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Imunogenicidade e segurança da vacina contra influenza A H1N1/2009 em pacientes com artrite idiopática juvenil

Tese apresentada à Faculdade de Medicina da Universidade de São Paulo para obtenção do título de Doutora em Ciências

Programa de Ciências Médicas
Área de concentração: Processos Imunes e Infecciosos

Orientador: Prof. Dr. Clovis Artur Almeida da Silva

São Paulo

2012

Dados Internacionais de Catalogação na Publicação (CIP)

Preparada pela Biblioteca da
Faculdade de Medicina da Universidade de São Paulo

Aikawa, Nádia Emi
Imunogenicidade e segurança da vacina contra influenza A H1N1/2009 em pacientes com artrite idiopática juvenil / Nádia Emi Aikawa. -- São Paulo, 2012.
Tese(doutorado)--Faculdade de Medicina da Universidade de São Paulo. Programa de Ciências Médicas. Área de concentração: Processos Imunes e Infecciosos.
Orientador: Clovis Artur Almeida da Silva.

Descritores: 1.Vírus da influenza A subtipo H1N1 2.Artrite juvenil idiopática 3.Vacinação/efeitos adversos 4.Imunidade humoral

USP/FM/DBD-309/12

DEDICATÓRIA

À minha família, pelo carinho e por compreender meus longos períodos de ausência após a vinda a São Paulo.

Em especial, aos meus pais, pelo incentivo incansável aos estudos.

À minha irmã, pela amizade e apoio, mesmo à distância.

AGRADECIMENTOS

Ao Prof. Dr. Clovis Artur Almeida da Silva, que sempre foi e será meu pai reumatológico e grande amigo. Agradeço pelo carinho, os ensinamentos e a dedicação a mim e a todos os seus alunos e por manter a Reumatologia Pediátrica do ICr sempre muito viva, alegre e acolhedora.

À Profa. Eloisa Bonfá, por ter permitido minha entrada no CEDMAC e pela confiança e carinho com que sempre me tratou. A admiro muito pela sua inteligência, entusiasmo e pela disponibilidade em nos conduzir cientificamente, apesar dos inúmeros compromissos.

À Dra. Adriana Maluf e Dra. Lucia Campos, a quem admiro por unirem sabedoria, bom humor, humildade e bondade, especialmente na relação com os pacientes. Aprecio muito sua amizade.

À amiga Adriana de Jesus, que teve grande influência em meu interesse pela Reumatopediatria. Você é um exemplo para mim, pois reúne muito profissionalismo, coragem e delicadeza.

Aos meus grandes amigos Aline, Carlos, Kátia e Renata, que fizeram da residência na Reumatopediatria uma lembrança de um período repleto de alegrias, aprendizado e companheirismo.

Aos amigos Cíntia Michelin, Erica Gomes, Guilherme Trudes e Vanessa Guissa pela imensa ajuda na coleta de dados e pela amizade.

Aos amigos do CEDMAC, que me receberam muito bem e estão sempre dispostos a me ajudar, com bom humor. Trabalhar com vocês é muito agradável.

Aos assistentes da Reumatologia do HC, especialmente Dra. Claudia Schainberg e Dra. Rosa Pereira, que também me acolheram com carinho e me ensinam muito.

Às funcionárias do Laboratório de Investigação Médica da Reumatologia, que trabalharam arduamente e tiveram uma contribuição preciosa neste estudo.

Aos pacientes, que tornaram possível este estudo.

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São Paulo: Serviço de Biblioteca e Documentação; 2005.

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S U M Á R I O

LISTAS

ABREVIATURAS

AIJ	Artrite idiopática juvenil
EVA	Escala visual analógica
CHAQ	*Childhood Health Assessment Questionaire*
VHS	Velocidade de hemossedimentação
PCR	Proteína C reativa
MGT	Média geométrica dos títulos
FA da MGT	Fator de aumento da média geométrica dos títulos
IH	Inibição da hemaglutinação
DMARDs	Drogas anti-reumáticas modificadoras de doença
EMEA	*European Medicines Agency*
FDA	*Food and Drug Administration*
ARE	Artrite relacionada a entesite

TABELAS

RESUMO

Aikawa NE. Imunogenicidade e segurança da vacina contra influenza A H1H1/2009 em pacientes com artrite idiopática juvenil [tese]. São Paulo: Faculdade de Medicina, Universidade de São Paulo; 2012.

Introdução: A pandemia de gripe A H1N1 em junho de 2009 resultou em elevadas taxas de hospitalização entre pacientes imunodeprimidos, incluindo pacientes com artrite idiopática juvenil (AIJ). Embora a vacinação seja uma medida eficaz contra complicações da gripe pandêmica, não há estudos na literatura sobre seus efeitos na AIJ. **Objetivos:** Avaliar a resposta resposta da vacina contra influenza A H1N1/2009 sem adjuvante na AIJ, como uma extensão do estudo anterior de imunogenicidade e segurança em uma grande população de pacientes com doenças reumáticas juvenis. Além disso, avaliar a possível influência de dados demográficos, subtipos de AIJ, atividade da doença e do tratamento sobre a imunogenicidade e o potencial efeito deletério da vacina sobre a doença, particularmente sobre o número de articulações ativas e os marcadores inflamatórios. **Métodos:** 95 pacientes com AIJ e 91 controles saudáveis foram avaliados antes e 21 dias após a vacinação contra influenza A H1N1/2009 e a sorologia anti-H1N1 foi realizada por ensaio de inibição de hemaglutinação. A avaliação global de atividade da artrite por uma escala visual analógica (EVA) pelo paciente e pelo médico, o *Childhood Health Assessment Questionnaire* (CHAQ), o número de articulações ativas, as provas de fase aguda (VHS e PCR) e o tratamento foram avaliados antes e após a vacinação. Os eventos adversos foram também reportados. **Resultados:** Pacientes com AIJ e controles foram comparáveis em relação à média de idade atual (14,9 ± 3,2 vs. 14,6 ± 3,7 anos, p=0,182). A taxa de soroconversão após a vacinação foi significantemente menor nos pacientes com AIJ em relação aos controles (83,2% vs. 95,6%, p=0,008), particularmente no subtipo poliarticular (80% vs. 95,6%, p=0,0098). Os subtipos de AIJ, o número de articulações ativas, as provas de fase aguda, a EVA do paciente e do médico, o CHAQ e a frequencia de uso de DMARDs/imunossupressores foram semelhantes entre os pacientes que

soroconverteram *versus* os que não soroconverteram (p>0,05). Em relação à segurança da vacina, não foi observada piora no número de articulações ativas e nas provas de fase aguda durante o período de estudo. **Conclusão:** A vacinação contra influenza A H1N1/2009 na AIJ induziu uma resposta humoral reduzida com adequado efeito protetor, independente de parâmetros da doença e tratamento, e com um perfil adequado de segurança da doença.

Descritores: 1.vírus da influenza A subtipo H1N1, 2.artrite juvenil idiopática, 3.vacinação/efeitos adversos, 4.imunidade humoral

SUMMARY

Aikawa NE. Immunogenicity and safety of the influenza A H1H1/2009 vaccine in juvenile idiopathic arthritis patients [thesis]. São Paulo: “Faculdade de Medicina, Universidade de São Paulo”; 2012.

Introduction: The influenza H1N1 pandemic in June 2009 resulted in high hospitalization rates among immunocompromised patients, including patients with juvenile idiopathic arthritis (JIA). Although vaccination is an effective tool against pandemic flu complications, there are no studies in the literature on its effects in JIA. **Objectives:** To assess the immune response against the influenza A H1N1/2009 vaccine without adjuvant in JIA as an extension of previous observation of its immunogenicity and safety in a large population of patients with juvenile rheumatic diseases. Moreover to assess the possible influence of demographic data, subtypes of JIA, disease activity and treatment on the immunogenicity and the potential deleterious effect of vaccine on disease itself, particularly on the number of active joints and inflammatory markers. **Methods:** 95 JIA patients and 91 healthy controls were evaluated before and 21 days after vaccination against influenza A and serology for anti-H1N1 was performed by hemagglutination inhibition assay. The overall assessment of arthritis activity by a visual analogue scale (VAS) by patient and physician, the Childhood Health Assessment Questionnaire (CHAQ), the number of active joints, the acute phase reactants (ESR and CRP) and treatment were evaluated before and after vaccination. Adverse events were also reported. **Results:** JIA patients and controls were comparable regarding mean current age (14.9 ± 3.2 vs. 14.6 ± 3.7 years, p=0.182). After vaccination seroconversion rate was significantly lower in JIA patients compared to controls (83.2% vs. 95.6%, p=0.008), particularly in polyarticular subtype (80% vs. 95.6%, p=0.0098). JIA subtypes, number of active joints, acute phase reactants, patient and the physician VAS, CHAQ and frequency of use of DMARDs/Immunosuppressants were similar between patients with and without seroconversion (p>0.05). Regarding vaccine safety, no deterioration was observed in the number of active joints and the acute phase reactants during the study period. **Conclusion:**

Influenza A H1N1/2009 vaccination in JIA induces a lower but effective antibody response, probably independent of disease parameters and treatment with an adequate disease safety profile.

Descriptors: 1.H1N1 subtype influenza A virus, 2. juvenile rheumatoid arthritis, 3.vaccination/adverse effects, 4.humoral immunity

1 INTRODUÇÃO

A artrite idiopática juvenil (AIJ) é a principal causa de artrite crônica na faixa etária pediátrica, com alto impacto físico, mental e emocional nos pacientes e familiares. Sua incidência anual varia de 2 a 20 casos por 100.000 habitantes, com uma prevalência de 16 a 150 casos por 100.000 pessoas.[1]

Na última década grandes avanços foram feitos no tratamento da AIJ, resultando em uma melhora significativa no prognóstico da doença a longo prazo.[1] Por outro lado, a imunossupressão resultante da terapêutica com imunossupressores e agentes biológicos tornou esses pacientes mais propensos a complicações infecciosas.[2]

Em junho de 2009, uma nova pandemia de gripe H1N1 foi estabelecida, resultando em altas taxas de hospitalização (1 a 10%)[3] e mortalidade (2,6 a 7,6%),[4,5] particularmente entre pacientes imunossuprimidos.[5] De fato, as infecções são reconhecidas como importantes causas de aumento da morbidade em pacientes com doenças reumáticas pediátricas que utilizam de drogas imunossupressoras e agentes biológicos.[6,7]

Nesse sentido, a vacinação é uma medida preventiva de saúde pública estabelecida, eficaz contra uma variedade de agentes infecciosos,[8] incluindo o vírus da influenza, sendo recomendada para pacientes com doenças autoimunes. Entretanto, ainda há poucos estudos sobre imunização nesse grupo de pacientes, principalmente em situações epidêmicas.[9] Em 2010, baseada na previsão de que o vírus da influenza A H1N1/2009 continuaria a circular no ano seguinte, o *Advisory Committee*

on Immunization Practices (ACIP) recomendou que todas as crianças e adolescentes com idades entre 6 meses e 18 anos deveriam receber a vacina contra a gripe sazonal trivalente contendo a cepa A/California/7/2009(H1N1). De acordo com estas recomendações, a vacinação é particularmente importante para pacientes com risco aumentado de complicações graves, incluindo aqueles com condições crônicas, tais como AIJ, particularmente em terapia imunossupressora.[10] Mais recentemente, o *European League Against Rheumatism* (EULAR) reforçou a importância da vacinação em pacientes pediátricos imunodeprimidos com doenças reumatológicas.[11]

Além disso, para a aprovação de vacinas de gripe pandêmica, crianças, adolescentes e adultos devem preencher todos os três padrões imunológicos atualmente propostos pela *European Medicines Agency* (EMEA) e o *Food and Drug Administration* (FDA): soroproteção > 70%, soroconversão > 40% e um fator de aumento (FA) na média geométrica dos títulos de anticorpos (GMT) > 2,5.[12-14]

Existem poucos estudos na literatura médica sobre vacina contra a gripe H1N1 em pacientes com AIJ e todos eles são restritos à segurança e resposta global à vacina.[15-18] Malleson et al. avaliaram apenas 34 crianças com artrite crônica e 13 controles, e encontraram respostas imunes adequadas, independentemente do uso de glicocorticóides ou agentes imunossupressores.[18] Uma resposta vacinal reduzida, porém comparável à da vacina da gripe sazonal, foi evidenciada em 49 pacientes com doenças reumáticas, bem como por um grupo controle constituído por pacientes

com outras doenças crônicas.[17] O pequeno número de pacientes e controles saudáveis e a inclusão de crianças menores de 9 anos, um grupo com um padrão distinto de resposta imune à vacina,[17,18] impedem uma conclusão definitiva sobre seus resultados.

Recentemente, a Unidade de Reumatologia Pediátrica e a Disciplina de Reumatologia da Faculdade de Medicina da Universidade de São Paulo (FMUSP) realizaram um estudo prospectivo sobre a vacina anti-influenza A H1N1/2009 sem adjuvante em 237 pacientes com doenças reumáticas autoimunes juvenis, incluindo 93 pacientes com AIJ, demonstrando-se uma redução da resposta imune, especialmente associada à terapia com corticosteróides, com uma segurança adequada a curto prazo.[19]

No entanto, as médias de idades comparáveis entre pacientes e controles neste estudo não podem ser estendidas para cada grupo de doença.[19] Além disso, o possível papel das características demográficas, subtipos de AIJ e atividade da doença na resposta humoral à vacina anti-influenza A H1N1/2009 em pacientes com AIJ e o impacto da vacina sobre a segurança na doença, particularmente em relação ao número de articulações ativas e as provas de fase aguda, ainda não foram avaliados.

2 OBJETIVOS

1. Avaliar a imunogenicidade e segurança da vacina contra o vírus influenza A H1N1/2009 em pacientes com AIJ comparados com controles saudáveis.

2. Avaliar a possível associação entre reduzida ou adequada imunogenicidade da vacina com: dados demográficos, atividade clínica e laboratorial da doença e tratamento de pacientes com AIJ.

3. Avaliar os possíveis efeitos deletérios da vacina sobre a atividade evolutiva da doença.

3 MÉTODOS

População de estudo

No período de março a abril de 2010, todos os 169 pacientes com AIJ de acordo com os critérios da Liga Internacional Contra o Reumatismo (ILAR)[20] atendidos na Unidade de Reumatologia Pediátrica do Instituto da Criança e da Divisão de Reumatologia do Hospital das Clínicas da FMUSP foram convidados por carta e/ou por telefone para participar da campanha nacional para imunização com a vacina contra influenza A H1N1/2009 no centro de imunizações do mesmo hospital. Quarenta pacientes apresentavam idades acima de 21 anos e 27 pacientes, abaixo de 9 anos, sendo excluídos. Sete pacientes faltaram à campanha vacinal, resultando em 95 pacientes com AIJ entre 9 e 21 anos incluídos no estudo.

O grupo controle incluiu 91 crianças, adolescentes e jovens saudáveis voluntários entre 9 e 21 anos que procuraram o centro de imunização durante a referida campanha.

Todos os pacientes e controles saudáveis e seus respectivos responsáveis assinaram o Termo de Consentimento Livre e Esclarecido. O presente estudo foi aprovado pela Comissão de Ética para Análise de Projetos de Pesquisa do HCFMUSP (CAPPesq) (número 0114/10) e recebeu apoio da Fundação de Amparo a Pesquisa do Estado de São Paulo (2010/10749-0). Além disso, este estudo foi registrado no *clinicaltrials.gov* (NCT01151644).

Critérios de inclusão

1. Diagnóstico de AIJ segundo ILAR[20]

Critérios de exclusão:

1. Infecção prévia pelo vírus influenza A H1N1/2009, confirmada por sorologia
2. Alergia a componentes vacinais ou a ovo
3. Doença infecciosa aguda com febre acima de 38ºC no momento da vacinação
4. Síndrome de Guillain-Barré ou síndromes desmielinizantes
5. Insuficiência cardíaca descompensada
6. Imunização com vacina de vírus vivo até 4 semanas antes, vacina de vírus inativo até 2 semanas ou vacina anti-influenza até 6 meses antes da inclusão neste estudo
7. Hospitalização no momento da inclusão no estudo
8. Transfusão com hemoderivados até 6 meses antes do estudo

Vacina anti-influenza A H1N1/2009

Todos os pacientes com AIJ e controles saudáveis receberam uma dose única intramuscular (0,5 mL) da vacina monovalente anti-influenza A H1N1/2009 (A/California/7/2009/Instituto Butantan/Sanofi Pasteur). A vacina continha 15 µg do antígeno hemaglutinina equivalente à cepa A/California/7/2009 (H1N1) (NYMC X-179A) do vírus influenza,

fragmentada e inativada, sendo o vírus da vacina recombinante recomendado pela OMS. Esta cepa foi propagada em ovos embrionados de galinha, utilizando as mesmas técnicas padronizadas para a produção da vacina sazonal. A vacina foi apresentada em frascos de 5 mL, utilizando timerosal como conservante (45 µg por dose de 0,5 mL).

Avaliação da imunogenicidade da vacina anti-influenza A H1N1/2009

Todos os pacientes e controles foram avaliados no dia da vacinação e após 3 semanas. Os níveis de anticorpos contra o vírus H1N1 A/California/7/2009 foram determinados através do teste de inibição da hemaglutinação (IH) no Instituto Adolfo Lutz, conforme descrito previamente.[21] As concentrações de vírus foram previamente determinadas por titulação do antígeno hemaglutinina e o teste de IH foi realizado após a remoção de inibidores não específicos do soro. Os estoques de vírus foram aliquotados e armazenados a -70 º C até sua utilização.

Os soros foram testados para anticorpos contra a cepa do vírus influenza H1N1 A/California/7/2009 fornecido por Butantan a uma diluição inicial de 1:10 e uma diluição final de 1:2560. Para fins de cálculo, um valor de 1:5 foi atribuído para títulos negativos, e um valor de 1:2560 para títulos superiores a 1:2560. Todas as amostras foram testadas em duplicata.

Os *end-points* da imunogenicidade após a vacinação foram as taxas de soroproteção (títulos de anticorpos ≥ 1:40), soroconversão (títulos pré-vacinais < 1:10 e pós-vacinais ≥ 1:40 ou títulos pré-vacinais ≥ 1:10 e pós-

vacinais com aumento ≥ 4 vezes), a média geométrica dos títulos (MGT) e o fator de aumento (FA) na MGT (MGT da relação entre os títulos pós e pré-vacinais).[22]

Avaliação da segurança vacinal

No dia da vacinação, os pacientes ou responsáveis receberam um diário pessoal de 21 dias contendo a seguinte lista pré-definida de eventos adversos: reações locais (prurido, dor, eritema e edema) e eventos adversos sistêmicos (cefaléia, mialgia, artralgia, febre, diarréia, tosse, dor de garganta, rinorréia e congestão nasal).

Os participantes foram orientados a marcar "sim" ou "não" em cada um dos eventos adversos listados e a devolver os seus diários no segundo dia de avaliação (21 dias após a vacinação). Os eventos adversos que não constassem na lista também poderiam relatados. Todas as reações locais foram consideradas como relacionadas à vacina anti-influenza A H1N1/2009, enquanto os eventos adversos sistêmicos foram analisados pelos pesquisadores para determinar a sua causalidade. Eventos adversos graves foram definidos como aqueles que resultassem em hospitalização ou em óbito.

Avaliação clínica, laboratorial e tratamento da AIJ

Todos os pacientes com AIJ foram avaliados pré e 21 dias após a vacinação para dados clínicos, laboratoriais e tratamento. A avaliação da atividade da doença incluiu a contagem do número de articulações com artrite (edema articular ou presença de limitação à mobilização articular com dor à mobilização ou à palpação articular), a medida da velocidade de hemossedimentação (VHS) de acordo com o método de Westergreen e da proteína C reativa (PCR) por nefelometria. A avaliação clínica também incluiu a avaliação global de atividade da AIJ pelo paciente e pelo médico por meio de uma escala visual analógica (EVA) horizontal de 100 mm e a versão brasileira validada do questionário de qualidade de vida relacionada à saúde (*Childhood Health Assessment Questionaire* - CHAQ).[23]

O tratamento medicamentoso atual da AIJ, incluindo o uso de prednisona, drogas anti-reumáticas modificadoras de doença (DMARDs) (metotrexate, leflunomide, sulfassalazina e cloroquina), ciclosporina, anti-TNF (adalimumabe, etanercepte ou infliximabe) e abatacepte, bem como a dose atual (em miligramas), no momento do estudo, de cada medicamento também foram analisados.

Análise estatística

A comparação da população de AIJ e controles utilizando o teste exato de Fisher forneceu ao estudo um poder de 80% para encontrar diferenças de pelo menos 12,7% nas taxas de soroconversão, dado um

erro tipo I de 5% (Graphpad StatMate 1,01). As análises de imunogenicidade e de segurança foram descritivas, e intervalos de confiança de 95% (IC) bicaudais foram calculados assumindo distribuições binomiais para variáveis dicotômicas e distribuição em log-normal para os títulos de anticorpos por IH. As MGTs foram comparadas entre pacientes com AIJ e controles saudáveis utilizando o teste t-Student bicaudal ou o teste de Mann-Whitney sobre os títulos transformados em log10. As variáveis categóricas (taxas de soroproteção e soroconversão, uso de prednisona e drogas imunossupressoras e os efeitos adversos) foram comparadas utilizando o teste exato de Fisher. Os parâmetros de atividade da doença antes e após a vacinação foram analisados com o teste de Wilcoxon signed ranks. A significância estatística foi fixada em $p < 0,05$.

4 RESULTADOS

4.1 Dados demográficos e características da AIJ

Nenhum dos pacientes ou controles preencheram os critérios de exclusão propostos para o estudo.

Os 95 pacientes com AIJ e os 91 controles saudáveis foram comparáveis em relação à média de idade atual (14,9 ± 3,2 vs. 14,6 ± 3,7 anos, p=0,182) e a freqüência do sexo feminino (55,8 vs. 51,6%, p=0,659). A duração média da doença foi de 7,6 ± 4,6 anos. Em relação às formas de início da AIJ: 45 (47,4%) eram do subtipo poliarticular, 24 (21%) oligoarticular, 18 (18,9%) sistêmica e 8 (8,4%) apresentaram outros subtipos (Tabela 1).

Sessenta e três (66,3%) pacientes estavam em uso de pelo menos um DMARD/imunossupressor (prednisona, metotrexate, leflunomide, ciclosporina e/ou sulfassalazina) e 16 (16,8%) estavam sob terapia anti terapia-TNF (14 etanercepte, 1 adalimumabe e 1 infliximabe), com uma mediana de tempo de uso desta de 1,2 (0,1-4,2) anos. Nenhum paciente estava recebendo abatacepte, rituximabe ou tocilizumabe (Tabela 1).

Tabela 1 – Dados demográficos, duração de doença, formas de início e terapia atual em pacientes com artrite idiopática juvenil (AIJ) que receberam a vacina anti-influenza A H1N1/2009

Variáveis	AIJ (n=95)
Dados demográficos	
Sexo feminino, n (%)	53 (55,8)
Idade atual, anos	14,9 ± 3,2
Duração da doença, anos	7,6 ± 4,6
Forma de início da AIJ	
Poliarticular, n (%)	45 (47,4)
Oligoarticular, n (%)	24 (21)
Sistêmica, n (%)	18 (18,9)
Artrite relacionada a entesite, n (%)	8 (8,4)
Tratamento	
Prednisona, n (%)	9 (9,5)
Dose de prednisona, mg/dia	5 (2,5 - 20)
Metotrexate, n (%)	47 (49,5)
Dose de metotrexate, mg/sem	25 (5 - 50)
Ciclosporina, n (%)	14 (14,7)
Lelfunomide, n (%)	6 (6,3)
Anti-TNF, n (%)	16 (16,8)

Dados expressos em n (%) e mediana (variação) ou média ± desvio padrão, TNF – *Tumor Necrosis Factor.*

4.2 Imunogenicidade da vacina

As taxas de soroproteção e soroconversão, a MGT e o FA na MGT dos pacientes com AIJ e controles estão apresentados na Tabela 2.

No início do estudo, as taxas de soroproteção foram comparáveis entre pacientes e controles (20 vs. 20,9%, p=1,0) (Tabela 2).

Após 21 dias, a taxa de soroconversão foi significantemente menor nos pacientes *versus* controles (83,2% IC95% 75,6-90,7% vs. 95,6%, IC95% 91,4-99,8%, p=0,008). Porém, ambos os grupos apresentaram respostas adequadas de acordo com as normas da EMEA/FDA, visto que a soroproteção foi > 70%, a soroconversão > 40% e o FA da MGT > 2,5.

As avaliações dos subtipos de AIJ evidenciaram que apenas os pacientes com forma poliarticular obtiveram soroconversão estatisticamente reduzida comparada aos controles (80%, IC95% 68,2-91,8% vs. 95,6%, IC95% 91,4-99,8%, p=0,0098), enquanto nenhuma diferença foi evidenciada em pacientes com forma oligoarticular (p=0,157), sistêmica (p=0,087) e artrite relacionada a entesite (ARE) (p=0,35). Tanto os doze pacientes com AIJ poliarticular fator reumatóide positivo (p=0,033) quanto os 33 com fator reumatóide negativo (p=0,022) apresentaram menores taxas de soroconversão em comparação aos controles saudáveis (Tabela 2). A freqüência de uso de drogas imunossupressoras foi significantemente maior em pacientes com AIJ forma poliarticular comparada à de pacientes com forma oligoarticular (80% vs. 41,7%, p=0,0027) e semelhante à sistêmica (80% vs 55,6%, p=0,063). Em relação à influência do tratamento, não foi observada diferença em parâmetros de imunogenicidade entre pacientes com e sem drogas imunossupressoras, bem como entre os indivíduos com e sem metotrexato e bloqueadores de TNF (Tabela 2).

Tabela 2 – Imunogenicidade da vacina contra influenza A H1N1/2009 em pacientes com artrite idiopática juvenil (AIJ) e controles saudáveis

	Pré vacinação		Pós vacinação			
	MGT	**SP, %**	**MGT**	**SP, %**	**FA**	**SC, %**
Controles (n=91)	12,4 (9,8 - 15,7)	20,9 (12,5 - 29,3)	250,8 (197 - 319,3)	95,6 (91,4 - 99,8)	20,3 (15,6 - 26,3)	95,6 (91,4 - 99,8)
AIJ (n=95)	10,6 (8,3 - 13,5)	20 (11,9 - 28,1)	215,8 (159,2 - 292,5)	88,4 (82 - 94,9)	20,4 (15 - 27,6)	**83,2*** **(75,6 - 90,7)**
Subtipos de AIJ						
Oligoarticular (n=24)	7,9 (5,9 - 10,7)	12,5 (0 - 26)	195,8 (110,2 - 348,1)	87,5 (74 - 101)	24,7 (13,6 – 44,7)	87,5 (74 – 101)
Poliarticular (n=45)	11,7 (8 – 17,1)	22,2 (9,9 – 34,5)	198,5 (125,5 – 314)	88,9 (79,6 – 98,2)	17 (10,8 – 26,8)	**80*** **(68,2 – 91,8)**
FR-positivo (n=12)	22,4 (8,4 – 60,2)	25 (0 – 50,6)	285,1 (114,4 – 710,6)	91,7 (75,3 – 108)	12,7 (5,1 – 31,4)	**75*** **(49,4 – 100,6)**
FR-negativo (n=33)	9,2 (6,5 – 13,1)	21,2 (7 – 35,4)	174 (102,3 – 296)	87,9 (76,6 – 99,2)	18,9 (11,1 – 32,1)	**81,8*** **(68,5 – 95,2)**
Sistêmico (n=18)	9,3 (5,5 – 15,5)	16,7 (0 – 34,4)	201,6 (102,4 – 396,7)	88,9 (73,9 – 103,8)	21,8 (11 – 42,9)	83,3 (65,6 – 101)
ARE (n=8)	20 (5,8 – 68,5)	38 (1,6 – 73,4)	**538,2*** **(194,3 – 1490,8)**	87,5 (63 – 112)	26,9 (9,4 – 77)	87,5 (63 – 112)
Uso de DMARDs/IS						
Sim (n=55)	10,5 (7,7 – 14,4)	16,4 (6,5 – 26,2)	230,6 (154,1 – 345,1)	89,1 (80,8 – 97,4)	21,9 (14,7 – 32,6)	85,5 (76,1 – 94,9)
Não (n=40)	10,7 (7,3 – 15,7)	25 (11,4 – 38,6)	197 (123,5 – 314,3)	87,5 (77,1 – 97,9)	18,4 (11,5 – 29,5)	**80*** **(67,4 – 92,6)**
Uso de MTX						
Sim (n=47)	11,1 (7,8 – 15,9)	17 (6,2 – 27,9)	211,7 (134,5 – 333,4)	87,2 (77,6 – 96,9)	19,1 (12,3 – 29,5)	**83*** **(72,1 – 93,8)**
Não (n=48)	10,1 (7,3 – 14,1)	22,9 (10,9 – 34,9)	219,8 (145,8 – 331,4)	89,6 (80,9 – 98,3)	21,7 (14,2 – 33,1)	**83,3*** **(72,7 – 94)**
Uso de Anti-TNF						
Sim (n=16)	11,4 (6,3 – 20,7)	18,8 (0 – 38,5)	306,4 (158,1 – 593,9)	100	26,9 (13,7 – 52,8)	93,8 (81,5 – 106)
Não (n=79)	10,4 (8 – 13,7)	20,3 (11,3 – 29,2)	201 (143,1 – 282,3)	**86,1*** **(78,4 – 93,8)**	19,2 (13,7 – 27)	**81*** **(72,3 – 89,7)**

Os dados estão expressos em % ou valor (intervalo de confiança de 95%), MGT – média geométrica dos títulos, SP – soroproteção, FA - fator de aumento na MGT após a vacina, SC – soroconversão, FR – fator reumatóide, ARE – artrite relacionada a entesite, MTX – metotrexate, TNF – *Tumor Necrosis Factor,* DMARDs/IS – drogas anti-reumáticas modificadoras de doença/imunossupressores (prednisona, MTX, leflunomide, ciclosporina, sulfassalazina, agentes anti-TNF e/ou abatacepte), * p <0,05 – comparado ao grupo control.

4.3 Influência dos parâmetros da doença e tratamento sobre a resposta vacinal em pacientes com AIJ

A análise dos dados demográficos revelou que o predomínio do sexo feminino (p=0,412), a média de idade atual (p=0,086) e a duração da doença (p=0,449) foram comparáveis em pacientes com e sem soroconversão. As frequências dos subtipos de AIJ foram semelhantes em ambos os grupos (p>0,05). Além disso, a mediana do número de articulações ativas, VHS, PCR, EVA do paciente, EVA do médico e CHAQ foram semelhantes em pacientes soroconvertidos e não soroconvertidos (p>0,05). Em relação ao tratamento também não foi observada diferença nas freqüências e doses de cada medicamento em ambos os grupos (p>0,05) (Tabela 3).

Tabela 3 – Dados demográficos, subtipos de artrite idiopática juvenil (AIJ), parâmetros de doença e tratamento de acordo com a soroconversão em pacientes com AIJ

	Com soroconversão (n=79)	Sem soroconversão (n=16)	P
Dados demográficos			
Sexo feminino, n (%)	44 (55,7)	11 (68,7)	0,412
Idade atual, anos	14,7 ± 3,2	16,2 ± 2,7	0,086
Duração da doença, anos	7,4 ± 4,5	8,4 ± 5,1	0,449
Subtipos de AIJ			
Oligoarticular, n (%)	21 (26,6)	3 (18,8)	0,754
Poliarticular, n (%)	36 (45,6)	9 (56,3)	0,584
Sistêmico, n (%)	14 (17,7)	3 (18,8)	1,0
ARE, n (%)	8 (10,1)	1 (6,3)	1,0
Parâmetros de doença			
Número de articulações ativas	0 (0-16)	0 (0-28)	0,441
VHS, mm/1ª hora	18 (1-83)	23 (2-55)	0,842
PCR, mg/dL	1,8 (0,1-137,3)	1,9 (0,2-25,4)	0,505
EVA do paciente, 0-100 mm	10 (0-80)	6 (0-80)	0,669
EVA do médico, 0-100 mm	10 (0-84)	12,5 (0-90)	0,718
CHAQ	0 (0-3)	0,125 (0-2)	0,588
Tratamento			
Imunossupressores, n (%)	47 (59,5)	8 (50)	0,582
Dose de prednisona, mg/dia	5 (2,5 – 20)	-	-
MTX dose, mg/semana	25 (7,5-50)	30 (5-50)	0,661

Os dados estão expressos em número (%), mediana (variação) ou media ± desvio padrão; ARE – artrite relacionada a entesite, VHS – velocidade de hemossedimentação, PCR – proteína C reativa, EVA – escala visual analógica, CHAQ - *Childhood Health Assessment Questionnaire*, MTX – metotrexate

Além disso, todos os parâmetros da doença e tratamentos foram semelhantes entre pacientes com AIJ soroprotegidos e não soroprotegidos

(p>0,05), assim como entre os pacientes com AIJ que atingiram FA na MGT > e ≤ 2,5 (p>0,05).

4.4 Segurança da doença

O número de articulações ativas [0 (0-28) vs. 0 (0-18), p=0,552)], os valores de PCR [1,9 (0,1-137,3) vs. 2,7 (0,2-122,8) mg/dL, p=0,073] e a pontuação no CHAQ [0,123 (0-3) vs. 0 (0-3), p=0,058] mantiveram-se estáveis ao longo do estudo. No entanto, a mediana de VHS [19 (1-83) vs. 15 (0-83) mm/1ª hora, p=0,016], EVA do paciente [10 (0-80) vs 8,5 (0-80), p=0,001] e EVA do médico [10 (0-90) vs. 6 (0-80), p=0,002] foram estatisticamente inferiores na avaliação pós-vacinação (Tabela 4).

Tabela 4 - Parâmetros de atividade da doença, VAS do paciente e do médico e CHAQ em pacientes com artrite idiopática juvenil (AIJ) antes e após a vacinação

Variáveis	Pré-vacina	Pós-vacina	P
Atividade de doença			
Número de articulações ativas	0 (0-28)	0 (0-18)	0,552
VHS, mm/1ª hora	19 (1-83)	15 (0-83)	**0,016**
PCR, mg/dL	1,9 (0,1-137,3)	2,7 (0,2-122,8)	0,073
EVA do paciente, 0-100 mm	10 (0-80)	8,5 (0-80)	**0,001**
EVA do médico, 0-100 mm	10 (0-90)	6 (0-80)	**0,002**
CHAQ	0,123 (0-3)	0 (0-3)	0,058

Os dados estão expressos em mediana (variação); EVA – escala visual analógica, CHAQ - *Childhood Health Assessment Questionnaire*, VHS – velocidade de hemossedimentação, PCR - proteína C-reativa

4.5 Segurança da vacina

Os eventos adversos foram relatados por 42,1% dos pacientes e 44% dos controles (p=0,882). Nenhum evento adverso grave foi relatado durante as três semanas de acompanhamento. Apenas artralgia aguda e leve após a vacinação foi significantemente mais elevada em pacientes com AIJ em comparação com os controles (12,6% vs. 2,2%, p=0,01), com mediana de duração de 1 dia (1-5) e mediana de tempo para aparecimento da artralgia de um dia (1-12) após a vacinação. Os eventos adversos mais freqüentes em pacientes e controles foram dor local (21,1% vs. 23,1%, p=0,86), cefaléia (14,7% vs. 19,8%, p=0,438) e mialgia (15,8% vs. 6,6%, p=0,063) (Tabela 5).

Tabela 5 – Eventos adversos da vacina anti-influenza A H1N1/2009 em pacientes com artrite idiopática juvenil (AIJ) e controles

	AIJ (n=95)	Controles (n=91)	P
Reações locais	22 (23,2)	21 (23,1)	1,0
Dor	20 (21,1)	21 (23,1)	0,85
Edema	2 (2,1)	2 (2,2)	1,0
Prurido	2 (2,1)	0 (0)	0,498
Reações sistêmicas	29 (30,5)	27 (29,7)	1,0
Cefaléia	14 (14,7)	18 (19,8)	0,438
Mialgia	15 (15,8)	6 (6,6)	**0,063**
Artralgia	12 (12,6)	2 (2,2)	**0,01**
Febre	4 (4,2)	3 (3,3)	1,0
Diarréia	2 (2,1)	2 (2,2)	1,0
Tosse	3 (3,2)	5 (5,3)	0,49
Dor de garganta	2 (2,1)	5 (5,3)	0,271
Coriza	1 (1,1)	3 (3,3)	0,36
Congestão nasal	1 (1,1)	3 (3,3)	0,36
Total	40 (42,1)	40 (44)	0,882

Dados expressos em n (%)

5 DISCUSSÃO

Para nosso conhecimento, este foi o estudo que incluiu a maior população de pacientes com AIJ, demonstrando que a vacina anti-influenza A H1N1/2009 sem adjuvante induz uma resposta humoral reduzida porém adequada provavelmente independente dos parâmetros da doença e tratamento. No entanto, não foram registradas as infecções por influenza a longo prazo após a vacina, sendo assim, somente parâmetros sorológicos foram medidos.

A vantagem deste estudo foi a inclusão de um grupo controle saudável de idade equivalente, uma vez que a resposta imune à vacina de influenza apresenta um padrão distinto em crianças e adolescentes[24] e os menores de 9 anos foram excluídos devido ao fato de necessitarem de duas doses para uma adequada imunogenicidade.[24,25] A inclusão de todos os subtipos de AIJ é, no entanto, uma limitação do estudo, dado que as características clínicas e genéticas, bem como o tratamento e as evoluções não são uniformes em cada subgrupo de pacientes.[1]

Para serem aprovadas na população pediátrica, vacinas de gripe pandêmica devem atender a todos os três padrões atuais propostos.[12-14] Portanto, apesar de uma redução da resposta imune em pacientes com AIJ, essa população alcançou todos os critérios que indicam uma resposta imunológica eficaz. Da mesma forma, uma imunogenicidade satisfatória também foi observada com a vacinação contra a gripe sazonal em estudos anteriores com doenças reumáticas juvenis,[16] incluindo pacientes com AIJ.[18] Por outro lado, um recente estudo em nosso serviço evidenciou uma

resposta humoral reduzida para a mesma vacina na artrite reumatóide do adulto, particularmente naqueles em tratamento com metotrexate.[26]

Embora sintomas gripais após a imunização tenham sido avaliados, a incidência de infecção pós-vacinal por influenza determinada por coleta de amostras respiratórias não foi avaliada. Portanto, a redução real de risco de infecção por influenza não pôde ser calculada.

No presente estudo, uma taxa de soroconversão reduzida foi demonstrada no grupo de AIJ poliarticular, que incluiu pacientes mais frequentemente tratados com terapias imunossupressoras. No entanto, o baixo número de pacientes com AIJ que não tiveram soroconversão, bem como o número limitado de pacientes em uso de prednisona impedem uma conclusão definitiva sobre o possível efeito deletério das drogas sobre a imunogenicidade.

No entanto, a segurança a curto prazo demonstrada no presente estudo sugere a aplicação de uma dose de reforço da vacina em pacientes não-respondedores. De fato, em um estudo anterior com pacientes infectados pelo HIV, uma segunda dose da vacina contra influenza A H1N1/2009 resultou em um aumento adicional da taxa de soroconversão.[27]

Nossos pacientes com AIJ obtiveram menores taxas de soroconversão comparados aos controles, embora apenas para o subtipo poliarticular, as diferenças tenham sido estatisticamente significativas. De fato, o tamanho da amostra para os outros subtipos pode ter sido muito pequeno para alcançar diferença estatística. Além disso, uma elevada MGT pós-vacinação foi observada em pacientes com artrite relacionada a

entesite (ARE). O número limitado de pacientes deste subgrupo pode ter dificultado a interpretação desse achado.

Identificamos que a terapia imunossupressora não parece influenciar a resposta imunológica à vacina contra influenza pandêmica em pacientes com AIJ, como também evidenciado em adultos com artrite reumatóide e espondilite anquilosante.[28] Da mesma forma, estudos anteriores relataram a ausência de efeito dessas drogas sobre a imunogenicidade da vacina anti-influenza sazonal em pacientes com doenças reumáticas juvenis, incluindo um pequeno número de pacientes com AIJ.[16-18] Em um estudo recente, Toplak e col. avaliaram a resposta imune à vacina anti-influenza anual 2008/2009 e observaram uma imunogenicidade reduzida em pacientes em uso de agentes anti-TNF. No entanto, o número bastante limitado de pacientes sob esta terapia impede conclusões definitivas sobre tal efeito.[15] Por outro lado, em pacientes adultos com lúpus eritematoso sistêmico, drogas imunossupressoras foram associadas com uma redução significante das taxas de soroproteção e de soroconversão após a vacina pandêmica.[29] Um efeito deletério global da corticoterapia sobre esta resposta imune também foi observada em uma grande coorte de pacientes com doenças reumáticas juvenis.[19] A análise específica da população com AIJ do presente estudo não confirmou esta associação, provavelmente devido ao número limitado de pacientes sob essa terapia.

Um outro estudo recente descreveu que os parâmetros da doença podem prejudicar a resposta à vacina contra gripe pandêmica em pacientes adultos com lúpus.[30] A exclusão de pacientes internados na presente

coorte dificultou a interpretação da potencial relevância da atividade da doença sobre a resposta humoral à vacina devido à pequena representação desses pacientes.

A segurança vacinal em relação à doença foi corroborada pela demonstração de estabilidade no número de articulações com artrite e nas provas de fase aguda (VHS e PCR) ao longo do estudo. Reforçando este achado, estudos anteriores com vacinação contra hepatite, sarampo, caxumba e rubéola não mostraram qualquer piora nos parâmetros de atividade da AIJ.[31,32] No entanto, a falta de um grupo controle de pacientes com AIJ não vacinados no presente estudo, dificulta a avaliação exata do efeito da vacinação anti-influenza H1N1/2009 sobre a atividade da doença.

A utilização da vacina sem adjuvante foi escolhida para este estudo a fim de se evitar uma doença autoimune relacionada.[33] A vacina de influenza A H1N1/2009 foi bem tolerada e segura em pacientes com AIJ e nenhum evento adverso grave a curto prazo foi evidenciado, como também relatado previamente em um número limitado de pacientes com AIJ que receberam a vacina contra influenza sazonal. [16,17] Apenas artralgia leve e aguda foi observada em nossos pacientes com AIJ, conforme relatado anteriormente em um estudo maior em nossa unidade com 237 pacientes pediátricos com doenças reumáticas autoimunes.[19]

Em conclusão, este estudo prospectivo de uma vacina de influenza A H1N1/2009 pandêmica em pacientes com AIJ sugere uma imunogenicidade adequada, aparentemente independente da terapia

imunossupressora atual e sem efeitos prejudiciais a curto prazo para a própria doença.

6 CONCLUSÕES

1. A vacina anti-influenza A H1N1/2009 foi segura, com uma resposta humoral reduzida porém suficiente, em pacientes com AIJ.

2. A imunogenicidade da vacina anti influenza A H1N1/2009 não foi influenciada pela atividade clínica, atividade laboratorial ou pelo tratamento de pacientes com AIJ.

3. A vacina não induziu piora ou reativação da doença.

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ANEXOS

Anexo I – "Effective seroconversion and safety following the pandemic influenza vaccination (anti-H1N1) in patients with juvenile idiopathic arthritis"
Aceito para publicação na revista *Scandinavian Journal of Rheumatology*

Anexo II – "Glucocorticoid: major factor for reduced immunogenicity of 2009 influenza A (H1N1) vaccine in juvenile autoimmune rheumatic disease patients"
Publicado na revista *The Journal of Rheumatology*

Anexo III – "Influenza A H1N1/2009 vaccine in juvenile dermatomyositis: reduced immunogenicity in patients under immunosuppressive"
Aceito para publicação na revista *Clinical and Experimental Rheumatology*

Anexo IV – "High Disease Activity: an Independent Factor for Reduced Immunogenicity of Pandemic Influenza A Vaccine in Patients with Juvenile Systemic Lupus Erythematosus"
Submetido à revista *Arthritis Care and Research* (*Hoboken*)

Effective seroconversion and safety following the pandemic influenza vaccination (anti-H1N1) in patients with juvenile idiopathic arthritis

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Objectives: To assess the vaccine response in juvenile idiopathic arthritis (JIA) as an extension of previous observation of immunogenicity and safety of a non-adjuvanted influenza A H1N1/2009 vaccine in a large population of juvenile rheumatic diseases. Moreover, to assess the possible influence of demographic data, disease subtypes, disease activity, and treatment on immunogenicity and the potential deleterious effect of the vaccine in the disease itself, particularly in the number of arthritis and inflammatory markers.

Methods: A total of 95 patients with JIA and 91 healthy controls were evaluated before and 21 days after vaccination, and serology for anti-H1N1 was performed by haemagglutination inhibition assay (HIA). Patient and physician visual analogue scales (VAS), Childhood Health Assessment Questionnaire (CHAQ), number of active joints, acute phase reactants, and treatments were evaluated before and after vaccination. Adverse events were also reported.

Results: JIA patients and controls were comparable regarding mean current age (14.9 ± 3.2 vs. 14.6 ± 3.7 years, $p = 0.182$). After vaccination, the seroconversion rate was significantly lower in JIA patients compared to controls (83.2% vs. 95.6%, $p = 0.008$), particularly in the polyarticular subtype (80% vs. 95.6%, $p = 0.0098$). Of note, JIA subtypes, number of active joints, acute phase reactants, CHAQ, patient and physician VAS, and use of disease-modifying anti-rheumatic drugs (DMARDs)/immunosuppressive drugs were similar between seroconverted and non-seroconverted patients ($p > 0.05$). Regarding vaccine safety, no deterioration was observed in the number of active joints and acute phase reactants during the study period.

Conclusion: Influenza A H1N1/2009 vaccination in JIA induces a lower but effective protective antibody response probably independent of disease parameters and treatment with an adequate disease safety profile.

In 2009, an H1N1 influenza pandemic was established resulting in high rates of hospitalization (1–10%) (1) and mortality (2.6–7.6%) (2, 3), particularly among immunosuppressed patients. Indeed, infection is recognized as an important additional cause of increased morbidity of paediatric rheumatic diseases under treatment with disease-modifying anti-rheumatic drugs (DMARDs) and anti-tumour necrosis factor (TNF) agents (4, 5).

In this regard, vaccination is a well-known effective tool against a variety of infectious agents including influenza infection (6); in 2010, the Advisory Committee on Immunization Practices (ACIP) recommended influenza A H1N1/2009 immunization for high-risk groups, including juvenile idiopathic arthritis (JIA) patients (7). More recently, the European League Against Rheumatism (EULAR) task force reinforced the importance of vaccination in immunosuppressed paediatric rheumatology patients (8).

Additionally, for pandemic influenza vaccines to be licensed, children, adolescents, and adults must meet all three current immunology standards proposed by the European Medicines Agency (EMEA) and the Food and Drug Administration (FDA): seroprotection (SP) > 70%, seroconversion (SC) > 40%, and a factor increase (FI) in the geometric mean titre (GMT) > 2.5 (9–11).

There are few data in the literature regarding the H1N1 influenza vaccine in JIA patients and all of them are restricted to overall safety and vaccine response (12–15). Malleson et al (14) evaluated 34 children with chronic arthritis and 13 controls, and found adequate immune responses regardless of the use of glucocorticoids or immunosuppressive agents. A low but comparable immunoresponse to seasonal influenza vaccine was achieved by 49 rheumatic disease patients as well as by a control group with chronic illnesses (13). The small number of patients and healthy controls and the inclusion of infants, a group with a distinct pattern of vaccine

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Accepted 2 July 2012

DOI: 10.3109/03009742.2012.709272

immune response (13, 14), precludes a definitive conclusion about their findings.

We recently performed a prospective study regarding the non-adjuvanted influenza A H1N1/2009 vaccine in 237 juvenile autoimmune rheumatic diseases, including 93 JIA patients, and showed a reduced immune response especially associated with glucocorticoid therapy, with short-term vaccine safety results (15). However, the overall comparable ages among patients and controls in that study may not be extended to each disease group (15). In addition, the possible role of demographic characteristics, disease subtypes, and disease activity in antibody response to the pandemic H1N1 vaccine in JIA patients and the impact of the vaccine in disease safety, particularly related to the number of arthritis and acute phase reactants, were not assessed.

Therefore, the aims of this study were to analyse the influenza A H1N1/2009 vaccine response in patients and age-balanced healthy controls with further stratification of certain variables that could influence immunogenicity. The potential deleterious effect in disease activity parameters was also evaluated.

Methods

All 169 JIA patients followed at the Paediatric Rheumatology Unit of the Children's Institute and the Rheumatology Division, Clinics Hospital, Faculty of Medicine, University of São Paulo were invited by letter to participate in the public health influenza A H1N1/2009 vaccine campaign at the Immunization Centre of the same hospital. Ninety-five patients with JIA according to International League Against Rheumatism (ILAR) criteria (16) agreed to participate in the study and fulfilled the inclusion criteria. Ninety-one healthy volunteers who came to this centre seeking vaccination in response to the Public Health National Campaign were included as the control group. All participants were ≥ 9 and ≤ 21 years old. This study was approved by the local ethical committee of our University Hospital and informed consent was obtained from all participants or their legal guardians. The study was registered at clinicaltrials.gov under number NCT01151644.

Vaccine

Vaccination was contraindicated in the following conditions: (a) anaphylactic response to vaccine components or to egg, (b) acute infection resulting in fever with a temperature > 38°C at the time of vaccination, (c) history of Guillain–Barré or demyelination syndromes, (d) and live virus vaccination 4 weeks before or any inactivated vaccine 2 weeks before the study, according to Centers for Disease Control and Prevention (CDC) recommendations (6). Furthermore, exclusion criteria for patients and controls included: hospitalization, blood transfusion in the past 6 months, previous immunization against seasonal influenza 2008, and confirmed infection by influenza A H1N1/2009.

All JIA patients and healthy controls received one dose of a monovalent inactivated anti-influenza vaccine containing 15 μg of haemagglutinin antigen equivalent to the A/California/7/2009 (H1N1) virus-like strain (NYMC X-179A) without adjuvant, propagated in embryonated chicken eggs provided by the Butantan Institute. Virus concentrations were determined by the haemagglutination assay titration as described previously (17) and virus stocks were aliquoted and stored at −70°C until used. The vaccine was stored in 5-mL multi-dose vials using thimerosal (45 μg per 0.5-mL dose) as the preservative.

Immunogenicity assessment

All JIA patients and controls were evaluated on the day of vaccination and then 3 weeks later. Serology against the H1N1 A/California/7/2009-like virus was performed by haemagglutination inhibition assay (HIA) at the Adolfo Lutz Institute on the day of vaccination and then 21 days later.

Sera were tested for antibodies against the H1N1 A/California/7/2009 influenza strain supplied by the Butantan Institute at an initial dilution of 1:10 and a final dilution of 1:2560. For calculation purposes, a value of 1:5 was assigned for negative titres and a value of 1:2560 for titres > 1:2560. Samples were tested in duplicate and geometric mean values were used in the analysis. Virus concentrations were determined previously by haemagglutinin antigen titration, and the HIA test was performed after removing naturally occurring non-specific inhibitors from the sera as described previously (17).

Appropriate endpoints included: seroprotection (percentage of subjects with HIA neutralizing antibody titre $\geq$ 1:40), seroconversion (percentage of subjects with either a pre-vaccination HIA titre < 1:10 and a post vaccination HIA titre $\geq$ 1:40 or a pre-vaccination HIA titre $\geq$ 1:10 and a minimum fourfold rise in post-vaccination HI antibody titre); and an increase in geometric mean titre (GMT) (18).

Safety assessment

On the day of vaccination, all participants received a 21-day personal diary card containing the following list of predefined adverse events to be registered: local reactions (itching, pain, redness, and swelling at injection site) and systemic reactions (fever, malaise, chills, headache, arthralgia, myalgia, diarrhoea, cough, expectoration, sore throat, nasal congestion, and rhinorrhoea) (15). All local reactions were considered to be related to the influenza A H1N1/2009 vaccine, while systemic adverse events were analysed by the investigators to determine their causality. Severe side-effects were defined as those requiring hospitalization or death.

Clinical, laboratory, and therapy evaluations of JIA

Clinical and laboratory assessments of JIA patients were evaluated on the day of vaccination and after 3 weeks and included: number of active joints (swelling within a joint, or limitation in the range of joint movement with joint pain or tenderness), patient and physician global assessment of arthritis activity measured in mm on a 100-mm horizontal visual analogue scale (VAS) and the validated Brazilian version of the Childhood Health Assessment Questionnaire (CHAQ) (19). Erythrocyte sedimentation rate (ESR) was performed according to the Westergreen method and C-reactive protein (CRP) according to nephelometry. Current treatment with prednisone, DMARDs [methotrexate (MTX), leflunomide, and chloroquine], immunosuppressive drugs (cyclosporin), and anti-TNF agents (adalimumab, etanercept, and infliximab) was determined.

Statistical analysis

The difference between seroconversion rates in JIA patients and controls was calculated by Fisher's test with $\alpha = 0.05$. The size sample provided a power of 80% to find differences of at least 1/8 (12.7%) (Graphpad StatMate 1.01). The immunogenicity and safety analyses were descriptive, and the two-sided 95% confidence intervals (CIs) were calculated assuming binomial distributions for dichotomous variables and log-normal distribution for HIA titres. The GMTs were compared between JIA patients and the healthy controls using a two-sided Student's t-test or the Mann–Whitney U-test on the $\log_{10}$-transformed titres. Categorical variables (rates of seroprotection and seroconversion, prednisone and immunosuppressive drug use, and adverse events) were compared using Fisher's exact test. The effects on disease activity before and after vaccination were analysed with the Wilcoxon signed ranks test. The statistical significance was set at p-value < 0.05.

Results

JIA patients and controls were comparable regarding mean current age (14.9 ± 3.2 vs. 14.6 ± 3.7 years, $p = 0.182$) and female gender frequency (55.8% vs. 51.6%, $p = 0.659$). Mean disease duration was 7.6 ± 4.6 years. Regarding JIA subtypes, 45 (47.4%) were polyarticular, 24 (21%) oligoarticular, 18 (18.9%) systemic, and eight (8.4%) others. Sixty-three (66.3%) patients were taking at least one DMARD/immunosuppressive agent (prednisone, MTX, leflunomide, cyclosporin, sulfasalazine, anti-TNF agents, and/or abatacept) and 16 (16.8%) were under anti-TNF therapy.

Vaccine immunogenicity

Seroprotection and seroconversion rates, GMT, and FI in GMT in JIA patients and healthy controls are shown in Table 1. After 21 days, the seroconversion rate was significantly lower in JIA patients *versus* controls [83.2% (95% CI 75.6–90.7) vs. 95.6% (95% CI 91.4–99.8), $p = 0.008$]; however, both JIA patients and controls had adequate responses according to the EMEA/FDA standards, given that seroprotection was > 70%, seroconversion > 40%, and a GMT increase of > 2.5. The subanalysis of JIA subtypes showed that only polyarticular onset patients had statistically reduced seroconversion compared to controls [80% (95% CI 68.2–91.8) vs. 95.6% (95% CI 91.4–99.8), $p = 0.0098$], whereas no difference was found in oligoarticular ($p = 0.157$), systemic ($p = 0.087$), and enthesitis-related arthritis (ERA) ($p = 0.35$) patients. The 12 (26.7%) rheumatoid factor (RF)-positive polyarticular JIA patients had lower seroconversion rates ($p = 0.033$) compared to controls, as did the 33 RF-negative polyarticular JIA patients ($p = 0.022$) (Table 1). The use of immunosuppressive drugs in polyarticular JIA patients was significantly higher than in oligoarticular patients (80% vs. 41.7%, $p = 0.0027$) and similar to that in systemic patients (80% vs. 55.6%, $p = 0.063$). Regarding treatment influence, no difference was observed in immunogenicity parameters between patients with and without immunosuppressive drugs, as well as between subjects with and without MTX and TNF blockers (Table 1).

Influence of disease parameters and treatment in the vaccine response of JIA patients

Demographic data analysis revealed that female gender predominance ($p = 0.412$), mean current age ($p = 0.086$), and disease duration ($p = 0.449$) were comparable in seroconverted and non-serocoverted patients. The frequencies of JIA subtypes were similar in both groups ($p > 0.05$). The median of number of active joints, ESR, CRP, patients' VAS, physicians' VAS, and CHAQ were similar in seroconverted and non-serocoverted patients ($p > 0.05$). Regarding treatment, no difference was observed in the frequencies and doses of each therapy in both groups ($p > 0.05$) (Table 2). Furthermore, all disease parameters and treatments were similar between seroprotected and non-seroprotected JIA patients ($p > 0.05$), as well as between JIA patients who achieved FI in GMT > 2.5 and those who achieved FI in GMT ≤ 2.5 ($p > 0.05$).

Disease safety

The median number of active joints [0 (0–28) vs. 0 (0–18), $p = 0.552$)], CRP values [1.9 (0.1–137.3) vs. 2.7 (0.2–122.8) mg/dL, $p = 0.073$], and CHAQ score [0.123 (0–3) vs. 0 (0–3), $p = 0.058$] remained stable throughout the study. However, the medians for ESR [19 (1–83) vs. 15 (0–83) mm/1st hour, $p = 0.016$], patient VAS [10 (0–80) vs. 8.5 (0–80), $p = 0.001$], and physician VAS [10 (0–90) vs. 6 (0–80), $p = 0.002$] were statistically lower in the post-vaccination evaluation (Table 3).

AQ1 Table 1. Immunogenicity of influenza A H1N1/2009 vaccine in juvenile idiopathic arthritis (JIA) patients and healthy controls.

	Pre-vaccination		Post-vaccination			
	GMT	SP %	GMT	SP %	FI	SC %
Controls (n = 91)	12.4 (9.8–15.7)	20.9 (12.5–29.3)	250.8 (197–319.3)	95.6 (91.4–99.8)	20.3 (15.6–26.3)	95.6 (91.4–99.8)
JIA patients (n = 95)	10.6 (8.3–13.5)	20 (11.9–28.1)	215.8 (159.2–292.5)	88.4 (82–94.9)	20.4 (15–27.6)	**83.2* (75.6–90.7)**
JIA subtypes						
Oligoarticular (n = 24)	7.9 (5.9–10.7)	12.5 (0–26)	195.8 (110.2–348.1)	87.5 (74–101)	24.7 (13.6–44.7)	87.5 (74–101)
Polyarticular (n = 45)	11.7 (8–17.1)	22.2 (9.9–34.5)	198.5 (125.5–314)	88.9 (79.6–98.2)	17 (10.8–26.8)	**80* (68.2–91.8)**
RF-positive (n = 12)	22.4 (8.4–60.2)	25 (0–50.6)	285.1 (114.4–710.6)	91.7 (75.3–108)	12.7 (5.1–31.4)	**75* (49.4–100.6)**
RF-negative (n = 33)	9.2 (6.5–13.1)	21.2 (7–35.4)	174 (102.3–296)	87.9 (76.6–99.2)	18.9 (11.1–32.1)	**81.8* (68.5–95.2)**
Systemic (n = 18)	9.3 (5.5–15.5)	16.7 (0–34.4)	201.6 (102.4–396.7)	88.9 (73.9–103.8)	21.8 (11–42.9)	83.3 (65.6–101)
ERA (n = 8)	20 (5.8–68.5)	38 (1.6–73.4)	**538.2* (194.3–1490.8)**	87.5 (63–112)	26.9 (9.4–77)	87.5 (63–112)
DMARD/IS use						
Yes (n = 55)	10.5 (7.7–14.4)	16.4 (6.5–26.2)	230.6 (154.1–345.1)	89.1 (80.8–97.4)	21.9 (14.7–32.6)	85.5 (76.1–94.9)
No (n = 40)	10.7 (7.3–15.7)	25 (11.4–38.6)	197 (123.5–314.3)	87.5 (77.1–97.9)	18.4 (11.5–29.5)	**80* (67.4–92.6)**
MTX use						
Yes (n = 47)	11.1 (7.8–15.9)	17 (6.2–27.9)	211.7 (134.5–333.4)	87.2 (77.6–96.9)	19.1 (12.3–29.5)	**83* (72.1–93.8)**
No (n = 48)	10.1 (7.3–14.1)	22.9 (10.9–34.9)	219.8 (145.8–331.4)	89.6 (80.9–98.3)	21.7 (14.2–33.1)	**83.3* (72.7–94)**
Anti-TNF use						
Yes (n = 16)	11.4 (6.3–20.7)	18.8 (0–38.5)	306.4 (158.1–593.9)	100	26.9 (13.7–52.8)	93.8 (81.5–106)
No (n = 79)	10.4 (8–13.7)	20.3 (11.3–29.2)	201 (143.1–282.3)	**86.1* (78.4–93.8)**	19.2 (13.7–27)	**81* (72.3–89.7)**

GMT, Geometric mean titre; SP, seroprotection; FI, factor increase in GMT after vaccination; SC, seroconversion; RF, rheumatoid factor; ERA, enthesitis-related arthritis; MTX, methotrexate; TNF, tumour necrosis factor; DMARD, disease-modifying anti-rheumatic drug; IS, immunosuppressive drug.
Data are expressed as % or value (95% confidence interval).
* $p < 0.05$ compared to control group.

Table 2. Demographic data, juvenile idiopathic arthritis (JIA) subtypes, disease parameters, and treatment according to seroconversion in JIA patients.

	Seroconverted (n = 79)	Non-seroconverted (n = 16)	p
Demographic data			
Female gender	44 (55.7)	11 (68.7)	0.412
Current age (years)	14.7 ± 3.2	16.2 ± 2.7	0.086
Disease duration (years)	7.4 ± 4.5	8.4 ± 5.1	0.449
JIA subtypes			
Oligoarticular	21 (26.6)	3 (18.8)	0.754
Polyarticular	36 (45.6)	9 (56.3)	0.584
Systemic	14 (17.7)	3 (18.8)	1.000
ERA	8 (10.1)	1 (6.3)	1.000
Disease parameters			
Number of active joints	0 (0–16)	0 (0–28)	0.441
ESR (mm/1st h)	18 (1–83)	23 (2–55)	0.842
CRP (mg/dL)	1.8 (0.1–137.3)	1.9 (0.2–25.4)	0.505
Patient VAS, 0–100 mm	10 (0–80)	6 (0–80)	0.669
Physician VAS, 0–100 mm	10 (0–84)	12.5 (0–90)	0.718
CHAQ	0 (0–3)	0.125 (0–2)	0.588
Treatment			
Immunosuppressive drugs	47 (59.5)	8 (50)	0.582
Prednisone dose (mg/day)	5 (2.5–20)	–	–
MTX dose (mg/week)	25 (7.5–50)	30 (5–50)	0.661

ERA, Enthesitis-related arthritis; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; VAS, visual analogue scale; CHAQ, Childhood Health Assessment Questionnaire; MTX, methotrexate.
Data are expressed as number (%), mean ± standard deviation, or median (range).

Table 3. Disease activity parameters, patient and physician VAS and CHAQ of juvenile idiopathic arthritis (JIA) patients before and after vaccination.

Variable	Before vaccination	After vaccination	p
Disease activity			
Number of active joints	0 (0–28)	0 (0–18)	0.552
ESR (mm/1st h)	19 (1–83)	15 (0–83)	0.016
CRP (mg/dL)	1.9 (0.1–137.3)	2.7 (0.2–122.8)	0.073
Patient VAS, 0–100 mm	10 (0–80)	8.5 (0–80)	0.001
Physician VAS, 0–100 mm	10 (0–90)	6 (0–80)	0.002
CHAQ	0.123 (0–3)	0 (0–3)	0.058

VAS, Visual analogical scale; CHAQ, Childhood Health Assessment Questionnaire; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.
Data are expressed as median (range).

Vaccine safety

Adverse events were reported by 42.1% patients and 44% controls ($p = 0.882$). No severe adverse event was reported during up to 3 weeks of follow-up. Only acute and mild arthralgia was significantly higher in JIA patients after vaccination compared to controls (12.6% vs. 2.2%, $p = 0.01$), with a median duration of 1 day (1–5) and median time for arthralgia appearance of 1 day (1–12) after vaccination. The most frequent adverse events in patients and controls were local pain (21.1% vs. 23.1%, $p = 0.86$), headache (14.7% vs. 19.8%, $p = 0.438$), and myalgia (15.8% vs. 6.6%, $p = 0.063$).

Discussion

To our knowledge this is the largest study in JIA patients demonstrating that the adjuvant-free influenza A H1N1/2009 vaccine induces a reduced but adequate humoral response probably independent of disease parameters and treatment. However, no influenza infections were recorded, so only surrogate endpoints (immunogenicity) were measured.

The strength of our study lies in the inclusion of an age-balanced healthy control group because vaccine immune response has a distinct pattern in children and adolescents (20); those aged < 9 years were excluded because they require two doses for adequate immunogenicity (20, 21). The inclusion of all JIA subtypes is, however, a limitation because clinical and genetic features as well as treatment and outcomes are not uniform in each subgroup of patients (22).

Importantly, for pandemic influenza vaccines to be approved in a paediatric population, all three current standards must be met (9–11). Therefore, despite a reduced immune response in JIA patients, this population fulfilled all of the three criteria indicating an effective

immune response. Similarly, a satisfactory immunogenicity was observed with seasonal influenza vaccination in previous studies with juvenile rheumatic diseases (12), including JIA patients (14). By contrast, our recent report evidenced reduced humoral immune response for the same vaccine in adult rheumatoid arthritis, particularly in those under MTX therapy (23).

Although influenza-like symptoms after immunization were evaluated, the incidence of post-vaccination influenza infection determined by collection of respiratory samples was not assessed. Therefore, the real reduction of influenza infection risk could not be calculated. The short-term efficacy demonstrated here suggests the necessity of a second boost of vaccination in non-responders. In fact, in a previous study with HIV-infected patients, a second dose of the pandemic H1N1/2009 influenza vaccine resulted in an additional increase in seroconversion rate (24).

In the present study, a reduced seroconversion rate was demonstrated in the polyarticular JIA group, which included patients most often treated with immunosuppressive therapies. However, the low number of non-seroconverted JIA patients as well as the limited number of patients on prednisone precludes a definitive conclusion about the possible deleterious effect of these drugs on immunogenicity.

Our JIA patients had lower seroconversion rates compared to controls, although only for the polyarticular onset was the differences statistically significant. The sample size for the other subtypes may be too small for the difference to reach statistical significance. Furthermore, a high post-vaccination GMT was observed in ERA JIA patients. The very limited number of ERA patients may hamper the interpretation of this finding.

We found that immunosuppressive therapy does not seem to influence the pandemic influenza vaccine antibody response in JIA patients, as also evidenced in adults rheumatoid arthritis and ankylosing spondylitis (25). Previous studies have also reported no effect of these drugs on the immunogenicity of seasonal vaccine in juvenile rheumatic diseases patients, including small numbers of JIA patients (12–14). On the contrary, in adult systemic lupus erythematosus patients, immunosuppressive drugs were associated with significantly diminished seroprotection and seroconversion rates for the pandemic vaccine (26). An overall deleterious effect of glucocorticoid therapy on this immune response was also observed in a large cohort of patients with juvenile rheumatic disease (15). The specific analysis of JIA population of the present study did not confirm this association probably because of the limited number of patients under this therapy.

A recent study has reported that disease parameters may impair the pandemic influenza vaccine response in adult lupus patients (27). The exclusion of hospitalized patients in the present cohort hampered the interpretation of the potential relevance of disease activity in the pandemic vaccine antibody response because of the low representation of these patients.

Disease safety was supported by our findings of a stable number of patients with arthritis and acute phase reactants throughout the study. Reinforcing this finding, previous studies with hepatitis, measles, mumps, and rubella vaccination did not show any increase in JIA activity parameters (28, 29). However, the lack of a non-vaccinated JIA control group in the present study hampers the accurate assessment of the effect of H1N1 vaccination on JIA disease activity itself.

The use of a non-adjuvant vaccine was chosen in this study to avoid any autoimmune-related diseases (30). Influenza A H1N1/2009 vaccine was well tolerated and safe in the JIA patients and no serious short-term adverse events were found, as was reported previously in a limited number of JIA patients who received seasonal influenza vaccine (12, 13). Only mild and acute arthralgia was observed in our JIA patients, as reported previously in our large study with 237 paediatric patients with autoimmune rheumatic diseases (15).

In conclusion, this prospective study of pandemic influenza A H1N1/2009 vaccination in JIA patients suggests adequate immunogenicity probably independent of therapy with no short-term harmful effect on the disease itself.

Acknowledgements

This study was sponsored by grants from the Foundation for Research Support of the State of São Paulo (FAPESP 2009/51897-5 and 2010/10749-0 to EB), the National Council for Scientific and Technological Development (CNPQ302724/2011-7 to CAS and 301411/2009-3 to EB), the Federico Foundation (to CAS and EB), and the Butantan Foundation.

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Glucocorticoid: Major Factor for Reduced Immunogenicity of 2009 Influenza A (H1N1) Vaccine in Patients with Juvenile Autoimmune Rheumatic Disease

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ABSTRACT. ***Objective.*** To assess the immunogenicity and safety of non-adjuvanted influenza A H1N1/2009 vaccine in patients with juvenile autoimmune rheumatic disease (ARD) and healthy controls, because data are limited to the adult rheumatologic population.

Methods. A total of 237 patients with juvenile ARD [juvenile systemic lupus erythematosus (JSLE), juvenile idiopathic arthritis (JIA), juvenile dermatomyositis (JDM), juvenile scleroderma, and vasculitis] and 91 healthy controls were vaccinated. Serology for anti-H1N1 was performed by hemagglutination inhibition assay. Seroprotection rate, seroconversion rate, and factor-increase in geometric mean titer (GMT) were calculated. Adverse events were evaluated.

Results. Age was comparable in patients and controls (14.8 ± 3.0 vs 14.6 ± 3.7 years, respectively; p = 0.47). Three weeks after immunization, seroprotection rate (81.4% vs 95.6%; p = 0.0007), seroconversion rate (74.3 vs 95.6%; p < 0.0001), and the factor-increase in GMT (12.9 vs 20.3; p = 0.012) were significantly lower in patients with juvenile ARD versus controls. Subgroup analysis revealed reduced seroconversion rates in JSLE (p < 0.0001), JIA (p = 0.008), JDM (p = 0.025), and vasculitis (p = 0.017). Seroprotection (p < 0.0001) and GMT (p < 0.0001) were decreased only in JSLE. Glucocorticoid use and lymphopenia were associated with lower seroconversion rates (60.4 vs 82.9%; p = 0.0001; and 55.6 vs 77.2%; p = 0.012). Multivariate logistic regression including diseases, lymphopenia, glucocorticoid, and immunosuppressants demonstrated that only glucocorticoid use (p = 0.012) remained significant.

Conclusion. This is the largest study to demonstrate a reduced but adequate immune response to H1N1 vaccine in patients with juvenile ARD. It identified current glucocorticoid use as the major factor for decreased antibody production. The short-term safety results support its routine recommendation for patients with juvenile ARD. ClinicalTrials.gov; NCT01151644. (First Release Nov 15 2011; J Rheumatol 2012;39:167–73; doi:10.3899/jrheum.110721)

Key Indexing Terms:

VACCINE	SAFETY	IMMUNOGENICITY
PANDEMIC INFLUENZA A (H1N1)	CHILDREN	RHEUMATIC DISEASE

Infection remains a leading cause of morbidity and mortality in patients with juvenile autoimmune rheumatic diseases (ARD). The combined immunosuppressive effects of the disease itself and its treatment render the individual more susceptible to infections. Further, intercurrent infections may contribute to rheumatic disease flares[1,2,3]. The recent

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Supported by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 2010/10749-0 to Dr. Bonfa), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ 300248/2008-3 to Dr. Silva, 300665/2009-1 to Dr. Carvalho, 301411/2009-3 to Dr. Bonfa, and 300559/2009-7 to Dr. Pereira), the Federico Foundation (Dr. Silva, Dr. Carvalho, Dr. Bonfa, Dr. Pereira), and the Butantan Foundation.

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Accepted for publication August 22, 2011.

pandemic caused by the new influenza A H1N1/2009 virus led to a higher incidence of hospitalization and death than the annual rates associated with the seasonal influenza viruses[4], especially in immunosuppressed patients. Of note, vaccination is the most effective measure to control the spread of the virus and to reduce associated morbidity and mortality.

Based on concerns that influenza A H1N1/2009-like viruses would continue to circulate during the next influenza season, the 2010 Recommendations of the Advisory Committee on Immunization Practices stated that all children and adolescents aged between 6 months and 18 years should receive the trivalent seasonal influenza vaccine containing the A/California/7/2009(H1N1)-like virus[5]. According to these recommendations, vaccination is particularly important for patients at increased risk for severe complication, including those with chronic conditions, such as juvenile ARD, particularly in patients under immunosuppressive therapy[5].

However, a point of concern is whether the immune response to this vaccine is significantly impaired by rheumatic disease itself and/or its treatment. To date, no study had evaluated the efficacy and safety of the influenza A H1N1/2009 vaccine in patients with juvenile ARD. A few studies with small populations evaluated the immune response to other vaccines in these patients[6,7,8]. Kanakoudi-Tsakalidou, *et al* showed a satisfactory antibody response to the seasonal influenza immunization in patients with juvenile rheumatic diseases under immunosuppressive therapies[6]. In contrast, studies on immunosuppressed non-rheumatic children and adolescents, such as those with cancer and after kidney transplant, revealed a limited response to the influenza A H1N1/2009 vaccine[9,10].

The aim of our study was to evaluate the immunogenicity and safety of influenza A H1N1/2009 vaccine in patients with juvenile ARD compared to healthy controls.

MATERIALS AND METHODS

Patients and controls. A total of 237 outpatients routinely followed at the Pediatric Rheumatology Unit and the Rheumatology Division of Clinics Hospital, São Paulo, with juvenile ARD were included. All patients fulfilled the international classification criteria as follows: for juvenile systemic lupus erythematosus (JSLE)[11], juvenile idiopathic arthritis (JIA)[12], juvenile scleroderma (JScl)[13], juvenile dermatomyositis (JDM)[14], Behçet disease[15], Takayasu arteritis[16], granulomatosis with polyangiitis (previously denoted Wegener granulomatosis)[16], polyarteritis nodosa[16], and Henoch-Schönlein purpura or Kawasaki disease[17]. A total of 91 age-matched healthy subjects were concomitantly included in the control group. All participants were ≥ 9 and ≤ 21 years old, and exclusion criteria included previous proven infection by influenza A H1N1/2009; anaphylactic response to vaccine components or to egg; previous vaccination with any live vaccine 4 weeks before or any inactivated vaccine 2 weeks before the study; 2010 seasonal influenza vaccination; acute infection resulting in fever over 38˚C at the time of vaccination; Guillain-Barré syndrome or demyelinating syndromes; blood transfusion within 6 months; and hospitalization.

Study design. This was a prospective, open study conducted between March 2010 and April 2010. All patients with juvenile ARD were invited by letter to participate in the public health influenza A H1N1/2009 vaccine campaign at the immunization center of the same hospital. Healthy volunteers who came to this center seeking vaccination in response to the national public health campaign were included in the control group. This protocol was approved by the local institutional review board, and informed consent was obtained from all participants. The study was registered with clinicaltrials.gov under NCT01151644.

A single intramuscular dose (0.5 ml) of H1N1 A/California/7/2009-like virus vaccine (A/California/7/2009/Butantan Institute/Sanofi Pasteur) was administered to all participants. Patients and controls were evaluated on the day of vaccination (from March 22 to April 2) and after 3 weeks. Blood samples were obtained from each participant immediately before and 21 days after vaccination.

Vaccine. A novel monovalent, non-adjuvanted, inactivated, split-virus vaccine was supplied by Butantan Institute/ Sanofi Pasteur (São Paulo, Brazil). The vaccine contained an inactivated split influenza virus with 15 μg hemagglutinin antigen equivalent to the A/California/7/2009 (H1N1) virus-like strain (NYMCx-179A), one of the candidate reassortant vaccine viruses recommended by the WHO. Embryonated chicken eggs were employed using the same standard techniques for the production of seasonal, trivalent, inactivated influenza vaccine. The vaccine was presented in 5-ml multidose vials with thimerosal (45 μg per 0.5-ml dose) as a preservative.

Hemagglutination inhibition assay. Antibody levels against H1N1 A/California/7/2009-like virus were evaluated using the hemagglutination inhibition assay (HIA) at the Adolfo Lutz Institute.

Sera were tested for antibodies to the H1N1 A/California/7/2009 influenza strain supplied by Butantan Institute. Sera were tested at an initial dilution of 1:10, and at a final dilution of 1:2560. For the purposes of calculations, negative titers had an assigned value of 1:5, and titers > 1:2560 a value of 1:2560. Samples were tested in duplicate, and geometric mean values were used in the analysis.

Virus concentrations were previously determined by hemagglutinin antigen titration, and the HIA test was performed after removing naturally occurring nonspecific inhibitors from the sera as described[18].

The immunogenicity endpoints after vaccination were the seroprotection rate (titer ≥ 1:40), seroconversion rate (prevaccination titer < 1:10 and postvaccination HIA titer ≥ 1:40 or prevaccination titer ≥ 1:10 and postvaccination titer ≥ 4-fold increase), geometric mean titers (GMT), and factor-increase in GMT (ratio of GMT after vaccination to GMT before vaccination).

Safety assessment. At the day of vaccination, parents were given a 21-day personal diary card containing the following list of predefined adverse events: local reactions (pain, redness, swelling, and itching) and systemic adverse events (arthralgia, fever, headache, myalgia, sore throat, cough, diarrhea, rhinorrhea, and nasal congestion). Participants were asked to give "yes/no" responses for each side effect and to return their diary cards at the second evaluation day (21 days after vaccination). Adverse events that were not on the list were also reported.

All local reactions were considered related to the influenza A H1N1/2009 vaccine, while systemic adverse events were analyzed by the investigators to determine causality. Severe side effects were defined as those requiring hospitalization or death.

Statistical analysis. The sizes of the juvenile ARD population and controls gave the study a power of analysis > 95%.

The immunogenicity and safety analyses were descriptive, and 2-sided 95% CI were calculated assuming binomial distributions for dichotomous variables and log-normal distribution for hemagglutination inhibition titers. For prednisone and immunosuppressant drug use, seroprotection rate, seroconversion rate, and adverse events, Fisher's exact test was used. GMT were compared between each subgroup of patients with juvenile ARD and the control group using a 2-sided Student t test or Mann-Whitney U test on the $\log_{10}$-transformed titers. The factor-increase in GMT was also calculated for all participants. Spearman's correlation was used to compare the $\log_{10}$-transformed titers and $\log_{10}$-transformed factor-increase with gluco-

corticoid dose. Multivariate logistic regression analysis was performed using seroconversion rate as the dependent variable and the variables with $p < 0.2$ in the univariate analyses as independent variables (JSLE, JIA, JDM, primary vasculitis, glucocorticoid use, concomitant glucocorticoid and immunosuppressant use, and lymphopenia). All tests were 2-sided, and significance was set at a p value < 0.05.

RESULTS

In total, 237 patients with the following juvenile ARD were studied: 99 JSLE, 93 JIA, 18 JDM, 11 JScl [5 systemic sclerosis (SSc) and 6 localized scleroderma], and 16 primary vasculitis (5 Henoch-Schönlein purpura, 3 Takayasu arteritis, 3 granulomatosis with polyangiitis, 3 polyarteritis nodosa, 1 Kawasaki disease, and 1 Behçet disease), and 91 healthy controls (Table 1).

Patients and controls were comparable regarding mean current age (14.8 ± 3.0 yrs vs 14.6 ± 3.7 years, respectively; p = 0.47), with a predominance of females among patients with ARD (66% vs 52%; p = 0.02). Mean disease duration was 5.8 ± 3.7 years. Ninety patients (38%) were taking glucocorticoids, with a mean dose of prednisone 17.4 ± 14.2 mg/day (0.36 ± 0.32 mg/kg/day), and mean glucocorticoid duration of 43.1 ± 34.5 months, and 84.5% of patients had a diagnosis of JSLE. Sixty percent (60.3%) of patients were treated with immunosuppressive agents, and more than half (51.7%) were under methotrexate (MTX) therapy (Table 1).

Influenza A H1N1/2009 vaccine immunogenicity. Seroprotection and seroconversion rates of patients and controls are shown in Table 2. At baseline, seroprotective antibody titer ≥ 1:40 was seen in 22.4% (n = 53) of patients with juvenile ARD and 20.9% (n = 19) of controls (p = 0.882; Table 2). After 21 days, the vaccine seroprotection rate was 81.4% (95% CI 76.5%–86.4%) in patients with juvenile ARD, significantly lower than in controls (95.6%; 95% CI 91.4%–99.8%; p = 0.0007). Moreover, following vaccination, the seroconversion rate was significantly lower in patients with juvenile ARD compared to controls [74.3% (95% CI 68.7%–79.9%) vs 95.6% (95% CI 91.4%–99.8%); p < 0.0001]. As for immunogenicity in each rheumatic disease, seroprotection rates prior to vaccination were comparable between patients and controls. The postvaccination seroprotective rate was lower in patients with JSLE compared to controls (p < 0.0001), and a tendency of a reduced rate was observed in those with primary vasculitis (p = 0.067). Of note, seroconversion rates were reduced in patients with JSLE (p < 0.0001), JIA (p = 0.008), JDM (p = 0.025), and primary vasculitis (p = 0.017) compared to controls (Table 2).

Table 1. Distributions of rheumatic diseases and therapies in 237 patients. Data are the mean ± SD or n (%).

Feature	
Disease	
Juvenile systemic lupus erythematosus	99 (41.8)
Juvenile idiopathic arthritis	93 (39.2)
Juvenile dermatomyositis	18 (7.6)
Juvenile scleroderma	11 (4.6)
Primary vasculitis	16 (6.8)
Treatment	
Prednisone	90 (38)
Dose, mg/day	17.4 ± 14.2
Dose, mg/kg/day	0.36 ± 0.32
Dose ≥ 20 mg/day	36 (40)
Duration of glucocorticoid therapy, mo	43.1 ± 34.5
Immunosuppressant	143 (60.3)
Methotrexate	74 (51.7)
Azathioprine	43 (30.1)
Cyclosporine	23 (16.1)
Mycophenolate mofetil	13 (9.1)
Leflunomide	6 (4.2)
Cyclophosphamide	3 (2.1)

The GMT values in patients with juvenile ARD and controls are illustrated in Table 3. GMT after immunization [147.2 (95% CI 119.7–181.1) vs 250.8 (95% CI 196.3–320.3); p = 0.011] and the factor-increase in GMT [12.9 (95% CI 10.7–15.7) vs 20.3 (95% CI 15.6–26.4); p = 0.012] were significantly lower in the ARD group compared to the control group. Disease evaluations for specific patient subgroups revealed lower GMT after immunization and also a lower factor-increase in GMT only in patients with JSLE compared to controls (p < 0.0001; Table 3).

Further analysis of the influence of therapy on immunogenicity revealed a lower percentage of seroconversion among patients using glucocorticoids compared to those without this medication (60.4% vs 82.9%; p = 0.0001). There was no difference in rates for seroprotection (p = 0.247) or seroconversion (p = 0.279) between patients taking prednisone < 20 mg/day and those taking ≥ 20 mg/day. However, a trend for lower GMT and factor-increase in GMT after vaccination was observed among patients taking prednisone > 20 mg/day [49.4 (95% CI 28.9–84.7) vs 95.2 (95% CI 63.4–143.1), p = 0.076, and 5.3 (95% CI 3.4–8.3) vs 9.3 (95% CI 6.6–13.2), p = 0.054, respectively]. Also, a significant negative correlation was observed regarding glucocorticoid dose and $\log_{10}$-transformed titers (r = –0.36, p < 0.0001), as well as glucocorticoid dose and $\log_{10}$-transformed factor-increase of GMT (r = –0.30, p < 0.0001).

Concerning immunosuppressant use, no differences in the seroconversion rate (76.4% vs 75.5%; p = 0.763), seroprotection rate (80.4% vs 83%; p = 0.733), or GMT [130.3 (95% CI 99.3-170.8) vs 177.4 (95% CI 129.7-242.6); p = 0.151] were observed comparing patients taking and not taking these drugs. The specific analysis of MTX, azathioprine, cyclosporine, mycophenolate mofetil, leflunomide, and cyclophosphamide revealed no effects on seroconversion and seroprotection (p > 0.05) in patients taking and not taking these drugs. A reduced postvaccination GMT was observed only for patients taking azathioprine (p = 0.019) and mycophenolate mofetil (p = 0.01). Concomitant use of immunosuppressive therapy and glucocorticoid resulted in a

Table 2. Seroprotection and seroconversion rates of influenza A (H1N1) 2009 vaccine in patients with rheumatic disease and controls.

	N	Seroprotection Rate (titer ≥ 1/40) Before Immunization, % (95% CI)	Seroprotection Rate (titer ≥ 1/40) After Immunization, % (95% CI)	Seroconversion Rate, % (95% CI)
Control	91	20.9 (12.6–29.3)	95.6 (91.4–99.8)	95.6 (91.4–99.8)
JARD	237	22.4 (17.1–27.7)	81.4 (76.5–86.4)*	74.3 (68.7–79.9)*
JSLE	99	20.2 (12.3–28.1)	73.7 (65.0–82.4)*	63.6 (54.1–73.1)*
JIA	93	20.4 (12.2–28.6)	88.2 (81.6–94.8)	82.8 (75.1–90.5)*
JDM	18	38.9 (16.4–61.4)	83.3 (66.1–100.5)	77.8 (58.6–97.0)*
JScl	11	27.3 (1.0–53.6)	90.9 (73.9–107.9)	90.9 (73.9–107.9)
Primary vasculitis	16	25.0 (13.8–46.2)	81.3 (62.2–100.4)	75 (53.8–96.2)*

* $p < 0.05$. JARD: juvenile autoimmune rheumatic diseases; JSLE: juvenile systemic lupus erythematosus; JIA: juvenile idiopathic arthritis; JDM: juvenile dermatomyositis; JScl: juvenile scleroderma.

Table 3. Geometric mean titers and factor-increases in the geometric mean titer after influenza A (H1N1) 2009 vaccination in patients with juvenile autoimmune rheumatic disease and controls.

	N	Geometric Mean Titer Before Immunization, % (95% CI)	Geometric Mean Titer After Immunization, % (95% CI)	Factor-increase in Geometric Mean Titer (95% CI)
Control	91	12.4 (9.7–15.7)	250.8 (196.3–320.3)	20.3 (15.6–26.4)
JARD	237	11.4 (9.7–13.3)	147.2 (119.7–181.1)*	12.9 (10.7–15.7)*
JSLE	99	10.9 (8.5–13.9)	91.1 (66.0–125.8)*	8.4 (6.3–11.2)*
JIA	93	10.8 (8.4–13.8)	217.2 (159–296.7)	20.2 (14.8–27.5)
JDM	18	15.3 (8.9–26.3)	201.6 (95.4–425.8)	13.2 (7.2–24.1)
JScl	11	12.1 (6.0–24.2)	181.5 (70.2–469.4)	15.0 (6.3–35.9)
Primary vasculitis	16	14.1 (6.8–29.2)	182.2 (68.1–487.4)	12.9 (5.9–28.2)

* $p < 0.05$. JARD: juvenile autoimmune rheumatic diseases; JSLE: juvenile systemic lupus erythematosus; JIA: juvenile idiopathic arthritis; JDM: juvenile dermatomyositis; JScl: juvenile scleroderma.

lower seroconversion rate compared to patients without immunosuppressive or glucocorticoid therapy (64.8% vs 78.3%; $p = 0.0352$).

In the analysis of lymphocyte count, patients with juvenile ARD with lymphopenia (lymphocyte count < 1000/mm^3) showed a significantly lower seroconversion rate compared to those without this complication (55.6% vs 77.2%, respectively; $p = 0.012$).

Multivariate logistic regression was performed to determine possible deleterious factors for the seroconversion rate [i.e., disease (JSLE, JIA, JDM, primary vasculitis), lymphopenia (lymphocyte count < 1000/mm^3), or glucocorticoid use or concomitant glucocorticoid and immunosuppressant]. Only glucocorticoid use remained significant (OR 0.20, 95% CI 0.06–0.70, $p = 0.012$; Table 4). Reinforcing this finding, a significant negative correlation was observed between glucocorticoid dose and $\log_{10}$-transformed titers ($r = –0.36$, $p < 0.0001$), as well as between glucocorticoid dose and $\log_{10}$-transformed factor-increase of GMT ($r = –0.30$, $p < 0.0001$).

Vaccine safety. Local and systemic adverse events reported within 21 days of vaccination are summarized in Table 5.

Table 4. Multivariate logistic regression analyses including current treatment and lymphopenia as independent variables for seroconversion in patients with juvenile autoimmune rheumatic diseases after influenza A (H1N1) 2009 vaccination.

Variable	OR (95% CI)	p
JSLE	0.36 (0.039–3.33)	0.368
JIA	0.45 (0.05–3.83)	0.47
JDM	0.51 (0.05–5.70)	0.586
Primary vasculitis	0.60 (0.05–7.21)	0.691
Glucocorticoid use	0.20 (0.06–0.70)	0.012
Concomitant use of glucocorticoid plus immunosuppressant	2.71 (0.90–8.20)	0.077
Lymphopenia	0.61 (0.27–1.38)	0.235

JSLE: juvenile systemic lupus erythematosus; JIA: juvenile idiopathic arthritis; JDM: juvenile dermatomyositis.

Local itching was reported exclusively by patients with juvenile ARD ($p = 0.003$). The only systemic reaction more frequently observed in patients was arthralgia (13.1% vs 2.2% in controls; $p = 0.002$), with a median duration of 1 (range 1–9) days and median time of appearance after vac-

Table 5. Adverse events following influenza A (H1N1) 2009 vaccination in patients with juvenile autoimmune rheumatic diseases (JARD) and controls. Data are n (%).

Adverse Events	JARD, n = 237	Control, n = 91	p
Local reactions	60 (25.3)	21 (23.1)	0.78
Pain	43 (18.1)	21 (23.1)	0.35
Redness	9 (3.8)	2 (2.2)	0.73
Swelling	3 (1.3)	2 (2.2)	0.62
Itching	19 (8)	0 (0.0)	0.003
Systemic reactions	84 (35.4)	27 (29.7)	0.36
Arthralgia	31 (13.1)	2 (2.2)	0.002
Fever	13 (5.5)	3 (3.3)	0.57
Headache	41 (17.3)	18 (19.8)	0.63
Myalgia	27 (11.4)	6 (6.6)	0.22
Sore throat	9 (3.8)	5 (5.5)	0.54
Cough	16 (6.8)	5 (5.5)	0.8
Diarrhea	8 (3.4)	2 (2.2)	0.73
Rhinorrhea	19 (8)	3 (3.3)	0.15
Nasal congestion	13 (5.5)	3 (3.3)	0.57

cination of 0 (range 0–12) days. No severe side effects were observed in patients or controls (Table 5).

DISCUSSION

Our study is the largest analysis in patients with juvenile ARD to demonstrate that the non-adjuvanted influenza A H1N1/2009 vaccine is safe and exhibits a reduced immunogenicity associated with glucocorticoid therapy.

This was the first report that evaluated the influenza A H1N1/2009 vaccine response in a cohort of pediatric patients with rheumatic diseases. All patients who agreed to participate were included regardless of disease activity status or current treatment, to closely represent the real-life situation. Also, all patients fulfilled the international criteria for juvenile ARD, and the study benefited from the inclusion of a large patient population, an essential requirement to accurately define vaccine immunoresponse and safety, which was not met by previous studies of seasonal influenza vaccine[6,8]. Moreover, age-matching of the control group is essential because effectiveness of vaccine has a distinct pattern in children and adolescents[19]. Our report included only patients over age 9 years, excluding younger children, who have a lesser humoral response to influenza A H1N1/2009 vaccine[19,20].

This study design provided strong evidence that the immunoresponse to influenza A H1N1/2009 vaccine was impaired in the juvenile ARD population, in contrast to previous studies on seasonal influenza vaccination[6,7,8]. In this regard, Malleson, *et al* evaluated 34 children with chronic arthritis (91% JIA) and observed similar seasonal vaccine immunogenicity in patients and 13 controls, independent of the use of prednisone or immunosuppressive agents[7]. The lack of age-matching to controls hampers the interpretation of their findings due to the inclusion of extremes of age[7]. In addition, the adequate humoral response reported for children with JIA, JSLE, JDM, and other rheumatic diseases was also not conclusive due to overrepresentation of JIA in the sample and the lack of a healthy control group[6]. On the other hand, in the study of Ogimi, *et al*, the 49 patients with rheumatic disease and 36 with juvenile chronic diseases in the control group had unexpectedly low immunoresponses to the seasonal influenza, although it was comparable between groups[8]. Again, the inclusion of infants and the vaccination protocol used in that study may account for the impaired response that was observed[8].

Of note, our disease subgroup analysis revealed a reduced protective immunogenicity against the pandemic influenza A H1N1/2009 vaccine in all rheumatic autoimmune conditions except JScl. Similarly, we have recently observed an adequate response for this vaccine in adult patients with SSc[21], and effective humoral and cellular responses to an adjuvanted virosomal nonpandemic flu vaccine were also reported in others with this disease[22].

The immunoresponse was considerably compromised in our patients with JSLE, as indicated by the inadequate postseroprotection and postseroconversion rates, deficient increase in GMT, and low factor-increase in GMT, suggesting a more severely impaired immune state in persons with this illness that may ultimately affect the response to antigenic challenge[23]. The well-known lupus intrinsic antibody and cellular dysfunction[24] may account for this finding, which is reinforced by the observation of decreased antibody response[25] and cell-mediated response to influenza vaccination in adult SLE[26].

With regard to JIA, a diminished vaccine response, determined by the significantly lower seroconversion rate, was observed, although it was higher than that in juvenile lupus, in spite of comparable postimmunization seroprotection, GMT, and factor-increase in GMT. The preimmunization rate cannot account for this finding because it was similar to that of the control group. In contrast, previous reports suggest apparently adequate vaccine responses for seasonal influenza[8] and hepatitis[27] in persons with JIA. The inclusion of patients or controls younger than age 9 years[8,27] and 3 years old[8] precludes a definitive conclusion about their findings, as vaccine responses in these 2 age brackets are expected to be much lower than in older children.

Patients with JDM had a deficient seroconversion rate, which is in accord with a report for the same vaccine in adult DM[21]. This finding may be associated with the underlying pathology of this disease, which is known to involve the humoral endotheliopathy initiated by complement deposition in intramuscular blood vessels[28].

The lower immune response to vaccine that we observed in the primary vasculitis group contrasts with the adequate response in reports concerning adult patients with granulomatosis with polyangiitis immunized with seasonal[29] and pandemic H1N1 vaccine[21]. The most likely explanation for

this discrepancy is the limited number of children with primary vasculitis analyzed in our study and the underrepresentation of granulomatosis with polyangiitis in our sample.

Alternatively, a vaccine response may be affected by immunosuppressive therapy, and we determined by multivariate analysis that glucocorticoid therapy was the main contributing factor to a reduced immunoresponse in patients with juvenile ARD. There are conflicting data regarding this drug[30], with a few reports describing no effects on influenza vaccine response in children with rheumatic diseases[6,7,8]. However, the prednisolone dose was described in only 1 of these studies, and it was quite low (0.21 ± 0.16 mg/kg), making it difficult to determine the influence of this drug on vaccine immunogenicity[8]. In contrast, others have reported an attenuated immune response to seasonal influenza vaccination in patients with SLE and asthma under glucocorticoid therapy[25,31]. Indeed, Holvast, *et al* found that glucocorticoid and/or immunosuppressant was associated with lower humoral and cell-mediated responses against the H1N1 strain of seasonal influenza vaccine in adult SLE[25,26].

Interestingly, in our study the seroconversion rate was not affected by the use of immunosuppressive drugs other than glucocorticoid. However, this analysis was uncertain because MTX represented more than half of the immunosuppressive drugs used, and there was a clear bias of indication by disease. In this regard, an extensive separate analysis of disease activity and drug influence in JSLE and JIA is under way. Nevertheless, previous studies with pediatric and adult rheumatic patients have suggested no deleterious effect of immunosuppressive drugs on antibody responses to seasonal influenza vaccine[6,32,33].

We observed that lymphopenia also reduced seroconversion to unadjuvanted influenza A H1N1/2009 vaccine in patients with juvenile ARD. The response to influenza vaccine depends on adequate antigen processing and presentation, and normal interaction between T and B cells and their activation[25,26]. Studies in patients infected with HIV-1 have shown that anti-influenza-specific antibody responses correlated with the CD4 T cell count[34]. Indeed, HIV-1 infected patients generated poorer responses to monovalent influenza A H1N1/2009 vaccine compared to healthy subjects[35,36].

For pandemic influenza vaccines to be licensed they must meet all 3 current immunologic standards established for seasonal vaccines, which include a percentage of seroprotection > 70%, a percentage of seroconversion > 40%, and a factor-increase in GMT > 2.5[37,38]. These criteria were established for healthy adults aged 18 to 60 years, but were also proposed to be used among the pediatric population[39]. Therefore, although our population of patients with juvenile ARD presented lower percentages of seroprotection and seroconversion and a lower factor-increase in GMT compared to healthy controls, these patients still achieved all of the 3 established immunologic thresholds, showing that the vaccine, while being less immunogenic, was effective in protecting them.

Influenza A (H1N1) vaccine was well tolerated and safe in patients with juvenile ARD, as no serious short-term adverse event was observed. Arthralgia was a more frequent complaint of patients with juvenile ARD compared to healthy controls. Studies on influenza A/H1N1 2009 vaccine in healthy children and adolescents have not reported musculoskeletal complaints[19,20], suggesting that the occurrence of this manifestation could be related to the patient's genetic background for rheumatic disease[40].

Our study revealed a reduced but adequate immune response to the unadjuvanted influenza A H1N1/2009 vaccine in patients with juvenile autoimmune rheumatic diseases, and identified current glucocorticoid use as the major factor for decreased antibody production. The short-term safety results support routine recommendation for vaccination for patients with juvenile ARD.

ACKNOWLEDGMENT

We thank the subjects for their critical roles in our study, and the staff of Hospital das Clinicas, Faculdade de Medicina da Universidade de São Paulo; Laboratorio de Investigação Médica (LIM-17), Faculdade de Medicina da Universidade de São Paulo; Adolfo Lutz Institute; and Butantan Institute.

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Influenza A H1N1/2009 vaccine in juvenile dermatomyositis: reduced immunogenicity in patients under immunosuppressive

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Abstract

Objectives

The aim of the present paper is to assess the influence of demographic, muscle enzymes, JDM scores and treatment on non-adjuvanted influenza A H1N1/2009 vaccine immunogenicity in juvenile dermatomyositis (JDM) patients.

Methods

Thirty JDM patients and 81 healthy age-matched controls were vaccinated. All participants were evaluated pre- and 21 days post-vaccination and serology for anti-H1N1 was performed by haemagglutination inhibition assay. Muscle enzymes, JDM scores and treatment were evaluated before and after vaccination. Adverse events were reported.

Results

After immunisation, seroconversion rates were significantly lower in JDM patients compared to age-matched controls (86.7 vs. 97.5%, p=0.044), whereas seroprotection (p=0.121), geometric mean titres (GMT) (p=0.992) and factor increase (FI) in GMT (p=0.827) were similar in both groups. Clinical and laboratorial evaluations revealed that JDM scores and muscle enzymes remained stable throughout the study (p>0.05). A higher frequency of chronic course was observed in non-seroconvert compared to seroconverted (100% vs. 27%, p=0.012). Regarding treatment, a lower rate of seroconversion was observed in patients under prednisone>20mg/day mg/day (50% vs. 4%, p=0.039), and in those treated with a combination of prednisone, methotrexate and cyclosporine (50% vs. 4%, p=0.039). Local and systemic vaccine adverse events were mild and similar in patients and controls (p>0.05).

Conclusion

This study identified that chronic course and immunosuppressive therapy is the major factor hampering seroconversion in JDM, suggesting that a specific protocol may be required for this subgroup of patients. In spite of that, a single dose of non-adjuvanted influenza A/H1N1 2009 vaccine was generally seroprotective in this disease with no evident deleterious effect in disease itself (ClinicalTrials.gov, no. NCT01151644).

Key words

vaccine, immunogenicity, influenza A H1N1/2009, children, juvenile dermatomyositis

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Received on December 27, 2011; accepted in revised form on March 14, 2012.

Funding: This study was supported by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP no. 2009/51897-5 to EB and no. 2010/10749-0 to EB), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ no. 300559/2009-7 to RMRP, no. 2011/12471-2 to CAS and no. 301411/2009-3 to EB), Federico Foundation (to RMRP, CAS and EB) and and Butantan Foundation.

Competing interests: none declared.

Introduction

Improvements in the diagnosis and management of juvenile dermatomyositis (JDM) have significantly enhanced survival over the last decades (1-4). The treatment used in these patients and disease itself may induce immunosuppression with a consequent increase in infection susceptibility (5-7). Therefore, vaccination emerges as an essential prevention tool in pediatric rheumatologic disease (5, 8).

Recently, the European League Against Rheumatism (EULAR) task force has reinforced the relevance of vaccination in immunosuppressed pediatric rheumatologic patients, due to high risk of severe infection (8). Accordingly, the influenza A H1N1/2009 vaccination was recommended for all immunosuppressed patient (9), due to the high incidence of hospitalisation and death in this particular group of patients reported during the 2009 pandemic (10).

There are scarce data in the literature regarding H1N1 influenza vaccine in JDM patients and all of them are restricted to overall immunogenicity and safety (11-13). Ogimi *et al*. evaluated the immune response of influenza vaccine in small cohort of juvenile autoimmune rheumatic diseases, including only 6 JDM patients, and reported immune response comparable to controls (11). Only 3 JDM patients were evaluated in the study of Kanakoudi-Tsakalidou *et al*., thus precluding a definitive conclusion about their findings (12). We have recently assessed immunogenicity and safety of the non-adjuvanted influenza A H1N1/2009 vaccine in 237 juvenile autoimmune rheumatic diseases, including only 18 JDM patients, and showed an overall short-term safety with reduced immune response associated with glucocorticoid use (13), without a specific analysis of this subgroup patients.

Moreover, the possible role of demographic, disease and therapy factors in vaccine antibody response and the potential impact of vaccine in JDM disease parameters need to be determined. Gender and age are relevant for immunogenicity, since female gender has higher antibodies titers to a large number of viral vaccine (14) and patients younger than 9 years old may induce lesser humoral response to influenza A H1N1(15, 16). Treatment was also identified to contribute to vaccine response in lupus patients (17) and there were reports suggesting that the vaccine may induce flare in systemic lupus erythematosus patients (18).

Therefore, the objectives of this study were to assess the possible association between seroconversion rate with demographic data, muscle enzymes, JDM scores, lymphopenia and treatment in JDM patients, as well as the possible deleterious effect of the non-adjuvanted influenza A H1N1/2009 in the disease itself.

Methods

Thirty consecutive JDM outpatients, including 18 JDM patients of our previous study (13), routinely followed at the Pediatric Rheumatology Unit and the Rheumatology Division of Clinics Hospital, São Paulo, Brazil, were included in this study. All patients fulfilled the international classification criteria for JDM (19). A total of 81 age-matched healthy subjects were concomitantly included in the control group. All participants were ≥9 and ≤21 years old, and exclusion criteria included previous proven infection by influenza A H1N1/2009, anaphylactic response to vaccine components or to egg, previous vaccination with any live vaccine four weeks before or any inactivated vaccine two weeks before the study, 2010 seasonal influenza vaccination, acute infection resulting in fever over 38°C at the time of vaccination, Guillain-Barré syndrome or demyelinating syndromes, blood transfusion within six months, and hospitalisation (13).

Study design

This was a prospective, open study conducted between March 2010 and April 2010. All JDM patients were invited by letter to participate in the Public Health influenza A H1N1/2009 vaccine campaign at the Immunisation Centre of the same hospital. Healthy volunteers who came to this centre seeking vaccination in response to the Public Health National Campaign were included as control group. This protocol was ap-

proved by the Local Institutional Review Board, and informed consent was obtained from all participants or their legal guardian. The study was registered at clinicaltrials.gov under no. NCT01151644.

A single intramuscular dose (0.5 ml) of H1N1 A/California/7/2009-like virus vaccine (A/California/7/2009/Butantan Institute/Sanofi Pasteur) was administered to all participants. Patients and controls were evaluated on the day of vaccination (from March 22nd to April 2nd) and after three weeks. Blood samples were obtained from each participant immediately before and 21 days after vaccination.

Vaccine

A novel monovalent, non-adjuvanted, inactivated, split-virus vaccine was supplied by Butantan Institute/Sanofi Pasteur (São Paulo, Brazil). The vaccine contained an inactivated split influenza virus with 15 μg of haemagglutinin antigen equivalent to the A/California/7/2009 (H1N1) virus-like strain (NYMCx-179A), one of the candidate reassortant vaccine viruses recommended by the WHO. Embryonated chicken eggs were employed using the same standard techniques for the production of seasonal, trivalent, inactivated influenza vaccine. The vaccine was presented in 5-ml multi-dose vials with thimerosal (45 μg per 0.5-ml dose) as a preservative.

Haemagglutination inhibition assay

The antibody levels against H1N1 A/California/7/2009-like virus were evaluated using the haemagglutination inhibition assay (HIA) at the Adolfo Lutz Institute. Sera were tested for antibodies to the H1N1 A/California/7/2009 influenza strain supplied by Butantan Institute at an initial dilution of 1:10, and at a final dilution of 1:2560. For calculation purpose, negative titers had an assigned value of 1:5, and titers greater than 1:2560 a value of 1:2560. Samples were tested in duplicate, and geometric mean values were used in the analysis. Virus concentrations were previously determined by haemagglutinin antigen titration, and the HIA test was performed after removing naturally occurring nonspecific inhibitors from the sera as previously described (20).

The immunogenicity end-points after vaccination were the seroprotection (SP) rate (antibody titre ≥1:40), seroconversion (SC) rate (pre-vaccination titre <1:10 and post-vaccination HIA titre ≥1:40 or pre-vaccination titre ≥1:10 and ≥4-fold increase in post-vaccination titre), geometric mean titres (GMTs), and factor increase in GMT (GMT of the ratio of antibody titres after and before vaccination).

Safety assessment

On the day of vaccination, patients or parents were given a 21-day personal diary card containing the following list of pre-defined adverse events: local reactions (pain, redness, swelling, and itching) and systemic adverse events (arthralgia, fever, headache, myalgia, sore throat, cough, diarrhoea, rhinorrhoea, and nasal congestion). Participants were asked to give 'yes/no' responses to each side effect and to return their diary cards at the second evaluation day (21 days after vaccination). Adverse events that were not on the list were also reported. All local reactions were considered related to the influenza A H1N1/2009 vaccine, while systemic adverse events were analysed by the investigators to determine their causality. Severe adverse events were defined as those requiring hospitalisation or death.

Disease activity, JDM clinical course, muscle strength and treatment in JDM patients

JDM activity was assessed by disease activity score (DAS) (21) (range 0–20), and muscle strength was evaluated by childhood myositis assessment scale (CMAS) (22) (range 0–52) and manual muscle testing (MMT) (23) (range 0–80). The JDM clinical course was classified in monophasic, recurrent and chronic (24). The serum muscle enzymes performed were aspartate aminotransferase (AST) (normal value <41 IU/L), alanine aminotransferase (ALT) (normal value <37 IU/L), lactate dehydrogenase (LDH) (normal range 240–480 IU/L), creatine phosphokinase (CK) (normal range 39–308 IU/L) and aldolase (normal value <7.6 IU/L). Data concerning the current JDM treatments included: prednisone, methotrexate, azathioprine, chloroquine, cyclosporine, cyclophosphamide, mycophenolate mofetil, intravenous immunoglobulin and rituximab.

Statistical analysis

The immunogenicity and safety analyses were descriptive, and the two-sided 95% confidence intervals (CI) were calculated assuming binomial distributions for dichotomous variables and log-normal distribution for haemagglutination inhibition titres. The analysis of continuous variables was based on distributional assumptions. The GMTs and FI in GMT were compared between JDM patients and the healthy controls using a two-sided Student's *t*-test or Mann-Whitney U-test on the $\log_{10}$-transformed titres. Mann-Whitney U-test was also used to compare demographic data, muscle enzymes, JDM scores and prednisone current dose between patients with and without seroconversion. For categorical variables, statistical summaries included the rates of seroconversion that were compared using Fisher's exact test. All tests were two-sided with a 0.05 significance levels.

Results

Demographic data

JDM patients and healthy controls had similar current age (15.5 [9–21] *vs*. 15 [9–21] years, p=0.511) and frequencies of female gender (63% *vs*. 41%, p=0.286). The median disease duration of JDM was 5.5 (2–17) years.

Response to immunisation in JDM patients and controls

Table I illustrates seroprotection, seroconversion, GMTs and factor increases in the GMTs in JDM patients and controls before and after influenza A H1N1/2009 vaccination. Prior to immunisation, the seroprotection rate and GMT were comparable between JDM patients and healthy controls (p=0.457, p=0.817; respectively). After immunisation, the seroconversion rate was significantly lower in JDM patients compared to healthy controls (86.7%, 95% CI 74.9% to 99.3% *vs*. 97.5%, 95% CI 94.1% to 100.9%, p=0.044),

whereas the seroprotection rate was similar in both groups (90%, 95% CI 79.6% to 101.1% *vs*. 97.5%, CI 94.1% to 100.9%, p=0.121). In addition, GMT after immunisation and factor increase in GMT were alike in the two groups (p=0.992 and p=0.827 respectively). None of JDM patients and three (3.7%) healthy controls received previous immunisation with seasonal 2008/2009 influenza vaccine (p=0.562).

Immunisation response and disease parameters in JDM patients

Demographic data, muscle enzymes, JDM scores, lymphopenia and treatment at vaccination according to presence or absence of seroconversion in JDM patients after influenza A H1N1/2009 vaccination are shown in Table II.

Demographic data were comparable in the two groups (p>0.05) (Table II). The clinical courses of 19 JDM patients under any immunosuppressive agents were monophasic in 3 (15.8%), recurrent in 7 (36.8%) and chronic in 9 (47.4%). A higher frequency of chronic course was observed in non-seroconverted compared to seroconverted patients (100% *vs*. 27%, p=0.012). None of the patients had moderate or severe clinical activity or muscle weakness and seroconverted and non-seroconverted groups had comparable levels of JDM scores (p>0.05). Lymphopenia was not observed in patients that did not seroconverted. Muscle enzymes were also alike in both groups, except for a higher median level of aldolase in the non-seroconverted patients (7.4 [4.9–9.1] *vs*. 4.4 [2.1–7.2] IU/L, p=0.026). Regarding therapy, the four JDM patients that did not seroconvert had chronic course of disease and were more often under higher dose of prednisone (>20 mg/day) compared to those that seroconverted (50% *vs*. 4%, p=0.039). Likewise, a higher frequency of methotrexate (100% *vs*. 38%, p=0.036) and combination of prednisone, methotrexate and cyclosporine use (50% *vs*. 4%, p=0.039) was observed in patients that did not seroconvert (Table II).

Further analysis of the possible effect of vaccine in disease parameters revealed that the median of pre- and post-vaccination DAS (0 [0–11] *vs*. 0 [0–14], p=0.954), CMAS (52 [45–52] *vs*. 52 [41–52], p=0.803) and MMT (80 [74–80] *vs*. 80 [79–80], p=0.987) remained largely unchanged. Likewise, no significant differences were observed in muscle enzymes before and after immunisation: AST (20 [10–45] *vs*. 23 [11–36] IU/liter, p=0.246), ALT (32.5 [12–72] *vs*. 31 [11–63] IU/liter, p=0.825), LDH (187 [93–469] *vs*. 179 [83–446] IU/liter, p=0.906), CK (124 [49–533] *vs*. 102 [33–481] IU/liter, p=0.339) and aldolase (4.8 [2.1–9.1] *vs*. 4.8 [0–7.5] IU/liter, p=0.333). Frequencies of lymphopenia before and after immunisation were comparable (7% *vs*. 0%, p=0.492). Fur-

Table I. Seroprotection (SP), seroconversion (SC), geometric mean titers (GMT) and factor increases in the GMT (FI in GMT) in juvenile dermatomyositis (JDM) patients and controls before and after influenza A/H1N1/2009 vaccination.

Variables	JDM (n=30)	Controls (n=81)	p-value
SP			
Before immunisation	30 (12.5–45.5)	22.2 (13.1–31.3)	0.457
After immunisation	90 (79.6–101.1)	97.5 (94.1–100.9)	0.121
SC	86.7 (74.9–99.3)	97.5 (94.1–100.9)	**0.044**
GMT			
Before immunisation	13.8 (9.1–21)	13 (10.1–16.9)	0.817
After immunisation	259.9 (155.5–434.4)	260.6 (204.4–332.2)	0.992
FI in GMT	18.8 (11.4–31.1)	20 (15.2–26.3)	0.827

Values expressed in % (95% confidence interval).

Table II. Demographic data, muscle enzymes, juvenile dermatomyositis (JDM) clinical courses and scores, lymphopenia and treatment at vaccination according to seroconversion (SC) to influenza A H1N1/2009 vaccine in JDM patients.

Variables at vaccination (reference values)	Without SC (n=4)	With SC (n=26)	p-value
Demographic data			
Current age, years	15 (12–16)	15.5 (9–21)	0.646
Disease duration, years	4.9 (4–12)	7.2 (2–17)	0.806
Female gender	2 (50)	17 (65)	0.611
Muscle enzymes			
AST, IU/liter (<41)	26 (10–35)	19 (10–45)	0.471
ALT, IU/liter (<37)	41 (32–57)	31 (12–72)	0.155
LDH, IU/liter (240–480)	196 (168–211)	183 (93–469)	0.858
CK, IU/liter (39–308)	223 (65–533)	124 (49–387)	0.647
Aldolase, IU/liter (<7.6)	7.4 (4.9–9.1)	4.4 (2.1–7.2)	**0.026**
JDM clinical course			
Monophasic	0 (0)	11 (42)	0.267
Recurrent	0 (0)	8 (31)	0.550
Chronic	4 (100)	7 (27)	**0.012**
JDM Scores			
DAS (0–20)	3 (0–11)	0 (0–7)	0.126
CMAS (0–52)	51.5 (48–52)	52 (45–52)	0.894
MMT (0–80)	80 (80–80)	80 (74–80)	0.621
Lymphopenia (<1000/mm^3)	0 (0)	2 (7.7)	1.0
Treatment			
Prednisone	4 (100)	11 (42)	0.097
Current dose, mg	5.8 (2.5–12.5)	4 (1–35)	0.646
Prednisone > 20mg/day	2 (50)	1 (4)	**0.039**
Immunosuppressor (any)	4 (100)	15 (58)	0.267
MTX	4 (100)	10 (38)	**0.036**
Cyclosporine	2 (50)	4 (15)	0.169
Prednisone, MTX and cyclosporine	2 (50)	1 (4)	**0.039**
Azathioprine	0 (0)	2 (8)	1.0
Chloroquine	3 (75)	4 (15)	0.169

Values expressed in median (range) or n (%), AST: aspartate aminotransferase; ALT: alanine aminotransferase; LDH: lactate dehydrogenase; CK: creatine kinase; DAS: disease activity score; CMAS: childhood myositis assessment scale; MMT: manual muscle testing; MTX: methotrexate.

Table III. Adverse events of influenza A/H1N1/2009 vaccination in juvenile dermatomyositis (JDM) patients and controls.

Variables	JDM (n=30)	Controls (n=81)	*p*-value
Local reactions			
Pain	9 (30)	19 (23)	0.472
Redness	0	2 (2)	1.0
Swelling	0	2 (2)	1.0
Itching	0	1 (1)	1.0
Systemic reactions			
Arthralgia	1 (3)	2 (2)	1.0
Fever	1 (3)	3 (4)	1.0
Headache	5 (17)	18 (22)	0.606
Myalgia	1 (3)	6 (7)	0.671
Sore throat	0	5 (6)	0.321
Cough	0	5 (6)	0.321
Diarrhea	0	2 (2)	1.0
Rhinorrhea	4 (13)	3 (4)	0.083
Nasal congestion	0	3 (4)	0.561

Values expressed in n (%).

thermore, therapy was stable throughout the study in all patients.

Adverse events

Local and systemic vaccine adverse events were mild and had similar frequencies in JDM and controls ($p>0.05$) (Table III). None of them had severe adverse events.

Discussion

This study revealed that the non-adjuvanted influenza A H1N1/2009 virus immunisation is effective in JDM patients and identified that JDM chronic course and immunosuppressive therapy may hamper the vaccine induced antibody production.

The advantage of the present study was the inclusion of a homogenous group of patients that fulfilled the criteria for JDM (19) and the comparison with an age-matched control group, since vaccine efficacy has a distinct pattern in pediatric population (15). We also included only patients over 9 years of age, excluding the group of infants and children who had reduced humoral response to influenza A H1N1/2009 vaccine and required two doses of this vaccine (15, 16). Additionally, the use of non-adjuvant vaccine was chosen to avoid an autoimmune-related disease (25). The prospective design of this rare disease resulted, however, in a limited number of participants, and to our knowledge our study encompasses the largest JDM population that received influenza vaccine (11-13).

After immunisation with influenza A H1N1/2009 vaccine, the immunoresponse was impaired in JDM patients, as also observed in our recent report for the same vaccine in adult DM (26). Similarly, we evidenced reduced seroconversion rates for the same vaccine in a cohort of 99 of juvenile systemic lupus erythematosus (JSLE) and 93 juvenile idiopathic arthritis (JIA) patients (13). Further studies will be performed to assess the influence of influenza A H1N1/2009 vaccine in disease parameters and the potential deleterious effect of therapy in immunoresponse treatments in each of these diseases.

In contrast, previous studies in juvenile rheumatic diseases (27), including a very limited number of JDM populations (11, 12), demonstrated satisfactory immunogenicity with seasonal and pandemic influenza vaccination, independent of glucocorticoid and immunosuppressive therapies. In addition, the lower seroconversion rates in JDM patients cannot be explained by previous immunisation with seasonal influenza vaccine.

The four patients without seroconversion had chronic course of JDM and therefore, they were still under immunosuppressants combination in spite of mild disease activity parameters. Glucocorticoid was the major factor for the reduced overall immune response of pandemic vaccine in our recent study with juvenile autoimmune rheumatic diseases, mainly comprised by JSLE, JIA and 18 JDM also included in the present evaluation (13).

We have identified that immunosuppressive therapy may hamper vaccine antibody response in JDM patients. In our previous study including several pediatric autoimmune diseases, lymphopenia and immunosuppressants did not influence seroconversion against the same vaccine (13). Likewise, previous studies reported no effect of immunosuppressants in immunogenicity with seasonal (12, 27) and pandemic influenza vaccine (11) in patients with rheumatic diseases. In contrast, glucocorticoid and/or immunosuppressant use was associated with lower humoral and cell-mediated responses against the H1N1 strain of seasonal influenza vaccine in adult systemic lupus erythematosus (28, 29) and rheumatoid arthritis patients (30). In a recent study on pandemic influenza A H1N1/2009 vaccine in adult lupus, immunogenicity was improved in those under antimalarials therapy (17).

As regards the possible influence of other clinical and laboratorial parameters, lymphopenia was not a relevant finding in these patients and does not seem to interfere with immunoresponse to vaccine in JDM. Of note, in lupus, pandemic vaccination failure was significantly associated with reduced lymphocyte count (31).

The evaluation of the potential relevance of disease activity, as determined by JDM score, in pandemic vaccine antibody response was impaired by the small representation of patients with moderate or severe flares in our cohort that excluded hospitalised patients. Disease safety is reinforced by our findings of stable JDM scores and laboratorial muscle evaluation parameters throughout the study, including the borderline higher levels of aldolase in the non-seroconverted group. In this regard, studies with adult SLE have demonstrated no effect of seasonal influenza immunisation on disease flares (18).

Of note, influenza A H1N1/2009 vaccine was well tolerated and safe in JDM patients, as no serious short-term

adverse event was observed, as also reported previously in a limited number of JDM patients that received influenza vaccine (11, 12). In our large study with 237 pediatric autoimmune rheumatic diseases patients, only arthralgia was more frequently observed, comparing patients to healthy controls (13).

Notably, for pandemic influenza vaccines to be licensed, all children, adolescents and adults must meet all three current immunologic standards established: a percentage of seroprotection >70%, seroconversion >40%, and a factor increase in GMT >2.5 (29-31). JDM patients and healthy controls evaluated herein fulfilled all of the three criteria, indicating that the vaccine, while being less immunogenic, was effective.

In conclusion, this study identified that in JDM patients, chronic course and immunosuppressive therapy may hamper seroconversion, suggesting that a specific vaccination protocol may be required for this subgroup of patients. In spite of that, a single dose of non-adjuvanted influenza A H1N1/2009 vaccine was generally seroprotective and had no evident deleterious effect in disease itself.

Acknowledgments

We thank the subjects for their critical roles in this study, the staff of Hospital das Clinicas FMUSP, Laboratorio de Investigação Médica (LIM-17), Faculdade de Medicina da USP, Adolfo Lutz Institute and Butantan Institute.

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Original Article

High Disease Activity: an Independent Factor for Reduced Immunogenicity of Pandemic Influenza A Vaccine in Patients with Juvenile SLE

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Disclosure statement: The authors have declared no conflicts of interest

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Funding: This study was supported by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP #2009/51897-5 to EB and #2010/10749-0 to EB), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ #300248/2008-3 to CAS, #300559/2009-7 to RMRP and #301411/2009-3 to EB), Federico Foundation (to CAS, RMRP and EB) and Butantan Foundation.

ABSTRACT

Objective: Recent findings demonstrated a reduced immunogenicity of influenza A H1N1/2009 vaccine in juvenile rheumatic diseases. However, a point of concern is whether the vaccine could induce disease flares. The aim of this study is to assess disease safety and the possible influence of disease parameters and therapy in non-adjuvant influenza A H1N1 vaccine response of JSLE patients.

Methods: 118 JSLE and 102 healthy controls with comparable age were vaccinated. Seroprotection rate(SP), seroconversion rate(SC) and factor increase in geometric mean titre (FI-GMT) were calculated and effective immune response was defined by FDA and European Committee for Proprietary Medicinal Products vaccine immunologic standards. Disease parameters, treatment and adverse events were evaluated.

Results: Age was comparable in JSLE patients and controls (16.0±3.5 vs. 15.9±4.5 years, *p*=0.26). Three weeks after immunisation, SP(73.7 vs. 95.1%; *P*<0.001), SC(63.6 vs. 91.2%; *P*<0.001), GMT(90.8 vs. 273.3; *P*<0.001) and FI-GMT(8.1 vs. 19.9; *P*<0.001) were significantly lower in JSLE patients *versus* controls. Non-seroconversion was associated with higher frequency of patients with SLEDAI-2K≥8 (48.8 vs. 24% *P*=0.008) and higher mean of current glucocorticoid dose (18±21.4 vs. 10.5±12.5mg/day, *P*=0.018). Multivariate logistic regression including SLEDAI-2K≥8 revealed that only SLEDAI-2K remained a significant factor for non-SC (OR:0.42 95%CI 0.18-0.98, *P*=0.045). Disease parameters remained stable throughout the study and no severe vaccine adverse events were observed.

Conclusions: The present study demonstrated an adequate disease safety and is the first to discriminate that high disease activity impairs influenza A H1N1/2009 vaccine antibody production in JSLE, in spite of an overall immune response within recommended levels.

Keywords: Vaccine, disease activity, immunogenicity, pandemic influenza A (H1N1), systemic lupus erythematosus

SIGNIFICANCE AND INNOVATIONS

- High disease activity impairs antibody response to influenza A H1N1/2009 vaccine in JSLE patients
- Influenza A/H1N1 2009 vaccine is safe in JSLE patients

INTRODUCTION

Infections are recognized as an important cause of morbidity and mortality in patients with juvenile systemic lupus erythematosus (JSLE) and may also induce disease flares (1). Immunological abnormalities related to disease itself and its treatment seems to be a major contributing factor to this higher susceptibility to infections (2).

Children and adolescents were recognized as a risk group for hospitalisation and death in the recent influenza pandemic caused by the new influenza A H1N1/2009 virus, particularly in those with pre-existing chronic disorders (3). Vaccination is considered as the most effective measure to control the spread of the virus and to reduce associated morbidity and mortality (4,5). In fact, the Advisory Committee on Immunization Practices (ACIP) stated that all children and adolescents aged between 6 months and 18 years should receive the trivalent seasonal influenza vaccine containing the A/California/7/2009(H1N1) strain and this recommendation is particularly important for those with chronic conditions (6). More recently, the European League Against Rheumatism (EULAR) published their recommendations for vaccinations in paediatric patients with rheumatic diseases and reinforced that an annual influenza vaccination should be considered for these patients (5).

The efficacy and safety of the seasonal influenza vaccine in children with rheumatic diseases has been reported in previous studies with a limited number of patients (7,8). An appropriate response to the seasonal influenza vaccine in patients with juvenile rheumatic diseases, including eleven JSLE patients, regardless of their immunosuppressive therapy was reported by Kanakoudi-Tsakalidou et al.(7) Likewise, a satisfactory response for the seasonal influenza vaccine independent of treatment was observed in a population of pediatric rheumatic patients, twelve of them with JSLE (8).

With regard to the pandemic vaccine, we have recently published a study focusing solely in vaccine immunogenicity and safety in a large cohort of 237 juvenile autoimmune rheumatic diseases and demonstrated an overall reduced immunogenicity, particularly in those under glucocorticoid (GC) therapy (9). The inclusion of a heterogeneous group of illnesses in our cohort hampers the accurate interpretation of the possible influence of a specific disease and/or therapy. Moreover, we have not evaluated disease safety since another point of concern is whether the vaccine could induce flares (10).

Therefore, the aim of the present study was to evaluate disease safety and the possible influence of disease and therapy in JSLE immunized with non-adjuvant pandemic influenza A H1N1/2009 vaccine.

METHODS

Patients and controls

One hundred eighteen JSLE outpatients routinely followed at the Pediatric Rheumatology Unit and the Rheumatology Division of Clinics Hospital, São Paulo, Brazil, were included in this study. All patients fulfilled the American College of Rheumatology (ACR) classification criteria for juvenile systemic lupus erythematosus (JSLE) (11). A total of 102 healthy subjects were concomitantly included in the control group. All participants were nine to 21 years old. Exclusion criteria included: previous proven infection by influenza A (H1N1) 2009; anaphylactic response to vaccine components or to egg; previous vaccination with inactivated vaccines within two weeks or live vaccines in the last four weeks or even the 2010 seasonal influenza vaccination in the last six months before the study entry; acute

infection resulting in fever over 38°C at the time of vaccination; Guillain-Barré syndrome or demyelinating syndromes; heart failure; blood transfusion within six months; and hospitalisation.

Study design

An interventional, open label, phase IV study was conducted between March 2010 and April 2010. All JSLE patients were invited by letter to participate in the Public Health influenza A H1N1/2009 vaccine campaign at the Immunization Centre of the same hospital. Healthy volunteers who came to this centre seeking vaccination in response to the Public Health National Campaign were included as control group. This protocol was approved by the Local Institutional Review Board. All participants or their legal guardians signed the informed consent form. The study was registered at clinicaltrials.gov under #NCT01151644.

In the period comprised from 22 March 2010 to 2 April 2010, H1N1 A/California/7/2009–like virus vaccine (A/California/7/2009/Butantan Institute/Sanofi Pasteur) was administered to patients and controls as a single intramuscular injection (0.5 ml). All participants were evaluated on the day of vaccination and three weeks after that. Blood samples were obtained from each participant immediately before and 21 days after vaccination.

Patient demographic data, treatment and disease activity

The medical records of all patients were reviewed in terms of demographic data (disease duration) and treatment [glucocorticoid (GC) and immunosuppressant use). Disease activity was assessed by clinical and laboratorial parameters at study entry and

21 days after vaccination, including articular involvement (arthralgia or nonerosive arthritis), cutaneous lesions (malar or discoid rash, oral ulcers, vasculitis or photosensitivity), cardiopulmonary disease (serositis, myocarditis, restrictive lung disease and pulmonary hypertension), renal involvement (proteinuria > 0.5g/24h, cellular casts, persistent hematuria > 10 red blood cells per high power field, or renal failure), neuropsychiatric disease (seizure, psychosis, depression, or peripheral neuropathy) and hematologic abnormalities (hemolytic anemia, leukopenia with a white blood cell count < 4,000/mm^3, lymphopenia < 1,500/ mm^3, and thrombocytopenia with platelet count < 100,000/ mm^3). Complement levels were measured by radial immunodiffusion (SIEMENS Health Care, Marburg, Germany) and anti-dsDNA were detected by ELISA (INOVA Diagnostics Inc., San Diego, CA). The Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K)(12) was calculated at study entry and after 21 days.

Vaccine

A novel monovalent, non-adjuvant, inactivated, split-virus vaccine was supplied by Butantan Institute/Sanofi Pasteur (São Paulo, Brazil). The vaccine contained an inactivated split influenza virus with 15 µg of haemagglutinin antigen equivalent to the A/California/7/2009 (H1N1) virus–like strain (NYMCX-179A), one of the candidate reassortant vaccine viruses recommended by the WHO. Embryonated chicken eggs were employed using the same standard techniques for the production of seasonal, trivalent, inactivated influenza vaccine. The vaccine was presented in 5-ml multi-dose vials with thimerosal (45 µg per 0.5-ml dose) as a preservative.

Haemagglutination inhibition assay

The antibody levels against H1N1 A/California/7/2009–like virus were evaluated using the haemagglutination inhibition assay (HIA) at the Adolfo Lutz Institute. Sera were tested for antibodies to the H1N1 A/California/7/2009 influenza strain supplied by Butantan Institute. Titers were tested at an initial dilution of 1:10, and at a final dilution of 1:2560. For the purposes of calculations, negative titers had assigned a value of 1:5, and titers greater than 1:2560 a value of 1:2560. Samples were tested in duplicate, and geometric mean values were used in the analyses.

Virus concentrations were previously determined by haemagglutinin antigen titration, and the HIA test was performed after removing naturally occurring nonspecific inhibitors from the sera as previously (13).

The immunogenicity end-points after vaccination were the seroprotection (SP) rate (titre ≥ 1:40), seroconversion (SC) rate (pre-vaccination titre < 1:10 and post-vaccination HIA titre ≥ 1:40 or pre-vaccination titre ≥ 1:10 and post-vaccination titre ≥ 4-fold increase), geometric mean titres (GMTs), and factor increase in GMT (FI-GMT, ratio of the GMT after vaccination to the GMT before vaccination)

Safety assessment

At the day of vaccination, patients or legal guardians received a 21-day personal diary card containing the following list of pre-defined adverse events: local reactions (pain, redness, swelling, and itching) and systemic adverse events (arthralgia, fever, headache, myalgia, sore throat, cough, diarrhoea, rhinorrhoea, and nasal congestion). Participants were asked to give ‘yes/no’ responses to each side effect and to return their diary cards at the second evaluation day (21 days after vaccination). The participants were encouraged to report any other adverse events that were not on the list. All local reactions were

considered related to the influenza A H1N1/2009 vaccine, while systemic adverse events were analysed by the investigators to determine their causality. Severe side-effects were defined as those requiring hospitalisation or death.

Statistical analysis

The immunogenicity and safety analyses were descriptive, and the two-sided 95% confidence intervals (CI) were calculated assuming binomial distributions for dichotomous variables and log-normal distribution for haemagglutination inhibition titres. The GMTs and FI-GMT were compared between each subgroup of patients with JSLE and the healthy control group using a two-sided Student's t-test or Mann-Whitney U-test on the $\log_{10}$-transformed titres. Chi–squared or Fisher's exact tests were used for categorical variable. Multivariate logistic regression analysis was performed using seroconversion rate as the dependent variable and those with $P < 0.05$ in the univariate analyses as independent variables (SLEDAI-2K ≥8 and glucocorticoid current dose). All tests were two-sided, and significance was set at a P-value < 0.05.

RESULTS

Demographic data

One hundred and eighteen JSLE patients and 102 healthy controls were included in the study. Mean current age was comparable between patients and controls (16 ± 3.5 vs. 15.9 ± 4.5 years, *P* = 0.26), with a predominance of female gender in JSLE group (77.1% vs. 50%, *P* < 0.001) (table 1). Mean disease duration was 5.0 ± 3.6 years and the mean of SLEDAI-2K score was 6.0 ± 5.8. Renal involvement was observed in approximately half (50.8%) and lymphopenia in 27.1% of patients at study entry. Ninety two (78%) patients were under antimalarials, 83 (70.3%) under prednisone, with a mean dose of 18.8 ± 17 mg/day and 72 (61.0%) were taking immunosuppressive drugs [azathioprine (37.3%), mycophenolate mofetil (12.7%) and methotrexate (11.9%)] (Table 1).

Influenza A H1N1/2009 vaccine immunogenicity

Before immunization, seroprotection rates were comparable in patients and controls (*P* = 0.736), as well as GMT (*P* = 0.684). Three weeks after vaccination, all parameters were reduced in JSLE patients compared to controls: seroprotection rates (*P* < 0.001), seroconversion rates (*P* < 0.001), GMT (*P* < 0.001) and FI GMT (*P* < 0.001) (Table 2).

Comparison of seroconverted and non-seroconverted JSLE patients showed that the groups were similar regarding current age (*P* = 0.92) and female gender (*P* = 0.366). The non-seroconverted group showed higher median pre-immunization SLEDAI-2K score

(7.5 ± 5.8 vs. 5.2 ± 5.7, *P* = 0.035), higher frequency of SLEDAI-2K ≥ 8 (48.8 vs. 24 % *P* = 0.008) (Table 3).

Regarding the current treatment at study entry, the mean of prednisone dose was significantly higher in the non-seroconverted JSLE patients (18 ± 21.4 vs. 10.5 ± 12.5 mg/day, *P* = 0.018). In fact, the mean prednisone dose in patients with SLEDAI-2K score ≥ 8 was significantly higher compared to patients with low SLEDAI-2K scores (22.4 ± 21.5 vs. 8.7 ± 11.2, *P* < 0.001).The frequencies of antimalarial and immunosuppressant agents use were comparable in non-seroconverted and seroconverted patients (Table 3).

Multivariate logistic regression was performed to determine possible deleterious parameters for non-seroconversion, and included high SLEDAI-2K score (≥ 8) and glucocorticoid current dose, and only SLEDAI-2K score ≥ 8 (OR 0.42 95%CI 0.18-0.98, *P* = 0.045) remained a significant factor.

Disease safety

No change was observed in the mean SLEDAI-2K score [6.0 (5.0-7.1) vs. 5.2 (4.2-6.1), *P* = 0.23] before and 21 days after pandemic influenza A H1N1/2009 vaccine. The frequencies of articular involvement (5.2 vs. 2.6%, *P* = 0.49), renal involvement (51 vs. 40%, *P* = 0.11), neuropsychiatric disease (0 vs. 0.8%, *P* = 0.49) and hematologic abnormalities (4 vs. 7%, *P* = 0.41) were similar before and after pandemic influenza A H1N1/2009 vaccination whereas the frequency of mucocutaneous lesions were significantly higher before pandemic influenza A H1N1/2009 vaccination compared to after vaccination (15.6 vs. 3.5%, *P* = 0.003).

Vaccine safety

No serious adverse events were reported in both groups. JSLE patients presented higher frequencies of local redness and itching (11% vs. 2.0%, $P = 0.007$; and 16.9% vs. 0.0%, $P < 0.0001$, respectively), arthralgia (16.9% vs. 1.0%, $P < 0.0001$) and rhinorrhoea (12.7% vs. 3.9%, $P = 0.02$) when compared to healthy controls.

DISCUSSION

The present study is the first to discriminate that disease activity impairs non-adjuvant influenza A H1N1/2009 vaccine antibody production in JSLE patients, in spite of an overall immune response within recommended levels in these patients.

The analysis of solely lupus patients with a control group with comparable age was essential since we have previously demonstrated that vaccine immune response has a diversity related to disease (9) and age has been recognized as a major factor for this vaccine antibody production (14-16). The selection of sizeable number of patients regardless of disease activity status or immunosuppressive treatments, express better a real life situation and allows a more accurate interpretation of the influence of these factors in vaccine humoral response.

Interestingly, we have identified that an overall high disease activity score at immunization is a relevant factor for the pandemic vaccine non-seroconversion in JSLE possibly by a direct effect on humoral and cell-mediated immunity (17) that may ultimately affect the response to antigenic challenge (18). Further analysis of SLEDAI-2K parameters has not revealed a specific major organ involvement underlying this process. On the other hand, lymphopenia did not seem to influence this weaker response in JSLE as also reported for children with cancer (19) and adult SLE population (20), although Mathian et al., 2011 has observed such association in the later group (21). Additionally, we have identified a higher frequency of low complement levels and anti-dsDNA antibodies unrelated to renal involvement linked to low vaccine response, which appear to reflect the known correlation of immune inflammatory markers with global lupus activity (22).

Of note, the large enrollment of a single disease and the multidimensional comparison enabled a more precise definition that glucocorticoid was the only drug

associated with lower immunogenicity in JSLE, as also observed in preceding data that identified glucocorticoid as determinant of a vaccine weaker response in adult systemic lupus erythematosus (23,24), autoimmune rheumatic diseases (25) and juvenile rheumatic diseases (9). In contrast, a recent study with the adjuvanted influenza A H1N1/2009 vaccine in adult lupus showed no influence of therapy in immunogenicity (26). Additionally, none of the patients were under B cell depletion therapy and therefore the former deleterious effect of this biological agent in pandemic influenza vaccine response was not assessed in the present study (27). On the other hand, regarding antimalarials, the small representation of JSLE patients without this drug in the present study precludes an accurate interpretation of the absence of a beneficial effect previously reported in adult lupus patients (28).

We confirmed preceding observation of H1N1 pandemic vaccine disease safety by our findings of stable organ and system involvements, lupus biomarkers (29) and SLEDAI-2K scores (17,26,30) throughout the study in spite of the fact that immunization may induce B cell hyperactivity with a possible production of pathogenic autoantibodies and/or disease flare (10).

Furthermore, influenza A H1N1/2009 vaccine was well tolerated in JSLE patients without any severe short-term adverse event, as also reported previously by others evaluating a limited number of JSLE (7,8) and by our group analysing a large paediatric autoimmune rheumatic diseases patients (9).

Importantly, the vaccine reached all three current immunologic standards parameters for seroprotection (>70%), seroconversion (>40%) and factor increase in GMTs (>2.5) (31-32) regardless of the impaired antibody response to influenza A H1N1/2009 vaccine compared to healthy controls.

In conclusion, this large prospective study demonstrated an appropriate immune response to the pandemic influenza A/H1N1 2009 virus vaccine with an excellent disease safety profile in JSLE patients. Lower seroconversion rates were particularly associated with high disease activity scores and it was also possibly influenced by glucocorticoid use suggesting the need of a second boost in this subgroup of patients.

ACKNOWLEDGEMENTS

We thank the staff of Hospital das Clinicas FMUSP, Laboratório de Investigação Médica (LIM-17), Faculdade de Medicina da USP and Adolfo Lutz Institute and Butantan Institute for their contribution for the study.

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Table 1 – Demographic data, disease features and treatment in juvenile systemic lupus erythematosus (JSLE) patients and healthy controls at study entry

Variables	JSLE (n=118)	Controls (n=102)	P
Demographic data			
Age, years	16.0 ± 3.5	15.9 ± 4.5	0.26
Female gender	91 (77.1)	51 (50)	**< 0.001**
Disease duration, years	5.0 ± 3.6	-	-
Disease features			
SLEDAI-2K score	6.0 ± 5.8	-	-
Renal involvement	60 (50.8)	-	-
Neuropsychiatric involvement	0	-	
Lymphopenia	32 (27.1)	-	-
Treatment		-	-
Antimalarials	92 (78)	-	-
Prednisone	83 (70.3)	-	-
dose, mg/day	18.8 ± 17	-	-
dose ≥ 20mg/day	40 (48.2)	-	-
Immunosuppressant	72 (61.0)	-	-
Azathioprine	44 (37.3)	-	-
Mycophenolate mofetil	15 (12.7)	-	-
Methotrexate	14 (11.9)	-	-
Cyclophosphamide	3 (2.5)	-	-
Cyclosporine	2 (1.7)	-	-

Data are expressed as mean ± standard deviation or n (%), SLEDAI-2K - Systemic Lupus Erythematosus Disease Activity Index 2000.

Table 2 - Serological data before and after Influenza A pandemic (pH1N1) 2009 vaccine in juvenile systemic lupus erythematosus (JSLE) patients and healthy controls

	JSLE (n=118)	Controls (n=102)	P
Before immunization			
Seroprotection	18.6 (12.1-26.9)	20.6 (13.2-29.7)	0.736
GMT	11.2 (8.9-14.0)	11.9 (9.6-14.9)	0.684
After immunization			
Seroprotection	73.7 (64.8-81.4)	95.1 (88.9-98.4)	**< 0.001**
Seroconversion	63.6 (54.2-72.2)	91.2 (83.9-95.9)	**< 0.001**
GMT	90.8 (67.8-121.7)	237.3 (188.8-298.3)	**< 0.001**
FI-GMT	8.1 (6.3-10.5)	19.9 (15.6-25.4)	**< 0.001**

Data are expressed in % or values (95% confidence interval), GMT - geometric mean titre, FI-GMT - factor increase in GMT.

Table 3 – Demographic data, disease activity and treatment in seroconverted and non-seroconverted juvenile systemic lupus erythematosus (JSLE) patients at study entry

	Non-seroconverted (n=43)	Seroconverted (n=75)	P
Demographic data			
Age, years	16.5 ± 3.9	16.5 ± 3.3	0.92
Female gender	31 (72.1)	60 (80)	0.366
Disease duration, years	6.1 ± 3.9	5.6 ± 3.4	0.419
Disease characteristics			
SLEDAI-2K score	7.5 ± 5.8	5.2 ± 5.7	**0.035**
SLEDAI-2K score ≥ 8	21 (48.8)	18 (24.0)	**0.008**
Renal involvement	24 (55.8)	36 (48.0)	0.413
Neuropsychiatric involvement	0 (0)	0 (0)	1.0
Lymphopenia	15 (34.9)	17 (22.7)	0.197
C3, mg/dL	79 ± 27.7	83 ± 27.7	0.455
Anti-dsDNA	29 (67.4)	34 (45.3)	0.02
Treatment			
Antimalarials	34 (79.1)	58 (77.3)	1.0
Prednisone	32 (74.4)	51 (68)	0.533
dose, mg/day	18 ± 21.4	10.5 ± 12.5	**0.018**
dose ≥ 20mg/day	18 (41.9)	15 (29.3)	0.225
Immunosuppressant	25 (58.1)	47 (62.7)	0.696
Azathioprine	15 (34.9)	29 (38.6)	0.698
Mycophenolate mofetil	7 (16.3)	8 (10.6)	0.401
Methotrexate	4 (9.3)	10 (13.3)	0.571
Cyclophosphamide	1 (2.3)	2 (2.6)	1.0
Cyclosporine	2 (4.6)	0 (0)	0.131

Data are expressed as mean ± standard deviation or n (%), SLEDAI-2K - Systemic Lupus Erythematosus Disease Activity Index 2000.