6)	18936
#8	MeSH descriptor Anticoagulants explode all trees	3245
#9	MeSH descriptor Heparin explode all trees	3851
#10	hepar* OR UH OR UFH OR LMWH OR nadroparin* OR fraxiparin* OR enoxaparin OR Clexane OR klexane OR lovenox OR dalteparin OR Fragmin OR ardeparin OR normiflo OR tinzaparin OR logiparin OR Innohep OR certoparin OR sandoparin OR reviparin OR clivarin* OR danaproid OR danaparoid OR bemiparin	8548
#11	antixarin OR ardeparin* OR bemiparin* OR Zibor OR cy 222 OR embolex OR monoembolex OR parnaparin* OR "rd 11885" Or tedelparin OR Kabi-2165 OR Kabi-2165	279
#12	emt-966 or emt-967 or "pk-10 169" or pk-10169 or pk10169	17
#13	cy-216 or cy216 or seleparin* or tedegliparin or seleparin* or tedegliparin*	67
#14	wy90493 or "wy 90493"	5
#15	"kb 101" or kb101 or lomoparan or orgaran	50
#16	parnaparin or fluxum or lohepa or lowhepa or "op 2123" or parvoparin	45
#17	AVE5026	6
#18	anticoag* OR anti-coag*	5890

(*Continued*)

#19	(#8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR # 15 OR #16 OR #17 OR #18)	10133
#20	(#7 AND #19)	839

Appendix 2. Authors' LILACS search strategy 1

#1	Heparin OR Heparinic Acid OR Liquaemin OR Sodium Heparin OR Heparin, Sodium OR Heparin Sodium OR alpha-Heparin OR alpha Heparin OR Heparin, Low Molecular Weight OR LMWH OR Low Molecular Weight Heparin OR Low-Molecular-Weight Heparin OR Dalteparin OR Tedelparin OR Kabi-2165 OR Kabi 2165 OR Kabi2165 OR Fragmin OR Pharmacia Brand of Dalteparin Sodium OR Fragmine OR Pharmacia Spain Brand of Dalteparin Sodium OR Dalteparin Sodium OR Sodium, Dalteparin OR FR-860 OR FR 860 OR FR860 OR Enoxaparin OR Enoxaparine OR PK-10,169 OR PK 10,169 OR PK10,169 OR PK-10169 OR PK 10169 OR PK10169 OR EMT-967 OR EMT 967 OR EMT967 OR Lovenox OR Clexane OR EMT-966 OR EMT 966 OR EMT966 OR Nadroparin OR Nadroparine OR Nadroparin Calcium OR Calcium, Nadroparin OR Fraxiparin OR Fraxiparine OR CY 216 OR CY-216 OR CY216 OR LMF CY-216 OR LMF CY 216 OR LMF CY216 [Words]	922
#2	Heparin-induced thrombocytopenia OR Heparin induced thrombocytopenia OR Thrombocytopenia OR Thrombocytopenias OR Thrombopenia OR Thrombopenias OR Drug Monitoring OR Monitoring, Drug OR Therapeutic Drug Monitoring OR Drug Monitoring, Therapeutic OR Monitoring, Therapeutic Drug [Words]	514
#3	#1 AND #2	27

Appendix 3. Authors' LILACS search strategy 2

#1	Heparin OR Heparinic Acid OR Liquaemin OR Sodium Heparin OR Heparin, Sodium OR Heparin Sodium OR alpha-Heparin OR alpha Heparin OR Heparin, Low Molecular Weight OR LMWH OR Low Molecular Weight Heparin OR Low-Molecular-Weight Heparin OR Dalteparin OR Tedelparin OR Kabi-2165 OR Kabi 2165 OR Kabi2165 OR Fragmin OR Pharmacia Brand of Dalteparin Sodium OR Fragmine OR Pharmacia Spain Brand of Dalteparin Sodium OR Dalteparin Sodium OR Sodium, Dalteparin OR FR-860 OR FR 860 OR FR860 OR Enoxaparin OR Enoxaparine OR PK-10,169 OR PK 10,169 OR PK10,169 OR PK-10169 OR PK 10169 OR PK10169 OR EMT-967 OR EMT 967 OR EMT967 OR Lovenox OR Clexane OR EMT-966 OR EMT 966 OR EMT966 OR Nadroparin OR Nadroparine OR Nadroparin Calcium OR Calcium, Nadroparin OR Fraxiparin OR Fraxiparine OR CY 216 OR CY-216 OR CY216 OR LMF CY-216 OR LMF CY 216 OR LMF CY216 [Words]	922
#2	((Pt RANDOMIZED CONTROLLED TRIAL OR Pt CONTROLLED CLINICAL TRIAL OR Mh RANDOMIZED CONTROLLED TRIALS OR Mh RANDOM ALLOCATION OR Mh DOUBLE-BLIND METHOD OR Mh SINGLE-BLIND METHOD OR Pt MULTICENTER STUDY) OR ((tw ensaio or tw ensayo or tw trial) AND (tw azar OR tw acaso OR tw placebo or tw control$ OR tw aleat$ OR tw random$ OR (tw duplo and tw cego) OR (tw doble and tw ciego) OR (tw double AND tw blind)) AND tw clinic$)) AND NOT ((CT ANIMALS OR MH ANIMALS OR CT RABBITS OR CTMICE OR MH RATS OR MH PRIMATES OR MH DOGS OR MH RABBITS OR MH SWINE) AND NOT (CT HUMAN AND CT ANIMALS))	2197
#3	#1 AND #2	10

HISTORY

Protocol first published: Issue 1, 2009

Review first published: Issue 9, 2012

CONTRIBUTIONS OF AUTHORS

Daniela RG Junqueira was the lead author of this review. She was responsible for identifying potential studies, extracting data and for formatting the review in line with Review Manager 5 requirements. Daniela RG Junqueira also assessed the quality of trials selected for inclusion and performed analyses. She conceived the review, designed and wrote the protocol and the review.

Raphael RM Penholati read the reports identified for possible inclusion in the review and discussed their eligibility with Daniela RG Junqueira. He performed data extraction and checked the quality of trials.

Maria das Graças Carvalho conceived the review, revised and provided expert comments on the methodology and text of the review.

Edson Perini conceived the review, revised and provided expert comments on the methodology and text of the review. He also supervised the trial selection and data extraction process regarding the methodology of the trials assessed.

DECLARATIONS OF INTEREST

The authors received funds from the Minas Gerais State Research Foundation (Fapemig, Brazil) for their research. This review was completed as part of this research. Daniela RG Junqueira received a schollarship from CAPES during her Master degree program and she is supported by CNPq with a PhD fellowship.

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Internal sources

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Workplace and office supplies

- Coordination for the Improvement of Higher Level Education, CAPES, Brazil.
- National Council of Technological and Scientific Development, CNPq, Brazil.
- The Minas Gerais State Research Foundation (Fapemig), Brazil.

External sources

- Chief Scientist Office, Scottish Government Health Directorates, The Scottish Government, UK.

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DIFFERENCES BETWEEN PROTOCOL AND REVIEW

In the protocol of the review we planned to exclude participants younger than 18 years old of age. However, we realised that there was no need for this exclusion criterion and we did not consider it when evaluating trials. Nevertheless, none of the trials that were assessed would be excluded on the basis of this criterion.

We also planned to consider trials in which participants were randomly allocated to receive UFH versus LMWH or one type of heparin versus another anticoagulant therapy. However, this approach does not correspond to the title of the review. In order to keep the review faithful to its scope, we accepted only trials comparing UFH to LMWH.

We planned to extract data about 'death', as a secondary outcome, if it was confirmed by autopsy. However, as this is not a rigorous procedure adopted in clinical trials, we accepted extracting data related to this outcome without autopsy confirmation.

4.3 Atualização de conceitos sobre a trombocitopenia induzida por heparina destinada a produção de informação em saúde em nível nacional

As informações sobre a segurança da terapia com heparinas, as características da trombocitopenia induzida por heparina e o risco de trombocitopenia induzida por heparina produzidas nos dois primeiros estudos que compuseram a linha de pesquisa que integra a primeira parte dessa tese (*Epidemiologia das reações adversas a medicamentos*) foram organizadas num artigo com o intuito de promover a atualização dos profissionais de saúde do país sobre o tema e discutir os desafios enfrentados por esses profissionais no diagnóstico e tratamento da trombocitopenia induzida por heparina. O artigo está apresentado no formato de submissão e foi aceito para publicação na Revista da Associação Médica Brasileira:

- Daniela RG Junqueira, Maria das Graças Carvalho, Edson Perini. **Heparin-induced thrombocytopenia (HIT): a review of concepts on a dangerous adverse drug reaction.** Revista da Associação Médica Brasileira.

Title: Heparin-induced thrombocytopenia (HIT): a review of concepts on a dangerous adverse drug reaction

Portuguese title: Trombocitopenia induzida por heparina: revisão de conceitos de uma importante reação adversa a medicamentos

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Title: Heparin-induced thrombocytopenia (HIT): a review of concepts on a dangerous adverse drug reaction

Abstract

Heparin is a natural agent with antithrombotic action commercially available for therapeutic use as unfractionated heparin and low molecular weight heparin. The heparin-induced thrombocytopenia (HIT) is a serious adverse reaction to heparin which promotes antibody-mediated platelet activation. HIT is defined as a relative reduction in platelet count of 50% (even when the count of platelets at its lowest level is above >150 x 10^9/L) occurring within 5 to 14 days after initiation of the therapy. Thrombocytopenia is the main feature that directs the clinical suspicion of the reaction and the increased risk of thromboembolic complications is the most important and paradoxical consequence. The diagnosis is a delicate issue and requires a combination of clinical probability and laboratory tests for the detection of platelet activation induced by HIT antibodies. The absolute risk of HIT has been estimated between 1% and 5% under treatment with unfractionated heparin and less than 1% under exposure to low molecular weight heparin. However, high-quality evidence about the risk of HIT from randomised clinical trials is scarce. In addition, information on the frequency of HIT in developing countries is not widely available. This review aims to provide a better understanding of the key features of this reaction and updated information on its frequency to health professionals and other interested parties. The knowledge, familiarity and access to therapeutic options for the treatment of this adverse reaction are mandatory to minimize the associated risks, improving patient safety.

Keywords: drug toxicity, anticoagulants, heparin, thrombocytopenia

Resumo

A heparina é um agente natural com ação antitrombótica, sendo disponível para uso terapêutico a heparina não-fracionada e heparina de baixo peso molecular. A trombocitopenia induzida por heparina (TIH) é uma reação adversa grave às heparinas mediada por anticorpos que promovem ativação de plaquetas. A TIH é definida como uma redução relativa na contagem de plaquetas de 50% (mesmo se a contagem de plaquetas no seu nível mais baixo estiver acima 150 x 10^9/L) que pode ocorrer no período de 5 a 14 dias após o início da terapia com o medicamento. A trombocitopenia é a principal característica que direciona a suspeita clínica da reação, sendo que o aumento do risco de complicações tromboembólicas é a consequência mais importante e paradoxal. O diagnóstico é uma questão delicada e requer a combinação da probabilidade clínica com testes de laboratoriais para a detecção da ativação plaquetária induzida pelos anticorpos da TIH. O risco absoluto de TIH tem sido estimado entre 1% e 5% no tratamento com heparina não-fracionada e menor que 1% no uso de heparina de baixo peso molecular. No entanto, evidências de alta qualidade advindas de ensaios clínicos aleatorizados sobre a frequência dessa reação são escassas. Além disso, informações sobre a freqüência de TIH em países em desenvolvimento não são amplamente disponíveis. Esta revisão teve como objetivo fornecer aos profissionais de saúde e demais interessados um melhor conhecimento sobre a TIH e as principais características desta reação, bem como apresentar dados atualizados sobre a frequência da mesma. Conhecimento, familiaridade e acesso a opções terapêuticas para o tratamento dessa reação adversa são necessários para minimizar os riscos associados, melhorando a segurança do paciente.

Palavras-chave: toxicidade de drogas, anticoagulantes, heparina, trombocitopenia.

Introduction

Heparin is one of the most commonly used medications worldwide, with more than 1 trillion units utilised in the United States yearly [1]. It is an anticoagulant occurring naturally in the organism in small amounts whose activity is expressed through ligation to a plasma cofactor, the antithrombin, thus inactivating thrombin (factor IIa) and factors Xa, IXa and XIa [2]. For medicinal purposes, the drug is extracted from animal mucosal (suine or bovine) and used mainly in the treatment and prophylaxis of thromboembolic disorders.

There are two types of heparin drugs available comprising the unfractionated heparin (UFH), also called standard heparin, and the low molecular weight heparin (LMWH). The UFH is a heterogeneous mixture of glycosaminoglycans with molecular weight ranging from 3,000 to 30,000 in average. The LMWH is obtained by fractionation or depolymerisation of the standard heparin yielding fragments with mean molecular weight of 4,500 to 5,000 [2,3]. Therefore, LMWH constitutes a group of several drugs (e.g. enoxaparin, dalteparin, nadroparin, tinzaparin, etc) differing in some extent in their pharmacokinetic properties and anticoagulant profile since they are prepared by different methods of depolymerisation. The LMWH presents a more predictable dose-response relationship and an improved bioavailability after subcutaneous administration due to reduced binding to plasma proteins, macrophages and endothelial cells, then allowing a fixed-dose regime [2,4].

Along the range of adverse effects possibly occurring during treatment with heparin, haemorrhage is the main and most well-known recognised risk, occurring in 5 to 10% of exposed patients [3]. Another important adverse drug reaction faced by clinicians during treatment with heparin is a syndrome named heparin-induced thrombocytopenia (HIT), potentially the most morbid complication of heparin therapy. Formerly designated as white clot syndrome and HIT type II, HIT is a type of acquired hypercoagulability syndrome caused by an immune-mediated reaction induced by the heparin compound and commonly followed by venous or arterial thrombosis [5-7]. The first report of the association of HIT with thrombosis dates from 1958 and since then there has been a huge attempt to explain this intriguing syndrome.

Purpose of the review

Considering the potential consequences of a thrombotic event, HIT is an important and life-threatening adverse drug reaction following treatment with heparin. Therefore, we aimed to review the literature addressing key characteristics of this syndrome, its frequency and diagnosis issues in order to help HIT recognition in daily clinical practice.

Pathophysiology of HIT

The pathophysiology of the thrombocytopenia in HIT is still not complete understood [8]. According to the elucidated mechanism, following the administration of heparin, the platelet factor 4 (PF4), a small peptide stored in platelet α-granules, is released in blood circulation because of a transient and unspecific platelet aggregation induced by direct interaction of platelets with heparin [9]. Subsequently, heparin binds to PF4 enforced by charge differences then resulting in a macromolecular complex (PF4/heparin). The formation of this complex induces a conformational change in the molecules resulting in the formation of several neo-epitopes [2,10,11]. An immune response against these antigenic epitopes results in the production of antibodies of IgG, IgM and IgA classes.

The clinical importance of IgA and IgM antibodies remains uncertain since they appear unable to cause platelet activation in the presence of heparin [12,13], although in a few HIT cases (<10%) only IgA or IgM antibodies to PF4/heparin are detectable [11]. The IgG antibodies then react with PF4/heparin complex forming an immunocomplex of PF4/heparin/IgG antibodies (HIT antibodies) which has the ability to bind to platelets surface through their FcγRIIa receptor inducing platelet activation and aggregation [14,15]. The intensive platelet activation induced by HIT antibodies increases thrombin generation thus determining a hypercoagulability state [16,17].

Observational data about the prevalence of HIT in the setting of local or systemic inflammation have also raised the possibility that additional cell types are involved in the pathogenesis of thrombosis, including leukocyte-platelet aggregates and monocytes [8,18]. The HIT antibodies can bind to monocytes prompting their degranulation and release of several procoagulant and inflammatory substances [19].

Moreover, there is evidence that activated monocytes express tissue factor on their surface which reinforces the activation of the coagulation pathway [6]. The activated coagulation system also determines the release of vesicular platelet-membrane (platelet microparticles) which contains substances (GPIb, GPIIb and GPIIIa, P-selectin and thrombospondin) capable of increasing thrombin generation *in vivo* [10,20,21]. In addition, endothelial damage certainly plays a role in HIT pathogenesis since it can be caused by immunoglobulins, cytokines released by activated leukocytes, microparticles from activated platelets, adhesion molecules from both activated platelets and leukocytes, as well as by mechanical disruption due to the surgical process or pathological processes such as atherosclerosis [6]. Recently, in the setting of cardiothoracic patients, one study reported that HIT patients have positive antibodies to Adamts-13 and a reduced concentration of Adamts-13, which could be a complicating factor in HIT pathogenesis [22]. Considering that patients needing antithrombotic therapy with heparin may be bedridden at least to some extent, the procoagulant state together with vascular injury and stasis may be a central mechanism of the venous and arterial thrombosis associated with the development of HIT.

It has been shown that there is a dissociation between the development of HIT antibodies and the risk of HIT occurrence [23]. Therefore, not all patients who form HIT antibodies will develop thrombocytopenia or other sequelae of HIT [7,24]. The reticuloendothelial system may clear platelets coated with antibodies from circulation thus preventing the clinical manifestation of HIT in most patients [19]. However, it is currently unknown why some patients develop antibodies with ability to activate platelets (functional antibodies) and others do not [25].

Definitions and fundamental characteristics of HIT

The HIT is defined as a relative reduction in platelet count of 50% (even when the count of platelets at its lowest level is above >150 x 10^9/L) occurring within 5 to 14 days after starting heparin therapy [26]. Remarkably, this immune thrombocytopenia, HIT, differs from a nonimmune heparin-associated thrombocytopenia (HAT) which is secondary to a direct interaction of heparin with platelets and resolves spontaneously [10].

The time pattern of HIT may be of difficult recognition in the postoperative setting since platelet counts commonly decrease after a surgical procedure [26]. Therefore, in postoperative patients, thrombocytopenia in HIT may be defined as a drop in platelet counts of 50% or more from the maximum number of platelets achieved after the surgery and within the predefined time frame [27]. Noticeably, patients recently exposed to heparin may have circulating antibodies and then they can develop a rapid-onset of HIT within 24 hours after a new heparin administration [13]. A delayed-onset of HIT has also been described when HIT occurs months after the discontinuation of heparin [28,29]. The delayed-onset of HIT is typically recognised because of a thrombotic event and the possibility of an unsuccessful long-term anticoagulant therapy is a challenge to the diagnosis of the syndrome [12].

A clinical suspicion of this adverse drug reaction mainly occurs because of the thrombocytopenia which is the central feature of the syndrome. However, the clinical suspicion must be confirmed by the demonstration of antibodies with ability to induce platelet activation [26]. Thrombotic events may also prompt a suspicion of HIT since these complications can occur in an unpredicted way throughout the use of the drug and even before the thrombocytopenia status be reached [7,30].

Diagnosis and treatment of HIT

The diagnosis of HIT is a challenging issue. It requires the combination of clinical likelihood and laboratory tests to detect platelet activation induced by the HIT antibodies [31,32]. Some assays, known as functional assays, are able to demonstrate the presence of those clinically relevant antibodies [33]. These assays are the ^{14}C-serotonin released assay (SRA) and the heparin-induced platelet activation assay (HIPA) which also present the most favourable sensitivity/specificity trade-off [33,34].

The platelet aggregation assay has also been used but it lacks adequate sensitivity [35] and is not generally recommended. Another available procedure is to detect HIT antibodies in the patient sera by means of immunoassays. There are a number of commercial enzyme-linked immunoassays (ELISA) available to diagnose HIT. These immunoassays are able to detect pathogenic and non-pathogenic antibodies and commonly lead to a high rate of false positives [33]. However, despite their low

specificity, these assays represent an ideal test to rule out HIT and their combination with a functional assay can be an interesting procedure to screen negative cases. Thus functional assays should be reserved to just a small numbers of cases.

A clinical scoring system aiming to improve the clinical diagnosis of HIT was developed [32]. Using four clinical features of HIT (magnitude of Thrombocytopenia, Timing of thrombocytopenia regarding heparin exposure, occurrence of Thrombosis or other sequelae, and the absence of other explanations for the thrombocytopenia), the '4Ts' scoring system represents a risk assessment tool that classifies patients within low, moderate, and high probabilities for HIT. A number of studies have been investigating the usefulness of combining the 4Ts scoring system and laboratory testing in the diagnosis of HIT [36,37]. However, it may lack satisfactory ability to identify the probability of HIT in order to be widely used in clinical practice [38,39].

Considering the role of thrombin generation in HIT pathogenesis, all sources of heparin may be suspended when the reaction occurs. In case of a strong suspicion, even the results of the laboratorial assays should not be waited for [40]. However, the cost-benefit of introducing a treatment with an alternative anticoagulant must be considered in this clinical decision-process due to significant risk of bleeding [34]. There is a rationale for the use of direct thrombin inhibitors (argatroban, lepirudin or bivalirudin) and of an agent anti-factor Xa (fondaparinux) which inhibit thrombin generation to treat HIT. Treatment must continue until platelet count becomes normal and asymptomatic thrombosis may be investigated. In cases of complicated HIT by thromboembolic events, the therapy with alternative anticoagulants must be carefully followed by therapy with warfarin during 2 or 3 months [35]. An evidence-based guideline regarding the management of HIT is available [19] .

Some procedures may be avoided in the management of HIT. The LMWH is not a therapeutic option since it cross-reacts with circulating HIT antibodies [31,40]. Also, oral anticoagulant drugs must not be used because they reduce protein C and S levels then contributing to an increase in thrombin generation and resulting in a higher risk of thromboembolic complications [32]. Indeed, venous gangrene has developed in patients treated for HIT with oral anticoagulants. Therefore, warfarin therapy may be

carefully started only after the platelet count becomes normal. Platelet transfusion should not be given as it might induce or exacerbate thromboembolic complications [40].

Frequency of HIT

HIT may develop following any mode of heparin administration [10,34], including parenteral infusions [28], subcutaneous therapy [41], and even due to low-grade exposures such as heparin line flushes or following the insertion of heparin-bonded pulmonary artery catheters [42]. The development of HIT is influenced by the type of heparin used (UFH or LMWH) and the type of heparin-exposed patient population [34]. Also, the incidence of HIT seems to be higher with the use of bovine heparin when compared with porcine heparin. However, data regarding accurate values of the incidence of HIT are conflicting [34]. Overall, it has been generally accepted an absolute risk of HIT during treatment with UFH between 1 to 5% and during LMWH use between 0.1 to 1% [19,26,43]. The association of HIT with the type of heparin may be justified by the higher molecular weight and degree of sulphation of the UFH which determine a higher probability to induce the formation of HIT antibodies when compared to the LMWH.

The highest risk population is constituted of postoperative patients receiving UFH (incidence estimated lies between 1 to 5%) [34]. Postoperative patients receiving LMWH seem to show a lower risk of HIT (incidence estimated lies between 0.1 to 1%), together with medical and obstetrical patients exposed to UFH [41]. In other settings, as in the population constituted of medical and obstetrical patients exposed only to LMWH or receiving catheter flushes with UFH, HIT is described as a rare event, with an incidence <0.1% [34] despite higher frequencies having been observed [44]. Specific characteristics of patients submitted to certain surgeries have also been shown to influence the risk profile of HIT [18,23], but most studies have enrolled patients after orthopaedic surgery.

Recent investigations have shown weakness regarding the evidence supporting the generally accepted incidence of HIT. Although a lower incidence of HIT in postoperative patients under thromboprophylaxis with LMWH when compared with

UFH can be shown, randomised clinical trials which include HIT as an outcome are scarse [45]. Of note, the absolute risk (incidence) of HIT in patients subjected to major surgeries was found similar for both types of heparins (incidence >1% and <10%) in a recently published systematic review [45]. These findings are preliminary but can possibly impact clinical recommendations regarding platelet count monitoring during thromboprophylaxis with heparin.

Special concern may be addressed regarding the frequency of HIT in Brazil. To our knowledge, no information about the incidence of HIT neither in Brazil nor in Latin America is available and slight information about the frequency of HIT in other developing countries may be obtainable. This is a concerning issue because the specificities in the population composition and its genetics can clearly influence the effects of drugs [46] . Most importantly, bovine heparin has shown a higher potential to induce HIT when compared with porcine heparin. While most countries do not produce this kind of heparin anymore, an amount of 40% of the manufactured products containing heparin in Brazil is produced from the bovine heparin [47]. Therefore, there is a need for an improvement of knowledge and awareness regarding the occurrence of this adverse drug reaction in the clinical practice in our country. The poorly understood picture of HIT in Brazil may contribute to a delayed recognition of the syndrome, thus negatively impacting morbidity and mortality of patients.

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PARTE II
EPIDEMIOLOGIA GENÉTICA E ESTUDO DE GÊMEOS

5 EFICÁCIA E SEGURANÇA DAS INTERVENÇÕES FARMACOLÓGICAS PARA A DOR LOMBAR

A velocidade de geração de conhecimento para a produção de medicamentos inovadores, ou pretensamente inovadores, atende ao legítimo anseio humano em dispor de mais e melhores recursos terapêuticos. Percebemos na sociedade contemporânea uma ideia generalizada e dominante do medicamento como solução[37]. No entanto, muitos problemas de saúde não são efetivamente tratados com intervenções farmacológicas. A razão para isso reside, algumas vezes, em questões inerentes à atividade farmacológica do medicamento e, em outras, à complexidade de fatores causais das doenças e à amplitude de manifestações clínicas, questões que desafiam a identificação de medicamentos efetivos. Assim, mesmo considerando-se situações ideais de assistência e acesso a medicamentos, uma grande quantidade de pessoas enfrenta obstáculos para lidar com sua condição de saúde devido à ausência de tratamentos adequados e, nesse sentido, destaca-se uma série de problemas de saúde complexos que carecem de medicamentos com comprovação de efetividade, além de problemas de segurança.

Problemas de saúde complexos são condições em que uma combinação de fatores individuais, ambientais e genéticos contribui para a manifestação clínica.[38; 39] Como exemplos de problemas de saúde complexos podemos citar hipertensão, diabetes epilepsia, esquizofrenia, depressão, condições musculoesqueléticas, dentre outros. A dor, definida como uma experiência sensorial e emocional desagradável, associada a dano tecidual real ou potencial, consiste na produção de sinais dolorosos por receptores especializados, denominados nociceptores,[40] e consiste de um problema de saúde complexo e altamente prevalente em todo o mundo. As condições dolorosas estão entre os vinte e cinco principais problemas de saúde encontrados nos pacientes.[41] Dentre as diversas condições dolorosas, a dor lombar destaca-se como um problema de saúde tão frequente que se estima que oito em cada 10 pessoas apresentem ao menos um episódio ao longo da vida.[42]

A prevalência da dor lombar no mundo varia de 22% a 65%, dependendo de como a condição é definida.[43] Na Austrália, aproximadamente quatro milhões de pessoas sofrem de dor lombar em algum momento da vida e o custo total com tratamentos excede a A$1 bilhão por ano.[44] Somente nos Estados Unidos, os gastos com dor lombar atingem impressionantes US$32 bilhões anuais[45] e na Europa 90% do total dos gastos associados com a dor lombar são devidos a ausências no trabalho.[46] Um fator que aumenta os custos do manejo da dor lombar é a utilização de testes diagnósticos, já que se trata do problema de saúde mais comum para o qual um exame de imagem é solicitado pelo clínico geral.[47] A dor lombar é, na maioria dos casos (~90%), atribuível a nenhuma patologia ou alteração detectável e os exames de imagem são, portanto, desnecessários além de exporem os pacientes a doses de radiação.

O desafio para o tratamento da dor lombar é a identificação de intervenções terapêuticas eficazes. As atividades físicas são uma das mais populares formas no manejo dessa condição, no entanto, evidências demonstram que exercícios físicos oferecem apenas efeitos moderados no seu tratamento e nenhum tipo de atividade parece apresentar eficácia superior.[48; 49; 50; 51; 52] Ainda, a utilização de terapias de manipulação da coluna sob a forma de técnicas de velocidade variadas aplicadas às articulações da coluna vertebral, demonstra oferecer somente efeitos pequenos a moderados.[53; 54; 55; 56 57; 58] O efeito do calor,[59] de abordagens psicossociais,[60; 61] e da analgesia medicamentosa[58] são também limitados.

Notadamente, existe um número substancial de medicamentos disponíveis para o tratamento da dor lombar. No entanto, as intervenções farmacológicas oferecem apenas efeito moderado na redução da dor aguda e não demonstram redução da dor e nem da incapacidade associada a dor lombar crônica.[58; 62; 63; 64; 65] Adicionalmente, medicamentos comumente utilizados na dor lombar apresentam consideráveis problemas de segurança na utilização em longo prazo, como os anti-inflamatórios não-esteroidais, que apresentam um pobre balanço entre risco e benefício na utilização prolongada, e os analgésicos opiódes, que induzem uma grande quantidade de reações adversas graves. O paracetamol, medicamento considerado como primeira opção medicamentosa para o tratamento da dor lombar

aguda, apresenta um elevado risco de hepatotoxicidade se ingerido em doses superiores a 4g/dia.[66] Considerando-se que no Brasil são populares os comprimidos de paracetamol com 750mg, cinco comprimidos é a quantidade diária máxima segura para um indivíduo adulto. A dose total de paracetamol ingerida é fator prognóstico importante, e o evento pode ser fatal mesmo em uma única sobredosagem.[67] Além disso, o consumo contínuo do medicamento aumenta ainda mais o risco de uma reação adversa hepática já que a sobredosagem pelo consumo escalonado (sobredoses em dias consecutivos) está associada a risco aumentado de hepatotoxicidade e a menores taxas de sobrevivência.[68] Assim, para um indivíduo que evolui com dor lombar crônica, esse limiar de dose pode ser facilmente ultrapassado uma ou mais vezes, dependendo da experiência dolorosa do paciente. O consumo de paracetamol concomitantemente com bebidas alcoólicas é também fator de risco hepático pouco divulgado à população. Portanto, considerando que as opções terapêuticas atuais são limitadas, a realização de pesquisas investigando os fatores de risco para a dor lombar é de grande importância dado que a prevenção parece ser mais tangível que a cura.

Para a adoção de medidas preventivas é essencial o estudo dos fatores causais das doenças, particularmente em se tratando dos problemas de saúde complexos. Para o estudo dos fatores de risco, é fundamental o desenvolvimento de métodos que permitam a identificação de atributos, propriedades ou fatores que reconheçam grupos mais protegidos, ou mais vulneráveis, e esse é um interesse especial da Epidemiologia. Assim, a identificação de fatores de risco, em especial os modificáveis, apresenta-se como potencial solução para o problema da dor lombar. Esta abordagem epidemiológica é atraente e existem notáveis exemplos em que tal abordagem tem proporcionado melhorias significativas na saúde pública (manejo da doença coronariana, prevenção do câncer de pulmão, por exemplo).

5.1 Fatores de risco da dor lombar e o potencial da aplicação da epidemiologia genética e do estudo de gêmeos

Um grande número de fatores individuais e de estilo de vida tem sido investigado em suas relações com a causalidade da dor lombar ou com a evolução para a dor lombar crônica. No entanto, a provável relação causal entre os determinantes ambientais e a dor é questionável. Assim, fatores de risco anteriormente considerados determinantes no desenvolvimento de dor lombar como posturas estranhas, trabalho em pé ou andando continuamente, movimentação manual, elevação e transporte de cargas, não parecem estar associados à dor lombar, enquanto uma associação independente tem sido por vezes demonstrada entre a incidência de dor lombar, o sobrepeso e o tabagismo.[69; 70; 71; 72; 73]

Uma análise dos estudos investigando fatores de risco para dor lombar revela algumas falhas potenciais que poderiam explicar a escassez de fatores de causalidade identificados. Uma significativa variedade de definições e intervalos de tempo de prevalência para o desfecho é utilizada de forma aleatória nos diversos estudos epidemiológicos que buscam estudar os determinantes da dor lombar.[74] A utilização de definições diferenciadas e perguntas não-padronizadas limita a comparabilidade dos estudos e a utilização de diferentes pontos temporais de prevalência dificulta a avaliação do risco de viés das estimativas calculadas. Comumente, a observação da ocorrência da dor lombar é realizada no período compreendendo o último ano[74] e mudanças de estilo de vida e um significativo viés de memória podem impactar de forma significante os resultados mensurados. Além disso, em sua maioria, os fatores de risco identificados como potenciais causadores de dor lombar são derivados de estudos de baixa qualidade e com falhas metodológicas no ajuste para, pelo menos, um potencial fator de confusão. Assim, fatores importantes não são considerados, como *status* socioeconômico, natureza da ocupação dos participantes, sintomas de depressão e a influência genética (hereditariedade).[75; 76; 77; 78]

A ausência de controle para fatores genéticos em estudos investigando causas da dor lombar[79; 80] pode ser problemático considerando-se que o desfecho apresenta um componente hereditário estimado entre 40% a 44%,[81; 82] significando que fatores genéticos parecem ter uma forte influência na ocorrência de dor lombar. Não surpreendentemente, outros fatores de risco para dor lombar apresentam efeitos pequenos ou moderados sobre o risco de desenvolvimento da condição e isso pode, adicionalmente, explicar porque estratégias de prevenção dirigidas a esses fatores de risco de longo prazo apresentam sucesso limitado.

A interação entre fatores de risco genéticos e ambientais (fatores ambientais compreendidos como determinantes não-genéticos como dieta e estilo de vida, entre outros) contribui para uma série de problemas de saúde complexos como câncer, doenças coronarianas e doenças psiquiátricas, e representam um desafio para a pesquisa científica em saúde. Os recentes avanços na genética, como a conclusão do sequenciamento do genoma humano e uma maior compreensão da expressão gênica e da variabilidade *genética* ao nível de *sequência* de DNA, juntamente com o desenvolvimento de poderosas ferramentas estatísticas multivariadas, abriram novos caminhos de investigação em genética humana.[83] No entanto, sutis interações entre ambiente e a genética necessitam de métodos capazes de integrar diferentes tipos de dados e testar hipóteses aplicadas às chamadas características humanas complexas, em que uma combinação de fatores individuais, ambientais e genéticos contribui para o fenótipo expresso.[83; 84] Assim, a integração entre a pesquisa genômica e estudos epidemiológicos de fatores ambientais representa uma estratégia de pesquisa mais eficaz para a prevenção de doenças.[85]

Reconhecendo-se a necessidade de compreender a genética populacional e as propriedades biométricas humanas para a maximização da probabilidade de mapeamento de genes,[83; 86] é importante compreender a interação dos componentes ambientais e genéticos no desenvolvimento de problemas de saúde complexos ao invés da implementação de esforços no mapeamento direto de genes. Ainda, dado que a maioria desses problemas de saúde é influenciada por variantes na sequência genética de pouca penetrância, a investigação de fatores de risco modificáveis e suas interações com o componente genético oferece uma abordagem de maior

relevância na determinação do risco atribuível dessas doenças, e representa ainda uma estratégia para o desenvolvimento de intervenções de maior impacto em nível de saúde pública.[85]

Uma potencial alternativa para o estudo dos fatores de risco ambientais e genéticos é a utilização do método conhecido como Estudo de Gêmeos (*Twin Study Design*), delineamento epidemiológico que tem sido amplamente empregado na pesquisa em saúde, tradicionalmente nas áreas de oncologia, diabetes, envelhecimento e saúde mental. Em Epidemiologia Genética, os gêmeos são considerados como uma "oportunidade de experimento" porque eles permitem o estudo do papel da genética em conjunto com fatores ambientais na composição do desenvolvimento de uma doença.[87]

O estudo de gêmeos representa um método robusto para estimar os efeitos da hereditariedade e do ambiente sobre a prevalência e incidência das doenças. Os gêmeos são representativos da população em geral, apresentando taxas de mortalidade similares, bem como igual prevalência para uma série de doenças.[88; 89; 90; 91; 92] Considerando que os gêmeos monozigóticos compartilham de genes iguais e que os gêmeos dizigóticos possuem, em média, 50% dos seus genes semelhantes, pode-se comparar a concordância dos diferentes pares de gêmeos para um fenótipo de interesse (ex: dor lombar) e calcular a contribuição da genética e dos determinantes ambientais para a variabilidade do fenótipo em estudo.[93] Classicamente, os estudos de gêmeos quantificam os componentes ambientais e hereditários de uma doença a partir de métodos biométricos que comparam a suscetibilidade de gêmeos monozigóticos e dizigóticos para uma determinada característica (*classical twin study*).[83; 93; 94] Um desenho transversal ou longitudinal pode ser utilizado e, no caso de um estudo de coorte, estimativas de incidência e risco relativo são então calculadas para fornecer uma estimativa do risco de desenvolvimento da doença em gêmeos expostos e não expostos e esse risco pode ser estratificado de acordo com a zigosidade dos pares. Alternativamente, um desenho metodológico intitulado de "*co-twin control study*" também pode ser utilizado e, nesse caso, a exposição ao risco é analisada de forma retrospectiva em pares de gêmeos discordantes para a doença aonde um gêmeo não-afetado serve

de controle para o gêmeo que apresenta a doença.[83; 94] Esse desenho representa uma alternativa aos tradicionais estudos de caso-controle para a identificação de fatores associados a uma doença que diferem entre casos e controles e, nesse tipo de desenho, casos e controles são perfeitamente pareados para idade e genética e parcialmente pareados para influências ambientais precoces (circunstâncias pré e pós-natal da gestação e do crescimento).[94] Além disso, variações entre indivíduos para fatores de confusão estáveis são mais adequadamente controlados.

A hipótese de composição genética da dor lombar reside em evidências de controle genético influenciando a percepção da dor, sua sinalização e processamento psicológico.[95; 96] O controle da expressão de citocinas inflamatórias e de fator de crescimento neural também podem estar associados a dor lombar de diferentes maneiras.[97; 98; 99; 100]. Embora seja necessário um aprofundamento nos conhecimentos a respeito dos potenciais mecanismos genéticos que influenciam a expressão genotípica da dor lombar, pode-se hipotetizar que esses mecanismos sejam determinados por mutações de baixa penetrância e, portanto, a utilização do estudo de gêmeos oferece uma importante vantagem sobre os tradicionais estudos de fatores de risco na investigação do papel da interação genes-ambiente na etiologia dessa condição clínica. Evidências adicionais de que uma associação entre um fator ambiental e o risco de uma doença é causal são fornecidas quando uma interação entre o fator ambiental e o genótipo é documentada.[94]

Nesse sentido, foi desenvolvido recentemente um método de análise inovador derivado de modelos de regressão tradicionais para a interpretação de dados de estudos de gêmeos intitulado "Inferência causal com eliminação do confundimento do fator familiar" (‘*Inference on Causation from Elimination of Familial Confounding’ – ICE FALCON*)[101]. O princípio básico que dá suporte a essa abordagem é o estudo da associação entre o desfecho de um gêmeo (ex: dor lombar) com o seu *status* de exposição (ex: nível individual de aptidão física) e o *status* de exposição do seu gêmeo, estudo realizado com o objetivo de detectar interações entre o fator ambiental do gêmeo e do seu co-gêmeo no *status* de doença de um gêmeo. Se a exposição é causal, então o risco de um indivíduo desenvolver o desfecho vai depender da exposição do seu gêmeo, o que permite investigar se os dados são

consistentes com uma exposição que influencia o desfecho ou se trata-se de uma associação induzida por outros fatores compartilhados pelos pares de gêmeos que influenciam tanto a exposição quanto o desfecho. Num segundo caso, os dados são consistentes com uma associação sem base genética.[102]

A elegância dessa inovadora abordagem de análise reside em sua simplicidade e na possibilidade de eliminar ou controlar fatores de confusão familiar no desenvolvimento de inferências causais. Outra vantagem deste método é a possibilidade de utilizar dados de estudos transversais, desde que a relação temporal entre fator de risco que precede o desfecho seja contabilizada pelo uso de dados de um co-gêmeo. Estes atributos fazem desse método estatístico uma ferramenta útil para a pesquisa em dor lombar.

6 OBJETIVOS

O objetivo geral da linha de pesquisa em *Epidemiologia genética e estudo de gêmeos* foi investigar a contribuição da genética e de fatores de risco ambientais na dor lombar e desfechos associados (dor lombar crônica, incapacidade, intensidade da dor, etc).

6.1 Objetivos específicos

- Delinear um estudo-piloto utilizando gêmeos para a investigação de componentes genéticos e ambientais da dor lombar e desfechos associados;

- Determinar a influência genética e os determinantes ambientais da dor lombar crônica.

7 RESULTADOS

Os resultados dos estudos realizados na segunda linha de pesquisa que compõe essa tese, *Epidemiologia genética e estudo de gêmeos*, estão organizados em dois artigos originais submetidos para publicação em periódicos científicos.

7.1 Epidemiologia genética e o estudo de gêmeos na dor lombar – *Australian Twin Low Back Pain study* (estudo AUTBACK) [Protocolo]

Esse artigo apresenta o protocolo de pesquisa do estudo de gêmeos desenvolvido para a determinação de fatores de risco genéticos e ambientais da dor lombar. O estudo foi concebido em parceria como o *Australian Twin Registry* (o Registro de gêmeos da Austrália) e o artigo apresenta as etapas para formatação de um estudo de gêmeos com esse registro, dos aspectos metodológicos aos éticos. O estudo, intitulado AUstralian Twin Low BACK pain study e chamado estudo AUTBACK, utiliza o método de estudo de gêmeos de forma pioneira na dor lombar e testa adicionalmente a utilização de um questionário de preenchimento eletrônico (*online*). A definição da dor lombar (incluindo o aspecto temporal para a determinação da prevalência) seguiu um consenso para a padronização de definições de dor lombar em estudos observacionais estabelecido segundo a metodologia Delphi[37] e os fatores de risco investigados se concentraram na influência da atividade física, tabagismo e do consumo de álcool. O planejamento analítico do estudo prevê a utilização do método clássico de análise, do *co-twin control design* e da análise de *ICE FALCON.* Os resultados estão apresentados no formato do artigo submetido para a revista *Journal of Manual and Manipulative Therapy* como:

- Paulo H Ferreira, Daniela RG Junqueira, Christopher G Maher, John L Hopper, Manuela L Ferreira. **Genetic and lifestyle architecture underlying low back pain: profile of a web-based twin study.**

Genetic and lifestyle architecture underlying low back pain: profile of a web-based twin study

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Competing interests
The author(s) declare that they have no competing interests.

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Abstract

Objectives: Prevention in low back pain might be more achievable than cure and the investigation of modifiable lifestyle factors are warranted. In this regard, the twin study design is a powerful method to study the role of 'nature and nurture' for the development of low back pain because it allows for more precise estimates of risks after adjusting for genetic confounders. The main aims of the study are: i) to explore the relative genetic and environmental contribution on low back pain outcomes and ii) to explore possible causation paths between lifestyle factors and low back pain outcomes using a novel twin regression approach. **Methods:** This study was developed in collaboration with the Australian Twin Register and employs a web-based methodology to survey twins regarding low back pain outcomes and individual lifestyle risk factors. Low back pain outcomes will be the prevalence of low back pain, associated disability, frequency and pain intensity of episodes. Investigated risk factors include alcohol consumption, smoking habits and twins' engagement in physical activity. All register twins living in Australia aged 18 to 65 years old and with available email address will be invited to participate in the study. **Discussion:** This study will be the first one in the low back pain field to use a twin design and web-based approach. The unique feature of this study is the potential to identify a causal path between risk factors and low back pain using an innovative twin analysis after adjusting for possible familial confounding.

Keywords: Low back pain, heritable quantitative trait, risk factors, twin studies.

Background

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage. [1] Low back pain (LBP) is a highly prevalent cause of pain in the general population among all ages with a global mean one-month prevalence estimated at 29.1%.[2] Although common, treatment of this condition is challenging, with a wide range of conservative treatments failing to reduce pain and disability levels significantly. [3-6] A consensus in the field is that research efforts need to be allocated to the investigation of causes and risk factors for LBP, as prevention might be more achievable than cure.

Individuals' genetic susceptibility for LBP has been recently investigated as a contributing factor for back pain development. The study of genetic causes of a disease usually employs a familial or a twin design. In genetic epidemiology, twins are regarded as an 'experiment opportunity' because they allow the study of the role of genetics along with shared and non-shared environmental factors in the composition of a disease development. [7] Monozygotic twins (MZ) share all their genes and dizygotic twins (DZ) share, on average, half of their segregating genes. By analysing phenotypic (e.g. LBP) concordance rates across MZ and DZ twins a classic twin study enables the assessment of the contribution of genes and environment to phenotypic variability without direct mapping genes. [8] This method is valuable because the investigation of modifiable risk factors and their interactions with genetic components offers a more comprehensive approach in determining the attributable risk of the diseases than investigating genetics or lifestyle factors in isolation.[9]

Although twin and familial studies have been used to investigate the influence of genetic and environmental factors in LBP as a symptom [10,11] they are more commonly used in the study of genetic causes of spine degeneration, such as in intervertebral discs.[12] Additionally, a variety of individual lifestyle and environmental risk factors have been investigated in studies of the prevalence or risk of LBP. [13-19] In the context of LBP, the investigation of modifiable individual lifestyle or environmental risk factors is particularly appealing because of the paucity in effective preventative strategies. Unfortunately most of the original studies examining risk factors for LBP have limited applicability because they show some inherent methodological flaws.[20]

The most striking is the analysis of an isolated risk factor with no adjustment for additional or confounding risk factors. This is particularly common when risk factors such as alcohol consumption or smoking are investigated. [21,22] In this regard, twin studies could be a powerful method to study the role of 'nature and nurture' in the development or behavior of LBP particularly if a cluster of important lifestyle risk factors are considered, integrating genomic research with an epidemiological approach in a single well conducted study.

The Australian Twin Registry (ATR) was established in the late 1970s as a not-for-profit national volunteer registry organization of Australian twins with the aim of supporting medical and scientific studies which involve the participation of twins and/or their relatives. Currently, the ATR manages information of more than 30,000 twin pairs of all zygosity types and ages. [23] The ATR has supported over 450 twin research projects across a broad spectrum of medical conditions such as Alzheimer's disease and epilepsy. This impressive research initiative has resulted in more than 600 peer reviewed papers.[24] Currently, the ATR has 7 research projects recruiting participants in the fields of epilepsy, menopause, atrial fibrillation, pain disorders in childhood and invasive cervical cancer.

Research in collaboration with the ATR has been traditionally undertaken by the means of mailing questionnaires and live data collection. We chose to implement a web-based questionnaire as a pilot study given the potential for efficient data collection and processing. Previous clinical and epidemiological studies have showed the efficiency of web-based data collection, [25,26] however, to our knowledge, no research in LBP using twins has been conducted using web electronic resources.

The aim of this study will be to investigate the contribution of genetics and lifestyle factors on LBP related outcomes using Australian twins registered at the ATR using online data capture.

Methods

Electronic questionnaire and recruitment

A web-based structured questionnaire was developed following a recent consensus on the standardisation of LBP definitions for observational studies. [27] The questionnaire was based on participants' self-report with a recall period of one month and included questions regarding the prevalence of LBP, prevalence of chronic LBP (three months or more), LBP episode duration, limitation of daily activities because of LBP and pain intensity of the episodes (using a 0 to 10 scale). Demographic characteristics documented for this survey will include age, sex, and zygosity, which will be provided by the ATR. In order to investigate the association between lifestyle factors and LBP, data on alcohol consumption, smoking habits, daily time spent in a sitting position, and twins' involvement in physical activity such as light walking, moderate recreational physical activities (e.g. gentle swimming, social tennis, golf), vigorous recreational physical activity (e.g. jogging, cycling, aerobics, competitive tennis), and engagement in vigorous gardening or heavy work around the house will be collected. All questions related to physical activity will allow participants to report the total time spent doing the activity in the last week. [28] In total, the electronic survey comprised 21 questions.

Recruitment consists initially of an email approach, sent by the ATR, inviting twins to participate in the study. The email contains an electronic link directing twins to the web-based questionnaire, where twins are able to access the study information, consent form, and the options of accepting or declining participation. Twins will also be given the option to request a hard copy version of the questionnaire. A picture illustrating the electronic version of the survey is provided in Figure 1.

<<Figure 1>>

Completed questionnaires will be downloaded by the ATR team and reports sent to the study manager at scheduled intervals. A reminder email will be sent to twins who do not reply to the first survey invitation a month from the first email being delivered. After the launch of the second email wave the study manager will be able to

download responses from the ATR database. The recruitment process and twins' response will be monitored closely by study manager and the ATR team.

The ATR procedure

The survey was developed in collaboration with the ATR. The process consisted of several coordinated steps designed to assure that the study was in accordance with the methodology for partnership studies with the ATR. The ATR provided advice on sample availability, methods to approach twins, study costs, consent forms, follow-up approach, participant information sheets, and in the ethics' process. The proposal will also be peer-reviewed by external advisors. Once ethics and approval was granted from the ATR, the study was submitted and granted ethics from the University of Sydney Human Research Ethics Committee. A flowchart describing the ATR process is illustrated in Figure 2.

<<Figure 2>>

Participants

Twins living in all Australian states, aged 18 to 65 years old with available email address will be included in the study.

Data Analysis

The second phase of the study will comprise the analysis process which will be implemented in collaboration with the ATR. The analyses are designed to i) explore the relative genetic and environmental contribution on the different LBP outcomes, e.g., one-month prevalence of LBP, chronic LBP, limitation associated to LBP, frequency and pain intensity using a classical biometrial twin study; ii) investigate the association between lifestyle factors and LBP outcomes using a co-twin control design; and iii) explore possible causation paths between lifestyle factors and LBP outcomes using a novel twin regression approach developed by ATR researchers. [29]

For the analysis of the influence of genetics and environment on LBP outcomes we will use a classical model of twin resemblance correlation for heritability estimation. [30,31] The correlations for LBP will be expressed as correlations for dichotomous

outcome and a higher phenotypic similarity in MZ twins than in DZ twins is expected if there is a significant genetic component in the causes of the disease.[31]

To investigate the association between lifestyle factors and LBP we will use all complete monozygotic and dizygotic twin pairs discordant for the LBP outcome (twin pairs in which one twin reported suffering LBP and the co-twin reported no LBP) as matched pairs. The co-twin control design will be used to analyse the effect of lifestyles factors on the prevalence of LBP. [31] Conditional logistic regression and ORs with 95% confidence intervals (CIs) will be calculated from individual models for each variable adjusted for sex. Similar analysis will be carried out for the different LPB outcomes, e.g., chronic LBP, limitation associated to LBP and frequency of pain events.

In order to explore possible causation paths between lifestyle factors and LBP data will be analysed using a novel statistical approach developed by researchers from the ATR (named 'Inference on Causation from Elimination of Familial Confounding' – ICE FALCON).[29] The basis of this novel analysis is that evidence of a causal association between an environmental factor and a disease risk is provided when an interaction between the environmental factor and the relevant genotype is documented. [32] It is derived from classical regression models aiming to study the association between the outcome of a twin (e.g. LBP) with their exposure status (e.g. individual level of fitness) and the exposure status of his/her twin considered.

Four regression models will be used to analyse the data. The first two models consider the twin and co-twin's exposures (for example individual level of fitness) separately. In model I the association between the twin's exposure and his/her outcome (LBP) is estimated in isolation. In model II the association between the co-twin's exposure and the twin's outcome is estimated in isolation. In model III the association between the twin's exposure and his/her outcome is estimated along with the inclusion of the association between the co-twin's exposure and the twin's outcome. Model IV is similar to model III but in this instance the association of the co-twin is allowed to depend on his/her zigosity, that is, estimates are produced separately for mono (identical) and dizigotic (non identical) twins. If a twin's exposure

is associated with his/her outcome in isolation (model I) and the co-twin's exposure is associated with a twin's outcome in isolation (model II) but this association is attenuated when the association of both twins are analysed together (model III) then the data are consistent with a direct causal effect of the twin's exposure on his/her outcome. If the association between the co-twin's exposure and the twin's outcome is higher in identical twins (model IV) then the data are consistent with this association being driven by genetic factors. Figure 3 shows the path model for this statistical approach.

<<Figure 3>>

Discussion

This study will be the first one in the LBP field using an online approach and a twin design. It will provide estimates of the prevalence of LBP and related outcomes such as pain intensity, duration of symptoms, limitation associated with LBP with genetics and potentially modifiable lifestyle factors.

The hypothesis of the genetic composition of LBP lies on evidences of genetic control of pain perception, signaling and psychological processes.[33,34] The expression of inflammatory cytokines and nerve growth factor may also be associated with LBP in different control levels.[35-38] While the knowledge of the genetic mechanisms influencing the genotypic expression of LBP needs further research, it can be hypothesized that these mechanisms are determined by low penetrance gene variants and, therefore, the use of twin studies offers an important advantage over traditional risk factors studies in investigating the role of gene-environment interaction in the etiology of this condition.

We are collecting data on a range of important lifestyle risk factors such as smoking, alcohol consumption, and physical activity combined in single study. A unique feature of this study is the potential to identify a causation path between risk factors and LBP by using an innovative twin analysis that permits insights into whether the data are consistent with a measured exposure having an influence on an outcome, and/or whether this association is generated by other factors that are shared by twin pairs

that influence both the exposure and the outcome. We believe that by identifying potential modifiable lifestyle risk factors this information will be invaluable in designing future prevention strategies for LBP.

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Figures

Figure 1: Electronic version of the survey.

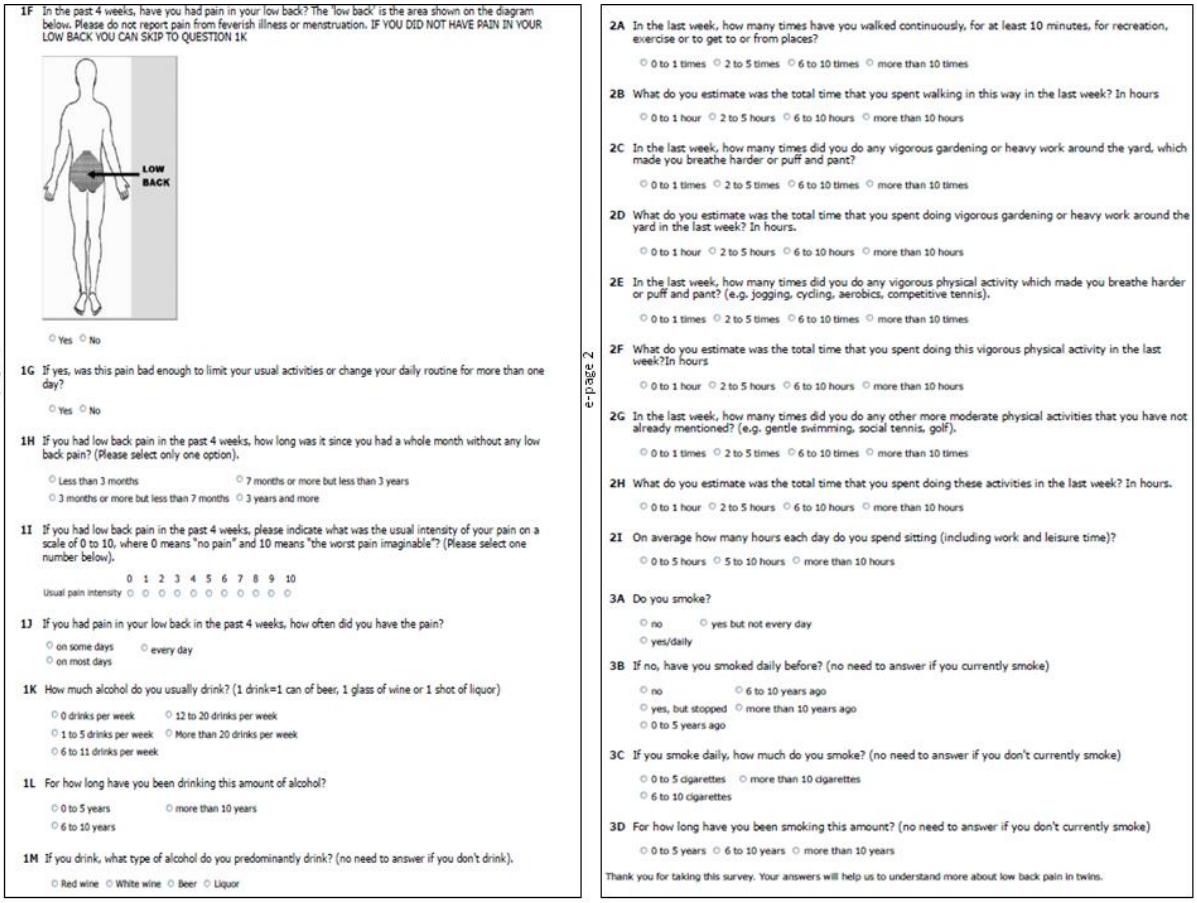


Figure 2: Flowchart showing the sequential procedures involved in the study implementation in collaboration with the Australian Twin Registry.

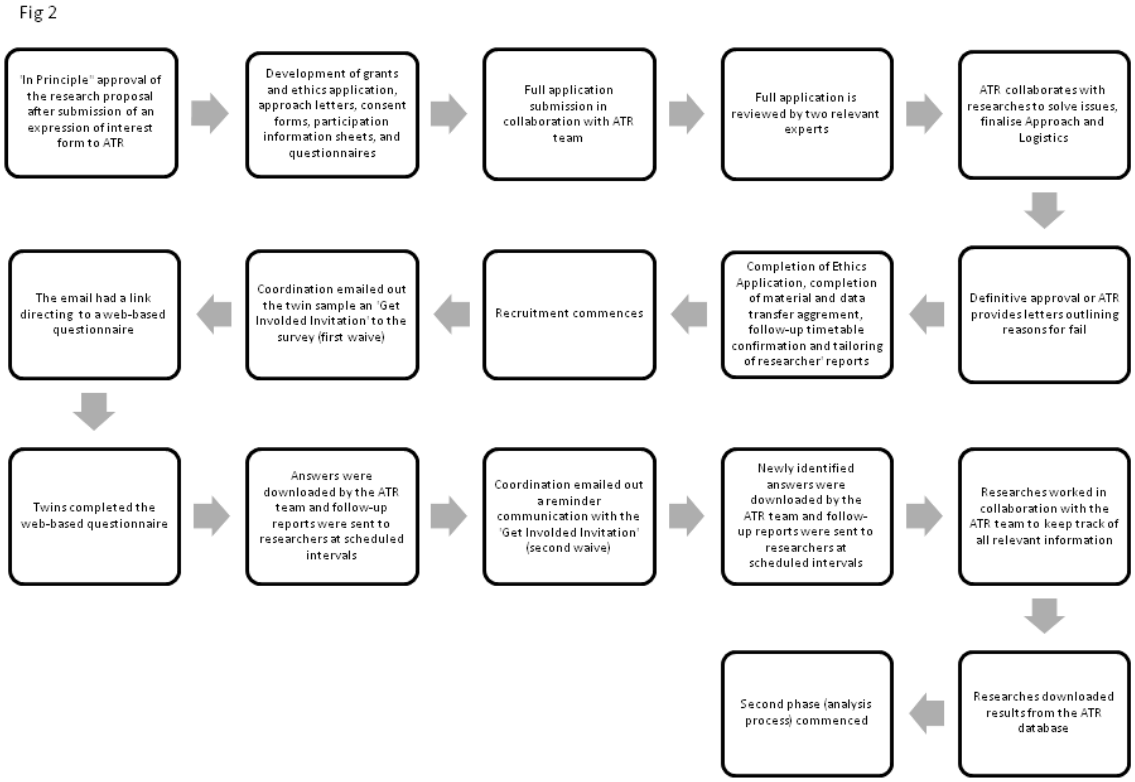
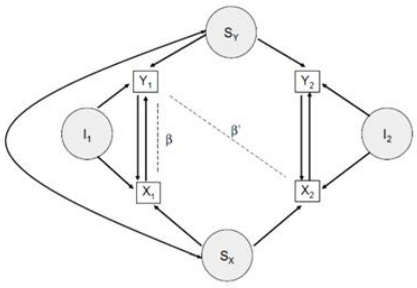


Figure 3: Path model illustrating the ICE FALCON analysis.

Fig 3



Legend: A path diagram showing the outcome variable (low back pain, Yi, and the corresponding causal exposures, X_i, e.g. physical fitness for each twin i = 1,2 in a pair); circles, potential causes (trait-specific causes common to or shared by twins, S_Y and S_X, and individual-specific between-trait confounders, I_1 and I_2). The single-headed arrow indicates the presumed direction of causation, whereas a double-headed arrow indicates that the causes are correlated. Dotted lines, the regression coefficients representing the associations on Y_1 of X_1 (β) and X_2 (β'). Adapted from Dite et al.[29]

7.2 Hereditariedade e fatores de risco de estilo de vida na determinação da dor lombar crônica

Esse artigo apresenta os resultados das primeiras análises realizadas com os dados coletados no estudo AUTBACK. Os resultados demonstram uma elevada influência genética na dor lombar crônica (~40%) e de uma associação, ainda que exploratória, entre atividades físicas leves e vigorosas com a dor lombar crônica. Essa relação não linear (em forma de U) entre atividade física e dor lombar é consistente com observações de outros estudos em outras populações e consiste de um interessante achado a ser melhor compreendido. Uma associação independente entre dor lombar crônica e a quantidade de tempo dedicada à prática de jardinagem e trabalho pesado ao redor da casa foi encontrada. Apesar de ser uma associação derivada de um estudo transversal, o valor da associação (*odds ratio*) foi elevado e, portanto, a realização de outros estudos com foco nessa atividade merece esforços. As atividades de jardinagem e trabalho ao redor da casa destacam-se ainda como um hábito população-específica (populações mais restritas a moradias verticais provavelmente apresentam níveis muito baixos desse tipo de atividade) e indicam a necessidade de que o estudo dos fatores de risco de estilo de vida da dor lombar seja realizado com foco nos hábitos e costumes de diferentes países.

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Heritability and lifestyle factors in chronic low back pain: results of the Australian Twin Low Back Pain Study (The AUTBACK study)

Running head: Genetic and lifestyle influences in chronic low back pain

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Conflict of Interest Statement

The authors state they have no conflict of interest to report.

What's already known about this topic?

- The effects of genetics in chronic low back pain rather than any low back pain have not been investigated in depth.
- The effects of lifestyle risk factors such as physical activity in chronic low back pain are contradictory.

What does this study add?

- Genetics have a major contribution in the prevalence of chronic low back pain.
- Domestic physical workload but not leisure physical activity has an important contribution to chronic low back pain.

Abstract

Background: Heritability and population-specific lifestyle factors are considered to significantly contribute to chronic low back pain (LBP) but traditional population studies fail to adjust for genetics and to use standard and validated definitions for LBP and for lifestyle factors. **Methods**: Using a classical and a co-twin control study design and validated definitions for chronic LBP and lifestyle variables, we explored the relative contribution of genetics and environment on the prevalence of chronic LBP in a sample of adult Australian twins. **Results:** Data from 105 twin pairs showed that the prevalence of chronic LBP is significantly determined by genetic factors (heritability=40%). Additionally, monozygotic twins were five times more likely to have chronic LBP than dizygotic twins when one of the siblings of the pair was affected. In a case-control analysis (n=38 twin pairs), an exploratory analysis showed higher prevalence of chronic LBP associated with light walking exercises and vigorous gardening or heavy work around the house. Daily time spent in sitting was also positively associated with chronic LBP but not moderate physical activities such as jogging, cycling, and gentle swimming. In the final multivariate model only time spent in vigorous gardening or heavy work around the house remained associated with chronic LBP (OR 6.5; 95%CI 1.47-28.8). **Conclusions**: The type, frequency, and duration of physical activity are important to understand contributing and risk factors for chronic LBP. The causation path between chronic LBP and people's engagement in activities involving frequent bending and twisting such as gardening and housework should be further investigated.

Key words: low back pain, risk factors, twin study

Introduction

The mean one-month prevalence of low back pain (LBP) is estimated at 29.1% (Hoy et al., 2012) with the direct health care costs estimated at A$1 billion per annum in Australia and A$50 billion per annum in United States (Walker et al., 2003). Recurrence is a significant characteristic of LBP (Stanton et al., 2008; Costa et al., 2009) and although interventions appear effective for reducing pain in acute LBP, treatments for chronic LBP fail to reduce pain and associated disability (Davies et al., 2008; Roelofs et al., 2008; Urquhart et al., 2008). Chronic and recurrent spinal pain may also lead to irrational use of drugs and invasive procedures such as spinal surgery. Since treatment offers only moderate effects, understanding risk factors for chronic LBP is crucial for effective prevention.

Genetics has been found to play a major role in a variety of pain conditions such as growing pain, widespread pain, migraine, and fibromyalgia (Violon, 1985; Campo et al., 2004; Roth-Isigkeit et al., 2005; Mikkelsson et al., 2008). Using a twin study design, it is possible to assess the contribution of genes and shared environment to phenotypic variability without directly measuring those factors by analyzing a phenotypic trait (e.g. LBP) across monozygotic (MZ) and dizygotic (DZ) twins (Hopper, 1992; Martin et al., 1997). Twin studies are also a powerful method to study the role of environmental risk factors taking into account the confounding effect of age and genetics.

Recently, genetics has been investigated as a contributing factor for LBP development and a significant genetic effect has been shown to be a component factor of chronic LBP (Hartvigsen and Christensen, 2007; Hartvigsen et al., 2009). Results from twin studies have also shown that twins' engagement in strenuous physical activity is protective for future episodes of LBP whilst lifestyle factors such as smoking and obesity, as well as lower levels of self-rated health and the presence of co-morbidities, are associated with higher prevalence of LBP (Hartvigsen et al., 2004; Hestbaek et al., 2004; Hartvigsen and Christensen, 2007). However, the majority of these data come from specific populations with LBP, such as older people or teenagers and definitions for LBP and the recall period of the outcome vary widely between studies.

A recent systematic review of twin studies in LBP (Ferreira et al., 2012) observed stronger associations and presence of dose-response relationships for risk factor related to long lasting or chronic LBP rather than “any” LBP. Additionally, genetics appears to have a stronger effect in highly disabling LBP presenting with a genetic component of 46% while for prevalence of “any” LBP the genetic influence was 27%.

We aimed to explore the contribution of genetic and environmental factors on the prevalence of chronic LBP in a sample of Australian adult twins and to investigate the association between a combination of lifestyle factors and chronic LBP using a classical biometrical twin model and a co-twin case-control design. We used comprehensive measures of physical activity in contrast to crude measures used in traditional twin studies.

Methods

Study Sample and Data Collection

This study is part of the AUstralian Twin low BACK pain (AUTBACK) study, an observational study with the main aim of investigating genetic and lifestyle risk factors for LBP. The AUTBAK study recruited participants from The Australian Twin Registry (ATR), a community-based volunteer twin register of Australian twins which manages information of more than 30,000 twin pairs of all zygosity types and ages (Hopper et al., 2006).The study population was all Australian twins aged 18 to 65 years with available email address registered at the ATR and who gave consent to participate in the web-based AUTBACK study. No exclusion criteria were applied. From January 2009 to December 2010, twins were invited to participate in the survey by means of an email approach sent by the ATR. The email contained an electronic link directing participants to the web-based questionnaire where they were able to access the study information sheet, consent form, and the option for choosing to participate. The study was approved by the University of Sydney Human Research Ethics Committee and the ATR.

Variables

Demographic characteristics including age, sex, and zygosity were provided by the ATR. The main outcome investigated in the survey was the occurrence of LBP, assessed as a dichotomous variable and based on self-report with a recall period of 4 weeks (one month). LBP definition followed a recent consensus on the standardisation of LBP definitions for observational studies (Dionne et al., 2008). The self-report of chronic LBP was the primary outcome for this study and was investigated by asking participants the following specific question: "If you had low back pain in the last 4 weeks, how long was it since you had a whole month without any low back pain?" Twins who stated they had a whole month without LBP longer than 3 months before completing the survey were considered chronic LBP participants.

Lifestyle risk factors included general factors such as current alcohol consumption and smoking habits, as well as more LBP specific factors such as daily time spent in a sitting position and engagement in recreational and work-related physical activity.

Alcohol consumption and smoking habits were collected as continuous variables (amount of alcohol intake or number of cigarettes smoked per week). Information regarding time spent in a sitting position was appraised as amount of hours on an average day.

Twins' engagement in physical activity was assessed by the Active Australia survey, a validated tool to assess people's engagement in physical activity in Australia (Australia, 1999; Bauman et al., 2002; Brown et al., 2004). This questionnaire included questions regarding twins' engagement in physical activity in the past week prior to completing the survey such as: a) light walking (continuous walking for 10 minutes for recreation, exercise or get to places); b) moderate recreational physical activities (e.g. gentle swimming, social tennis, golf); c) vigorous recreational physical activities (e.g. jogging, cycling, aerobics, competitive tennis); d) vigorous gardening or heavy work around the house (Brown et al., 2004). The intensity of participants' engagement in physical activity was evaluated by asking about the current frequency of practice of the specified activity in a week and the total hours spent on them.

Analysis

The twin cohort was demographically described in terms of proportions, means and standard deviations where applicable. Chronic LBP and lifestyle variables were described according to twins' zygosity status. Differences between MZ and DZ twins for percentage values for demographic and lifestyle variables were assessed using unpaired t-tests and z-tests whenever appropriate.

For analysis purposes, all variables related to lifestyle risk factors were dichotomized. Alcohol consumption and smoking were categorised in no alcohol consumption or smoking and compared to all exposure levels grouped. Time spent in sitting was stratified as "0 to 5 hours" or "more than 5 hours". The intensify of physical activity was analysed as "yes" or "no" for the engagement in each physical activity investigated and into "no practice" or "2 hours or more of practice per week" for the total time spent in each activity.

Using complete pairs (both twins in a pair answered the survey) irrespective of concordance or discordance of LBP, we explored the relative genetic and environmental contribution on chronic LBP using a classical model of twin resemblance correlation for heritability estimation (Neale and Cardon, 1992; Boomsma et al., 2002). A higher phenotypic similarity in MZ twins than in DZ twins is expected if there is a significant genetic component in the cause of the disease. Also, a logistic regression model was used to estimate the association between chronic LBP and a pair of individuals (Bonney, 1986; Hopper, 1993). This logistical model was stratified by zygosity to calculate the proportional increase in the disease chance of an individual given that the co-twin member of the pair was a MZ or DZ affected twin. A stronger disease chance for a MZ twin with an affected sibling than for a twin of an affected DZ twin pair is expected if there is significant genetic component acting in the causation path of chronic LBP.

A subsequent co-twin case-control model was employed using complete discordant pairs for chronic LBP (i.e., one twin reporting chronic LBP and the co-twin reporting no LBP in the previous month) as matched pairs in a model fitted to analyse the association between lifestyle factors and chronic LBP (Boomsma et al., 2002). A conditional logistic regression was used to estimate ORs with 95% confidence

intervals (CIs) for each variable. We used an exploratory univariate analysis (with p-value ≤0.2) to detect trends in relationships between the variables and the outcome. Finally, all variables associated with the outcome in the exploratory analysis were then incorporated in a multivariate model using a stepwise regression process until a final model was fitted. Significant statistical associations in the final model were accepted at a significance level of 0.05. All data analyses were performed using Stata Statistical Software: Release 12 (College Station, TX: StataCorp LP).

Results

We invited, by means of a web-based approach, 888 twins with available email address registered at the ATR. Four hundred and eighty six twins (243 pairs) responded to the questionnaire, accounting for an overall response rate of 54.7%. Data on chronic LBP from complete twin pairs (both twins responded, irrespective of concordance or discordance to LBP outcomes) were available from 105 pairs and were used for the heritability estimation analysis. A total of 38 twin pairs were discordant for chronic LBP and were used for the analysis of association between lifestyle factors and chronic LBP.

The overall prevalence of chronic LBP was 59.5%, with no evidence of difference in this prevalence between MZ and DZ twins (63.1% and 54.6% respectively; p= 0.212). Mean age of twins was 40.2 (SD=11.5) with MZ twins (mean=42.2; SD=11.0) being significantly older (p=0.003) than DZ twins (mean=37.5; SD= 11.8). Female participants accounted for 57.6% of the sample, with no difference in female proportion between MZ and DZ twins (p=0.252). Regarding the lifestyle factors investigated, more than half of the participants reported spending more than 5 hours sitting during one typical day (61.7%) and the majority of participants did not smoke (91.4%) and reported not consuming alcohol (70.0%). In general, twins tended to be more involved in light walking or in vigorous recreational physical activities (Table 1). Of note, MZ and DZ twins were similar for all lifestyle characteristics (Figure 1).

<<Table 1 approximately here>>

<<Figure 1 approximately here>>

Resemblance analysis of twin pairs investigating the relative genetic and environmental contribution on chronic LBP estimated concordant rates (r) of 0.35 (p-value= 0.0004) for MZ twins and of 0.15 (p-value = 0.33) for DZ twins. The calculated heritability factor was 40.0%. The estimated disease chance (OR) of an individual given that the co-twin member of the pair was an affected twin was 5.19 (95% CI 2.0-13.6) for MZ twins and 0.6 (95% CI 0.17-1.8) for DZ twins.

In the co-twin case-control exploratory analysis (n=38 twin pairs), we found a positive association between chronic LBP with low levels of physical activity (light walking and total time spent walking in a week) and with more strenuous activities (vigorous gardening or heavy work around the house and total time spent in this activity in a week), but not with moderately demanding physic activities such as gentle swimming, social tennis, golf, jogging, cycling, aerobics, competitive tennis). Additionally, more than 5 hours spent in sitting were found to be associated with chronic LBP (Table 2). The final multivariate model showed that the only variable that remained associated with chronic LBP, considering a significance level of 0.05, was the time spent in vigorous gardening or heavy work around the house (OR 6.5; 95%CI 1.47-28.8).

<<Table 2 approximately here>>

Discussion

Twin studies are a powerful method to study the role of environmental risk factors on the development of a number of different multifactorial diseases such as cardiovascular, neurological, malignant and others, since confounding effects of age and genetics are controlled for. Our study was designed to investigate the relationship of genetic and environmental factors with LBP in a cross-sectional survey of Australian twins. The study population was a sample of Australian twins who responded to a web-based questionnaire. Of note, this was the first fully web-based study conducted in collaboration with the ATR. Therefore, information regarding email address was available for a limited number of registered twins at the commencement of the study. Nevertheless, the achieved response rate enabled the construction of a sample where a cluster of LBP specific factors could be assessed in a single study in contrast with previous twin studies that tended to investigate factors in isolation (Hestbaek et al., 2006a; Hartvigsen and Christensen, 2007; Wright et al.,

2010). Our case-control analysis also allowed us to control for the influence of genetics and age in the investigation of factors associated with chronic LBP. We attempted to use contemporary validated definitions of LBP which could allow future comparison of data with other samples.

The results of our study showed a high prevalence of chronic LBP in a sample comprising middle-age adult twins with equal proportion of MZ and DZ twin pairs. The prevalence of chronic LBP in this study (59.5%) was higher than in other studies (prevalence ranging from 10.3% to 29.9% in a recent systematic review (Hoy et al., 2012)). However, comparisons with other study populations are difficult because the definition of back pain outcomes, including chronic LBP, and the recall period vary substantially among studies (Dionne et al., 2006; Dionne et al., 2008; Hoy et al., 2012). Comparison with other twin studies is also difficult since these studies usually define chronic LBP as symptoms lasting longer than 30 days within a recall period of one year, with the majority of studies focusing on specific age populations (Hestbaek et al., 2006a; Hestbaek et al., 2006b; Hartvigsen and Christensen, 2007; Hestbaek et al., 2008). One twin study defining chronic LBP as pain being experienced for at least 3 months within a recall period of 3 months showed a prevalence considerably lower than in our study (26.2%) (Wright et al., 2010). Although the prevalence of chronic LBP found in our study was similar to other study populations such as primary care patients (47.8%) (Gureje et al., 1998), we cannot rule out participant selection bias in our study given that twins suffering from chronic LBP would be more likely to participate in this study and the prevalence estimated needs to be interpreted with caution. Nevertheless, we demonstrated that both cohorts of MZ and DZ twins were similar for all variables under investigation, thus reducing the likelihood of selection bias based on the exposure variables.

We have shown that the prevalence of chronic LBP is significantly determined by genetic factors in men and women as the heritability factor was estimated as 40%. This estimated contribution of genetics to chronic LBP is similar to other high genetic influenced conditions such as autism (36%), the development of dependence on alcohol and other drugs (55%), and depression (42%) (Boomsma et al., 2002). Despite differences in LBP definitions between twin studies, our findings are similar

to studies that investigated the influence of genetics in LBP lasting >30 days out of the past year (heritability=32%) (Hartvigsen et al., 2009) and highly disabling LBP (heritability=57% (MacGregor et al., 2004) and 39% (Battie et al., 2007)). The analysis stratified by zygosity confirmed the contribution of genetics to chronic LBP since an increase in disease chance has been demonstrated for a MZ twin with an affected sibling compared to a DZ twin. This increased disease chance related to genetic similarity (MZ twins) shows that inheritance plays a significant role in the development of chronic LBP.

The mechanism underpinning the contribution of genetics to chronic LBP is intriguing. Hypotheses for the mechanisms of genetics in pain include the control of the expression of inflammatory cytokines such as interleukin-1, interleukin-6, and tumor necrosis factor-α (TNF). These cytokines have been linked to spinal disc degeneration and LBP and levels of their expression could be partially driven by genetics (Battie et al., 2007; Wang et al., 2008; Kraychete et al., 2010). Other experimental research suggests that genetic dependent nerve growth factor extracted from degenerative nucleus pulposus might have a role in pain transmission, because nerve growth factor promotes axonal growth and induces substance P production (Yamauchi et al., 2009). Genes related to pain perception, pain signalling and the psychological process involved in pain may also play a role in chronic LPB. Indeed, dysfunction in endogenous opioid activity (Bruehl et al., 2002) and several psychological factors such as alexithymia, coping and fear avoidance, have been related to chronic LBP (Pincus et al., 2002; Mehling and Krause, 2005).

The relationship between LPB and lifestyle risk factors such as smoking and alcohol intake have been explored in previous observational studies although they tend to be investigated in isolation in individual studies with no adjustment for potential confounders. A consistent association between alcohol intake and smoking with LBP has not been demonstrated in previous studies (Goldberg et al., 2000; Leboeuf-Yde, 2000). Our study did not show any association between alcohol intake and smoking habits with chronic LBP, although the majority of our recruited sample of Australian twins adopted a healthy lifestyle: the majority were engaged in low or moderate

levels of physical activity (>80%), were non-smokers (91.5%) and did not consume alcohol (70%).

The influence of physical activity on the prevalence of LBP remains an issue that is unresolved due to contradictory results from past research. Some studies have reported that physical activity may be protective whereas others have reported that physical activity increases the risk of developing LBP. This seeming paradox may be explained by the results of Heneweer and colleagues study (Heneweer et al., 2009) that demonstrated that both low and high activity levels were associated with chronic LBP (OR=1.31 and 1.22 respectively) with the authors suggesting that the relationship between physical activity and chronic LBP follows a U-shaped curve. In our study, an exploratory univariate analysis showed a positive association between chronic LBP and less demanding exercises (e.g.: recreational walking) or more laborious one (vigorous gardening or heavy work around the house). Higher frequencies of these activities in a week were also associated with higher prevalence of chronic LBP. These results reinforce the importance of detailing a spectrum of types, frequencies, and possibly population specific physical activities in studies investigating this lifestyle factor and LBP.

The only variable that remained associated with chronic LBP in the final multivariate regression model was time spent in vigorous gardening or heavy work around the house with a constant and strong association (OR=6.5). The frequency of manual handling, lifting and carrying involved in vigorous gardening and heavy work around the house makes it tempting to assume that this relationship is causal, that is, twins' engagement in vigorous gardening and heavy work around the house precedes and causes chronic LBP. However, the design of our study does not allow for such inferences. Alternatively, it is possible that twins could engage more frequently in physical activity as a self-management strategy for their chronic LBP and the causal relationship may actually be in the opposite direction. However, if the latter were true then it is likely that twins would also be more engaged in moderate or vigorous recreational physical activities and the lack of association between these variables and chronic LBP found in our study does not support this causation path.

Our research group is currently conducting a large observational study using a case-crossover design to investigate physical activity triggers for LBP and it is likely that the results of that study will shade some light into the causation relationship between physical activity and LBP (Steffens et al., 2012).

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Authors Contribution

Daniela Junqueira planned, analysed and interpreted data, and drafted the manuscript.

Manuela Ferreira designed the study, planned analysis, and edited the manuscript.

Kathryn Refshauge, Christopher Maher and John Hopper made substantial contributions to the study's conception, analysis and design.

Mark Hancock and Maria das Graças Carvalho gave expert advises for data analysis and the manuscript edition.

Paulo Ferreira designed and managed the study, planned analysis, and drafted the manuscript together with Daniela Junqueira.

All authors approved the final manuscript.

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Legends for illustrations

Table 1: Twins' engagement in physical activity stratified by zygosity.

Physical activity	**Yes**	
	MZ (%)	**DZ (%)**
Light walking	82.0	83.0
Vigorous recreational physical activity	55.7	50.0
Moderate recreational physical activity	16.4	15.9
Vigorous gardening or heavy work around the house	26.2	35.6
Total time spent in each activity per week	**2 hours or more per week**	
Light walking	68.0	71.3
Vigorous recreational physical activity	50.4	52.3
Moderate recreational physical activity	26.2	20.5
Vigorous gardening or heavy work around the house	27.9	37.2

Figure 1: Similarity between monozygotic and dizygotic twins for all investigated lifestyle variables.

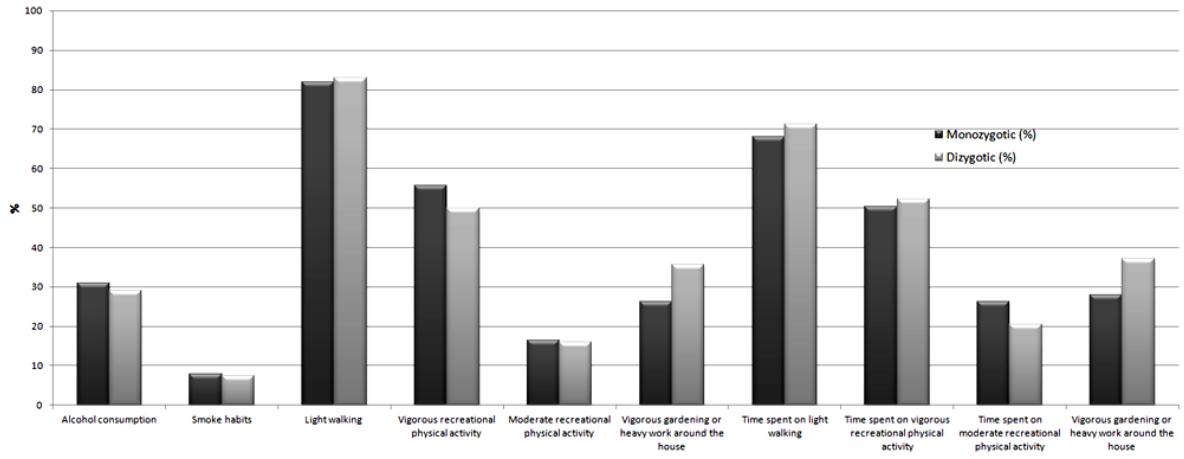

Table 2: Odds ratios (OR) for the co-twin case-control analysis including twins discordant for chronic LBP.

	Odds Ratio	95% CI
Exploratory Conditional Regression		
Alcohol consumption	0.7	0.19; 2.37
Smoke habits	0.7	0.11; 3.99
Total daily time spent in the sitting position	2.0	0.68; 5.85^
Vigorous physical activities	1.0	0.35; 2.86
Total time spent in vigorous activities	0.9	0.29; 2.55
Moderate physical activities	0.6	0.14; 2.52
Total time spent in moderate physical activities	1.0	0.37; 2.67
Light walking	3.0	0.61; 14.86^
Total time spent in light walking	2.4	0.85; 6.81^
Vigorous gardening or heavy work around the house	6.0	1.34; 26.81*
Total time spent in vigorous gardening or heavy work around the house	6.5	1.47; 28.81*
Multivariate Conditional Regression – Final Model		
Total time spent on vigorous gardening or heavy work around the house	6.5	1.47; 28.80*

^ p-value<0.2

* p-value<0.05

8 OUTRAS PUBLICAÇÕES RELEVANTES

Partes do trabalho apresentado nessa tese foram também publicados em periódicos, congressos e simpósios científicos, como sumarizado a seguir:

8.1 Publicações em periódicos

Junqueira, D.R.G. Perini, E. **Unfractionated heparin versus low molecular weight heparin for avoiding heparin-induced thrombocytopenia in postoperative patients**. Cochrane Database of Systematic Reviews (Online), v. 1, p. CD007557, 2009.

8.2 Apresentações em congressos com publicação no Annais

Pinto, I.V.L. Junqueira, D.R.G. Mendes, D.P. Perini, E. Carvalho, M.G. **Trombocitopenia induzida por heparina: relato de caso**. In: VII Congresso Brasileiro de farmácia Hospitalar, 2009, Belo Horizonte. VII Congresso Brasileiro de Farmácia Hospitalar, 2009.

Barros, F.C.R. Junqueira, D.R.G. Perini, E. Peixoto, E.R.M.. Carvalho, M.G. Viana, T.G. **Origem das preaparações de heparina no mercado brasileiro e o risco de trombocitopenia induzida por heparina**. In: XX Congresso Pan-Americano de Farmácia, 2010, Porto Alegre. Trabalhos do XX Congresso Pan-Americano de Farmácia, 2010.

Peixoto, E.R.M. Junqueira, D.R.G. Perini, E. Barros, F.C.R. Carvalho, M.G. Viana, T.G. **Intercambialidade de heparina subcutânea e intravenosa**. In: XX Congresso Pan-Americano de Farmácia, 2010, Porto Alegre. Trabalhos do XX Congresso Pan-Americano de Farmácia, 2010.

Junqueira, D.R.G. Carvalho, M.G. Perini, E. U**sing systematic reviews to study adverse drug reactions: the example of heparin-induced thrombocytopenia**. In: 19th Cochrane Colloquium, 2011, Madrid. Cochrane Colloquium Abstracts Journal, 2011.

9 CONSIDERAÇÕES FINAIS

Essa tese abordou aspectos interdisciplinares das intervenções farmacológicas, discutindo questões farmacoepidemiológicas da segurança da utilização de medicamentos em dois contextos clínicos e apresentando soluções metodológicas para o estudo de assuntos com potencial impacto no manejo da trombocitopenia induzida por heparina e da dor lombar.

A produção de conhecimentos sobre a eficácia e segurança de medicamentos tem preconizado cada vez mais a qualidade das evidências científicas e a aplicação de diferentes estratégias de refinado desenho epidemiológico para o estudo dessas questões. Tais conhecimentos representam um avanço na formação de recursos humanos e na promoção do uso racional e seguro dos medicamentos. Adicionalmente à pesquisa em intervenções terapêuticas, o estudo de fatores de risco é essencial para o avanço da ciência biomédica e, novamente, a aplicação de métodos refinados e inovadores apoiam a busca por evidências científicas de qualidade para a prática clínica e para a expansão das atividades científicas do país. Considerando essas questões, duas linhas de pesquisa se estruturaram ao longo do desenvolvimento dos estudos integrantes dessa tese: *Epidemiologia das reações adversas a medicamentos* e *Epidemiologia genética e o estudo de gêmeos* (Figura 2).

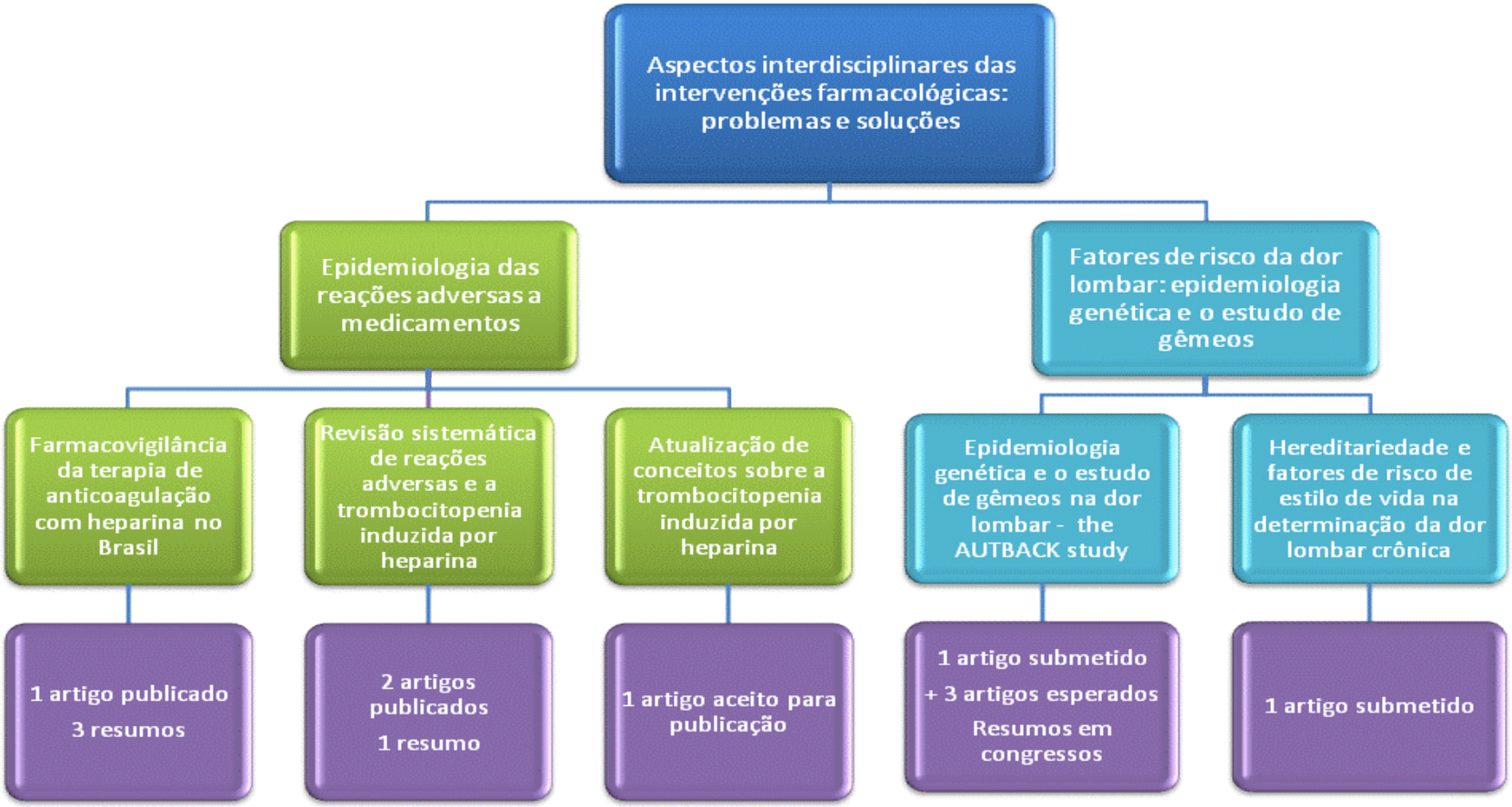

Figura 1 - Fluxograma ilustrando as linhas de pesquisa constituintes da tese intitulada: "Desafios metodológicos em epidemiologia: uma abordagem com foco na reação adversa da trombocitopenia induzida por heparina e na condição clínica da dor lombar.

Os estudos desenvolvidos dentro da linha investigativa em *Epidemiologia das reações adversas a medicamentos* demonstraram que, para proporcionar segurança na utilização de medicamentos, é necessário, inicialmente, contínua atenção a diferentes características dos fármacos e das populações que possam impactar o perfil de reações adversas a medicamentos. Nesse sentido, o artigo sobre a farmacovigilância das heparinas no Brasil contribui com informações a respeito das peculiaridades do mercado brasileiro desse anticoagulante e representa importante fonte para a tomada de decisões em saúde. Além disso, a segurança no uso de medicamentos deve se respaldar em estudos de qualidade em todas as fases de vida de um fármaco e, portanto, as reações adversas devem ser objeto de atenção tanto de ensaios clínicos realizados na fase de pré-comercialização quanto de estudos experimentais e observacionais conduzidos na fase de farmacovigilância. Em especial, os ensaios clínicos controlados e aleatorizados consistem do delineamento metodológico padrão para a comprovação da eficácia e segurança dos fármacos, sendo inclusive a base das atividades de regulamentação da comercialização de medicamentos. A revisão sistemática de ensaios clínicos controlados e randomizados sobre o risco de trombocitopenia induzida por heparina demonstra, no entanto, escassez de estudos dessa natureza sobre essa reação adversa. Considerando a importância do evento, esse resultado é um alerta para profissionais de saúde e para a comunidade científica sobre a lacuna existente em diversos ensaios clínicos na avaliação da segurança dos fármacos.

Considerado-se que a heparina é dos medicamentos mais utilizados em todo o mundo e que a trombocitopenia induzida por heparina é uma reação adversa a esse medicamento que oferece risco de vida, a escassez de ensaios clínicos controlados e randomizados incluindo a trombocitopenia induzida por heparina como desfecho, ainda que secundário, é surpreendente. O estudo demonstra ainda que estimativas de incidência dessa reação podem estar subvalorizadas, e que, com base nos ensaios clínicos identificados e incluídos na revisão sistemática, o evento pode se apresentar em subgrupos de expostos com risco superior àquele no qual estão baseados os protocolos clínicos da área. Em conjunto, os resultados apontam lacunas importantes no estudo e vigilância da segurança desse medicamento. Finalmente, os principais conhecimentos produzidos nessa linha de pesquisa foram

organizados numa revisão de conceitos com o objetivo de ser publicada em um periódico científico de circulação nacional. A produção de informação para profissionais de saúde que se encontram no ponto de cuidado ao paciente é atividade essencial para o desenvolvimento de um sistema de promoção do uso racional e seguro dos medicamentos.

Os estudos desenvolvidos no âmbito da linha de pesquisa em *Epidemiologia genética e o estudo de gêmeos* se inserem num grande projeto de pesquisa intitulado estudo AUTBACK (sigla originária do inglês *Australian Twin Low Back Pain study*). O projeto foi implementado utilizando gêmeos australianos e representa um avanço nos estudos dos determinantes da dor lombar, apresentando alto potencial de impactar os conhecimentos sobre esse problema de saúde. O conceito e as alternativas metodológicas que apoiaram o desenvolvimento desse projeto foram organizados e submetidos para publicação, dada a originalidade da proposta e das análises planejadas. Em especial, uma análise estatística inovadora do estudo de gêmeos, capaz de gerar inferências sobre causalidades a partir de dados transversais, será usada para identificar o papel de fatores de risco genéticos e de estilo de vida nos componentes da dor lombar. Os primeiros resultados desse projeto estão apresentados no artigo que discute a influência da hereditariedade e dos fatores ambientais na dor lombar crônica. Uma série de artigos está ainda em produção abordando outros resultados do projeto, os quais, em conjunto, delimitarão questões importantes para a compreensão dos mecanismos da dor lombar aguda e crônica.

Ambas as linhas de pesquisa apresentam perspectivas de estudos futuros e, certamente, uma contínua produção de conhecimentos deverá conferir maior impacto científico a essas linhas de investigação. Acreditamos também que os artigos produzidos apresentam potencial para contribuir positivamente para as respectivas áreas de conhecimentos de cada artigo no sentido de delinear novos protocolos clínicos e estratégias de saúde pública.

O desenvolvimento e aplicação de métodos refinados e originais permitiu o estabelecimento de parcerias internacionais, atributo marcante no desenvolvimento

dessa tese. Os resultados dos estudos referentes a linha de *Epidemiologia das reações adversas* representam parcerias com a Colaboração Cochrane via o *Cochrane Peripheral Vascular Disease Group* (Grupo de Doenças Vasculares e Periféricas da Cochrane) e o *Cochrane Adverse Effects Methods Group* (Grupo de Métodos em Efeitos Adversos da Cochrane), essenciais no desenvolvimento dos estudos concretizados. A linha de estudos em E*pidemiologia genética e o estudo de gêmeos* foi implementada pela *The University of Sydney* em parceria com o *Australian Twin Registry* e se desenvolveu dentro do âmbito dessa tese devido a inserção da autora no *Arthritis and Musculoskeletal Research Group* (grupo de pesquisas em artrite e condições musculoesqueléticas inserido na *The Faculty of Health Sciences* da *The Universtiy of Sydney*) sob a supervisão do Dr. Paulo Ferreira, líder do *Spinal Pain Research Group* (sub-grupo do *Arthritis and Musculoskeletal Research Group* dedicado a pesquisas de dores na coluna). Essas parcerias internacionais representam importantes perspectivas para um contínuo e duradouro intercâmbio científico.

Finalmente, os aspectos interdisciplinares e de original refinamento metodológico, característicos das linhas de pesquisas componentes dessa tese, apontam para a interessante intercambialidade dos métodos e desfechos, isto é, o desenvolvimento de pesquisas versando sobre os eventos adversos dos diferentes tratamentos atualmente propostos para a dor lombar e a concepção de estudo de gêmeos direcionados a temas de grande interesse na atualidade, como a epigenética e a farmacogenética. Ressaltamos ainda que ambas as linhas de pesquisas são incipientes no Brasil e, nessa perspectiva, a tese materializa as perspectivas de inovação científica e de colaboração internacional para o desenvolvimento de pesquisas competitivas como estratégia para o avanço científico do país.

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