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Review Article

Proposal for the development of biologics in pediatric rheumatology

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Abstract In order to assess the development, approval and early introduction into clinical practice of biologics in the pediatric field, we herein describe the current status of the development to approval of biologics as anti-rheumatic agents for children in Japan, discuss the present problems and provide a proposal for the future. It has become apparent that the duration of the review period required for the preparation of clinical trials and Pharmaceuticals and Medical Devices Agency approval is clearly reduced compared with the past. Thus, it was speculated that a rate-limiting step in the process from development to approval was the duration of clinical trials from start to end. Hence, we focused on the following key words with regard to promotion of the development of biologics and their early practical use: "registry", "centralization", and "global cooperation", all of which are related to the reduction of duration of a clinical trial. In conclusion, to reduce the duration of a clinical trial, it is essential to complete a world-scale registry system by developing the registry system established by the Pediatric Rheumatology Association of Japan. The next step is then to carefully plan to participate in the international network using the world-scale registry system, and develop global cooperative trials in which we can ensure a sufficient number of entries from Japan.

Key words biologic, centralization, international cooperation, pediatric rheumatology, registry.

Rheumatic diseases in childhood are regarded as incurable even now, and their pathogenesis has not been clarified. These are serious diseases that fulfill the following four conditions: (i) the pathogenesis has not been clarified; (ii) the therapeutic method has not been established; (iii) they are rare; and (iv) they need long-term medical treatment. Pediatric rheumatic diseases are systemic inflammatory diseases involving antoinflammation and autoimmunization, and their medication and treatment have dramatically advanced due to marked progress in diagnostic technology in inflammatory science and rheumatology. Therefore, favorable outcomes in the inflammatory state are expected without carrying over organ failure to adulthood if the principles of early diagnosis and early therapeutic intervention are maintained, and it is not too much to say that the advent of biologics facilitated this improvement. In the pediatric rheumatology field, four biologics (tocilizumab, etanercept, adalimumab and palivizumab) were approved in Japan by June 2017. In particular, new approval for pediatric indications for tocilizumab, etanercept and adalimumab – anti-rheumatic agents – has greatly changed medical treatment in the pediatric rheumatology field, by which many clinicians have realized that treatment has changed from care to cure.[1]

In this article, to assess the development, approval and early introduction into clinical practice of biologics in the pediatric field, we herein describe the current status of the development to approval of biologics as anti-rheumatic agents for children in Japan, discuss the present problems and provide a proposal for the future.

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Positioning of biologics as anti-rheumatic agents for children in Japan

In the early first decade of the 2000s, the major treatment for systemic-onset juvenile idiopathic arthritis (JIA) was steroid, and that for polyarticular JIA was methotrexate.[2] It was recognized, however, that 30–40% of pediatric patients did not respond to these treatments, nor did their symptoms easily resolve.[1]

Recently, indications for the use of biologics have been described according to guidelines for initial treatment[3,4] and guidelines for the use of biological preparations.[5–7] Namely, for systemic JIA, tocilizumab, an anti-interleukin (IL)-6

receptor monoclonal antibody, is started as early as possible in the following cases, when: (i) dose reduction of glucocorticoid is difficult during a current treatment course; (ii) no pathological change to macrophage activation syndrome has occurred, but is likely to occur; and (iii) the next step treatment is judged to be required because the treatment course is not satisfactory. For polyarticular JIA, in contrast, tocilizumab or anti-tumor necrosis factor (anti-TNF) preparations, etanercept and adalimumab, are used in the following cases when: (i) ≥3 months of treatment mainly with MTX is not effective, and clinical symptoms such as arthritis and laboratory data on inflammation are not improved; (ii) dose reduction of oral glucocorticoid is difficult or a patient is steroid dependent; and (iii) a patient shows poor tolerability to standard-dose MTX (nausea, hepatic dysfunction etc.).

Time lag from development to approval

In pediatric rheumatology, four biomedical preparations (tocilizumab, etanercept, adalimumab and palivizumab) have been approved for JIA in Japan. Therefore, the time lag from development to approval between inside and outside of Japan and between adult use and pediatric use was reviewed.

Tocilizumab

Tocilizumab is a biomedicine developed in Japan (Fig. 1; Table 1),[8,9] and, rarely, pediatric and adult indications (rheumatoid arthritis [RA]) were simultaneously approved in Japan ahead of other countries. In other countries (USA and EU), phase III trials of tocilizumab were started just after its approval in Japan, and approved 3 years later. It was thought that the interval between the clinical trial and approval of the clinically highly needed medicine was extremely short in the USA and EU.

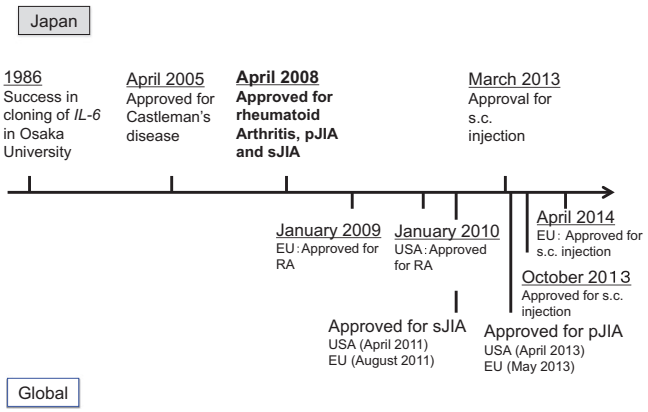

Fig. 1 Timeline of development of tocilizumab. Tocilizumab is a biologic developed in Japan, and it is a rare agent in that pediatric and adult indications (rheumatoid arthritis [RA]) were simultaneously approved in Japan ahead of other countries. In other countries (USA and EU), phase III trials of tocilizumab were started just after its approval in Japan and approved 3 years later. IL, interleukin; pJIA, polyarticular juvenile idiopathic arthritis; sJIA, systemic juvenile idiopathic arthritis.

Etanercept

Etanercept was approved in Japan 7 years after its approval for RA in the USA (Fig. 2; Table 2).[10] It was approved for JIA 1 year after its approval for RA in the USA, whereas it was approved for JIA in Japan 10 years after its approval for the same indication in the USA.

Adalimumab

After the world-first approval of adalimumab for RA in the USA, it took 6 years to be approved for JIA, probably because its developments for various related diseases were progressing

Table 1 Approval process for tocilizumab

Indication	Japan			Outside Japan			Time lag		
							Phase III approval		
	Start of phase III	Application	Approval	Start of phase III	Approval	Country/ Region	Outside Japan to in Japan	Outside Japan to in Japan	Application to approval in Japan
Castleman's disease		April 2003	April 2005	Not developed	Not developed	Not developed	Japan ahead	Japan ahead	Japan ahead
RA	March 2003	April 2006	April 2008	February 2005	January 2010/January 2009	USA/EU	Japan ahead	Japan ahead	Japan ahead
pJIA	November 2004	April 2006	April 2008	October 2009	April 2013/May 2013	USA/EU	Japan ahead	Japan ahead	Japan ahead
sJIA	May 2004	April 2006	April 2008	March 2008	April 2011/August 2011	USA/EU	Japan ahead	Japan ahead	Japan ahead
S.c. injection: RA	April 2010	March 2012	March 2013	September 2010	October 2013/ April 2014	USA/EU	Japan ahead	Japan ahead	Japan ahead

pJIA, polyarticular juvenile idiopathic arthritis; RA, rheumatoid arthritis; sJIA, systemic juvenile idiopathic arthritis.

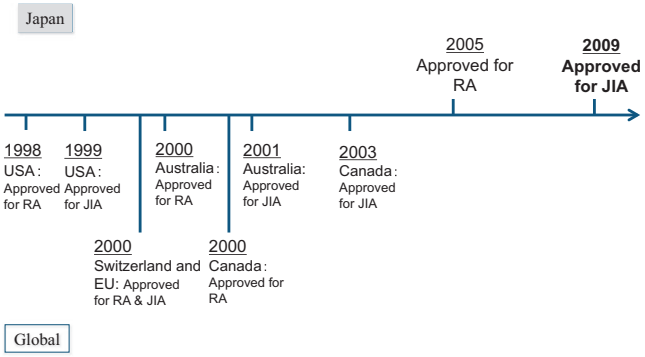

Fig. 2 Timeline of development of etanercept. Etanercept was approved in Japan 7 years after its approval for rheumatoid arthritis (RA) in the USA. It was approved for juvenile idiopathic arthritis (JIA) 1 year after its approval for RA in the USA, whereas it was approved for JIA in Japan 10 years after its approval for the same indication in the USA.

simultaneously (Fig. 3; Table 3).[11] In Japan, however, it was approved in an extremely short period of time after its approval in the USA; 3 year for RA and 2 years for JIA. In addition, it took only 1 year from application to approval in Japan. The duration of the review period in Japan was similar to that in other countries.

Palivizumab

Indications for palivizumab for premature infants and for congenital heart disease were approved in Japan with a delay of 2–4 years after the approval in the USA (Fig. 4; Table 4).[12] In 2013, additional indications for this medicine were approved in Japan for the first time in the world, and it will be interesting to see how these indications will be extended worldwide.

Both etanercept and adalimumab were biologics that were developed and approved outside Japan and then were approved in Japan after completing the domestic clinical trials, but there were large differences in the time to approval compared with overseas, or in the time from application to approval. The differences are understandable in the following context. The year 2011 corresponds to the final year of the "Five-Year Strategy for Creation of Innovative Pharmaceutical Products and Medical Devices" in Japan, and various programs were conducted to achieve the target review period (median) of 12 months for normal review items (administrative side, 9 months; applicant side, 3 months) and that of 6 months for priority review items (administrative side, 6 months; applicant side 3 months) that were set at the beginning of the project. As a result, the median development period and review period were 42.2 months (i.e. 3.5 years) and 10.1 months (i.e. 0.8 years), respectively, in 2011, and the review period in that year was 4.7 months shorter than that in 2010, and the shortest since 2000, when the survey started. The differences in time to approval between outside and inside Japan and the interval between application and approval in Japan between the aforementioned two biologics might reflect the difference in the timing of the review period (whether it was reviewed in 2011 or not). This indicates that the attempt to reduce the review period surely succeeded.

Proposal for the development of biologics and other agents for children

As mentioned, it is apparent that the duration of a review period required for the preparation of clinical trials and Pharmaceuticals and Medical Devices Agency approval clearly reduced in 2011 compared with the past. Thus, it was speculated that a rate limiting step in the process from development to approval was the duration of clinical trials from start to

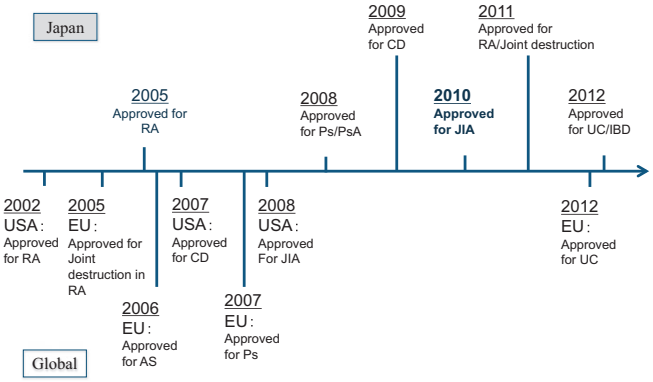

Fig. 3 Timeline of development of adalimumab. After the world-first approval of adalimumab for rheumatoid arthritis (RA) in the USA, it took 6 years to be approved for juvenile idiopathic arthritis (JIA), probably because its development for various related diseases were progressing simultaneously. In Japan, however, it was approved in an extremely short time after its approval in the USA: 3 years for RA and 2 years for JIA. In addition, it took a short time of 1 year from the application to approval of adalimumab in Japan. AS, ankylosing arthritis; CD, Crohn's disease; IBD, inflammatory bowel disease; PsA, psoriatic arthritis; UC, ulcerative colitis.

Table 2 Approval process for etanercept in Japan

	Indication	Application date	Review committee	Approval date	Time lag
1	RA (Use only for patients not effectively treated with existing treatment)	18 November 2002	October 2004	9 January 2005	–
2	pJIA (Use only for patients not effectively treated with existing treatment)	17 November 2006	April 2009	7 July 2009	4 years 6 months

pJIA, polyarticular juvenile idiopathic arthritis; RA, rheumatoid arthritis.

Table 3 Approval process for adalimumab in and outside Japan

Adalimumab 0.8 mL syringe for s.c. injection		Indication	Outside Japan			In Japan		
40 mg	20 mg		Start of phase III	Approval	Country/ Region	Start of phase III	Application	Approval
○		RA	Unknown	December 2002	USA	February 2004	December 2005	April 2008
○		Ps/PsA	Unknown	December 2007/ August 2005	EU	Unknown	May 2008	January 2010
○		As	January 2004	June 2006	EU	March 2008	October 2009	October 2010
○		CD	July 2002	February 2007	USA	January 2007	September2009	October 2010
○	○	JIA	September 2002	February 2008	USA	May 2008	August 2010	July 2011
○		Joint destruction in RA	December 2000	August 2005	EU	March 2009	September 2011	July 2012
○		IBD	–	–	–	October 2010	August 2012	May 2013
○		UC	November 2006	April 2012	EU	February 2009	March 2012	May 2013

Indication	Time lag			Remarks
	Phase III trial outside to in Japan	Approval outside to in Japan	Application to approval in Japan	
RA	Unknown	5 years 4 months	2 years 4 months	From the results of phase II and phase III trials conducted in Japan as bridging studies, it was judged that the results obtained from foreign trials can be extrapolated, and the manufacturer applied the manufacture and sales approval of ADA for RA as the indication
Ps/PsA	Unknown	2 years/4 years 4 months	1 year 8 months	Given that ADA was applied for Ps/PsA before 2008 at which its use for RA had not been approved yet, it was expected to be reviewed as a new drug. Therefore, ADA was re-applied after the approval of its indication for RA
AS	4 years 2 months	4 years 4 months	1 year	
CD	4 years 6 months	3 years 8 months	1 year 1 month	
JIA	5 years 8 months	3 years 5 months	1 year	There is no suitable animal disease model for JIA. A new pharmacologic study was not added, however, because it is judged not to be necessary from clinical studies in adults and toxicological studies in mature animals
Joint destruction in RA	8 years 4 months	6 years 11 months	10 months	Change in the indication: from "RA that has not been effectively treated with existing therapy", to "RA (including prevention of structural damage to the joint) "
IBD	–	–	9 months	ADA has not been approved for this indication outside Japan. In response to a request form from Behcet's disease *Tomo-no-kai* (patient circle) in June 2008, clinical studies for IBD were conducted
UC	2 years 4 months	1 year 1 month	1 year 2 months	A request form for early approval of ADA for UC was submitted by the Japanese Society of Mucosal Immunology and a patient organization in October 2012. The application was planned before the disclosure of the results of foreign clinical trials, the data obtained in foreign counties and Japan were comprehensively assessed after the end of trials in and outside Japan

ADA, adalimumab; AS, ankylosing spondylitis; CD, Crohn's disease; IBD, inflammatory bowel disease; JIA, juvenile idiopathic arthritis; Ps, psoriasis; PsA, psoriatic arthritis; RA, rheumatoid arthritis; UC, ulcerative colitis.

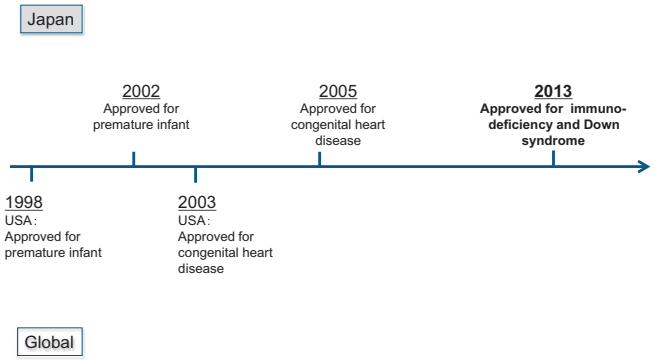

Fig. 4 Timeline of development of palivizumab. Indications for palivizumab for premature infants and for congenital heart disease were approved in Japan with a delay of 2–4 years after the approval in the USA. In 2013, additional indications for this medicine were approved in Japan for the first time in the world.

end. Therefore, it is necessary to consider the following three key words to promote the development of biologics and their early practical use: "registry", "centralization" and "global cooperation", all of which are related to the reduction of duration of a clinical trial.

Establishment of registry

The Pediatric Rheumatology Association of Japan finalized its registry system in April 2017. It was named Pediatric Rheumatology International Collaboration Unit Registry (PRICURE). We plan to register all pediatric and adolescent patients in Japan within the next 2 years and to participate in the international registry system. We asked several institutions that use a domestic networked registry system for adult patients with rheumatic diseases, about the current status of the registry system, and they replied that there is a problem in securing the cost of the registry maintenance. From this survey, it became apparent that the registry system could not be maintained if the funds are exhausted, because the huge cost of the maintenance is covered by funds personally collected by a responsible person in the institutions (mainly from the Health Labour Science Research Grant) in the aforementioned system. In addition, they replied that it was also necessary to secure the cost of the registry fee, which was 1,000 yen per person. This suggests that long-term fundraising is essential to establish a well-arranged registry system for children with rheumatic diseases that is similar to that in Western countries.

There are two international registry system for children with rheumatic diseases: the Pediatric Rheumatology International Trials Organization (PRINTO) in Europe and the Childhood Arthritis and Rheumatology Research Alliance (CARRA) in North America, both of which are managed by non-government organizations (non-profit organization, NPO) and are not directly supported by the government. These systems, however, are involved with official organizations and funds such as National Institutes of Health (https://www.printo.it and https://www.carragroup.org/). For example, CARRA invites Food and Drug Administration to its congress and PRINTO is associated with a network presided over by the European Medicines Agency. Thus, these systems are managed in a public nature, in cooperation with concerned patients/parents associations. Furthermore, CARRA is financially supported by an organization of seven private companies including large pharmaceutical companies in an open manner.

Fulfillment of centralization

Pediatric Rheumatology International Trials Organization was started as a small organization with personal fund raising by Alberto Martini and Nicolino Ruperto in 1996; it was developed into an international network supported by various national governments in the EU and other countries, and it consisted of 1,189 members from 490 institutions in 59 countries in 2014. In contrast, CARRA, a North American organization, consists of more than 425 members who cooperatively work toward better treatment of rheumatic diseases in children. When conducting a clinical trial, selection of a flagship/core medical institution that has many patients with the particular disease as "the trial center" and subsequent close cooperation by membership institutions enables smooth promotion of the clinical trial. To promote a clinical trial in Japan, it would be necessary to change the registry system to NPO or to construct a system officially approved by the government or for which the government is involved in fund-raising. It would be important to provide incentives to institutions that cooperate with us on centralization.

International cooperation: Taking part in a global clinical trial

At present, PRINTO and CARRA have registered information on approximately 8,000 and approximately 10 000 pediatric patients with rheumatic disease, respectively. The two organizations have been working together on the project to construct a worldwide cooperative registry system on pediatric rheumatic disease for 3 years, and expect completion in another 2 years. Given the present situation, in which Europe and the USA are taking big steps to construct a worldwide registry, we will miss the global wave if we do not construct a domestic registry system and take part in the worldwide cooperative registry system. In contrast, if we can successfully participate in this project, we will have the opportunity to actively take part in global cooperative trials. Participation in a global cooperative clinical trial and hence in the resulting global simultaneous approval using data obtained from these trials is a realistic and effective means by which to solve the time lag in the development and approval of medicines between the USA/EU and Japan, and the time lag between the approval of adult and pediatric indications for medicines in Japan. In short, we should aim for global cooperation: that is, to take part in global cooperative clinical trials and hence in the global simultaneous approval.

Table 4 Approval process for palivizumab outside and in Japan

Product name	Indication	Outside Japan			In Japan			Time lag in clinical trial (between outside and in Japan)		
		Phase I trial	Phase II trial	Phase III trial	Start of phase I	Start of phase II	Start of phase III	Start of phase I	Start of phase II	Start of phase III
Palivizumab for i.m. injection/solution for i.m. injection	Premature infant	December 1994–December 1997	November 1995–December 1996	November 1996–May 1997 (impact study)	May 1999–July 1999	February 2000–June 2000		5 years 1 month	4 years 3 months	
	Congenital heart disease			1998–2002 (cardiac study)			October 2003–March 2004			5 years
	Immunodeficiency	Not indicated	–	–	–	–	August 2011–April 2012	–	–	–
	Down syndrome	Not indicated	–	–	–	–	August 2011–April 2012	–	–	–

Product name	Indication	Approval date outside Japan	Approval date in Japan	Time lag of approval
Synagis for i.m. injection/solution for i.m. injection	Premature infant	June 1998 (USA)	January 2002	3 years 6 months
	Congenital heart disease	September 2003 (USA)	October 2005	2 years 1 month
	Immunodeficiency	–	August 2013	–
	Down syndrome	–	August 2013	–

In the field of pediatric rheumatology, two global cooperative trials are ongoing as of October 2015: (i) a trial evaluating canakinumab for periodic fever syndromes (familial Mediterranean fever, TNF-receptor-related periodic fever syndrome and hyper γ-globulinemia syndrome) except cryopyrin-associated periodic syndrome; and (ii) a trial evaluating belimumab for systemic lupus erythematosus. We were invited to participate in these trials, but only because a sufficient number of patients could not be recruited in the USA and EU. Therefore, there is a concern that we will be unable to participate in a global trial due to insufficient recruitment of subjects in Japan, if we cannot establish a registry and centralization in the future.

Future issues and perspectives

To reduce the duration of clinical trials, it is essential to take part in a world-scale registry system via the registry system PRICURE established by Pediatric Rheumatology Association of Japan. It is then important to carefully plan to participate in the international network of PRINTO and CARRA using the world-scale registry system, with the aim of taking part in global cooperative trials in which we can ensure a sufficient number of entries from Japan. If, however, we cannot take part in a global cooperative trial in the future, it is imperative that we identify the reasons for this and adjust our policy accordingly, with reference to global cooperation in the adult rheumatology field in Japan.

When all of the aforementioned measures are achieved, we should then present this proposal to the Japanese Pediatric Society and its sectional meeting groups to set up the policy for global cooperation.

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Author contributions

M.M. wrote the manuscript and designed this study; M.N., N.T., K.K., J.S., M.S., S.H. and K.S. critically reviewed the manuscript and supervised the whole study process; H.N. contributed to the conception and gave conceptual advice. All authors read and approved the final manuscript.

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PLOS ONE

RESEARCH ARTICLE

PADI4 and the HLA-DRB1 shared epitope in juvenile idiopathic arthritis

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Abstract

Objective

Both genetic and environmental factors are associated with susceptibility to juvenile idiopathic arthritis (JIA). Many studies have reported that both a 'shared epitope' (SE) encoded by several HLA-DRB1 alleles and the peptidyl arginine deiminase type 4 (PADI4) gene polymorphisms are associated with susceptibility to rheumatoid arthritis (RA). However, it is uncertain whether JIA and RA share the latter genetic risk factor. Therefore, here we investigated relationships between HLA-SE and PADI4 polymorphisms with clinical subtypes of JIA.

Methods

JIA patients (39 oligoarthritis, 48 RF-positive polyarthritis, 19 RF-negative polyarthritis and 82 systemic) and 188 healthy controls were genotyped for HLA-DRB1 by PCR-sequence-specific oligonucleotide probe methodology. Three PADI4 gene single nucleotide polymorphisms (SNPs), rs2240340, rs2240337 and rs1748033, were genotyped using TaqMan SNP Genotyping Assays.

Results

Frequencies of the HLA-SE were higher in RF-positive polyarticular JIA than in healthy controls. RF-positive polyarticular JIA was associated with HLA-SE (OR = 5.3, 95% CI = 2.5–11.9, pc < 0.001). No associations were found between clinical subtypes of JIA and PADI4 allele frequency. Nonetheless, rs2240337 in the PADI4 gene was significantly associated with anti-cyclic citrullinated peptide antibody (ACPA)-positivity in JIA. The A allele at rs2240337 was a significant risk factor for ACPA positivity in JIA (OR = 5.6, 95% CI = 1.71–23.7 pc = 0.03).

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Conclusion

PADI4 gene polymorphism is associated with ACPA-positivity in JIA. The association of HLA-SE with RF-positive polyarticular JIA as well as RA is confirmed in Japanese. Thus, HLA-SE and PADI4 status both influence JIA clinical manifestations.

Introduction

Juvenile idiopathic arthritis (JIA) is defined as a chronic arthritis developing in children <16 years of age and persisting for ≥6 weeks. According to the International League of Associations for Rheumatology (ILAR) classification criteria for JIA, it has 7 subtypes [1]. The 4 major subtypes are oligoarthritis, rheumatoid factor (RF)-positive polyarthritis, RF-negative polyarthritis and systemic arthritis. The major pathology of oligoarthritis and polyarthritis is articular inflammation and joint destruction. RF-positive polyarthritis is considered to be a counterpart of adult rheumatoid arthritis (RA) [2]. In contrast to the above forms of JIA, the major pathology of systemic JIA is systemic inflammation, which is considered similar to adult Still's disease [3,4].

In RA and JIA, both genetic and environmental factors are associated with disease susceptibility [5]. HLA class II gene polymorphisms are considered the most influential for RA susceptibility [6]. Many studies have reported the association of a 'shared epitope' (SE) encoded by several HLA-DRB1 alleles with RA susceptibility in adults [7]. Similarly, an association between HLA-SE and susceptibility to JIA has been reported in Caucasians [8]. We have previously reported that HLA-DRB1*04:05, a major SE-containing allele, is associated with polyarticular JIA also in the Japanese population [9].

More recently, a number of RA susceptibility genes outside of the HLA region have been identified by genome-wide association studies (GWAS) [10,11]. One of these, peptidyl arginine deiminase type 4 (PADI4) was first reported in Japanese RA patients [12,13], and subsequently confirmed in several Asian groups and subgroups of Europeans [14–17]. PADI4 is one member of PADI gene family. It codes for enzymes responsible for the posttranslational conversion of arginine residues into citrulline. It was indicated that an RA susceptibility haplotype in PADI4 was associated with increased stability of PADI4 mRNA [13]. And it could lead to accumulation of PADI4 protein, with subsequent increases in citrullinated proteins and enhanced production of autoantibodies against these citrullinated peptides [18].

PADI4 mRNA is detected in hematological cells and pathological synovial tissues [19,20]. And it was reported that PADI4 significantly overexpressed in the blood cells of RA patients [21]. Moreover, PADI4 have a nuclear localization signal, which affects the expression control of various genes [22]. PADI4 may have various role in the immune system and associated with development of autoimmune disease.

In each of the JIA subtypes, age of onset, clinical course and serological findings are different, which may be accounted for by different influences of the genetic background. However, it is uncertain whether JIA (particularly the RF-positive polyarthritic form) and RA share any genetic risk factors other than HLA-SE. There are no reports that PADI4 risk alleles are involved in JIA disease susceptibility. In the present study, which includes our previous cohort [9], we investigated relationships between HLA-SE and PADI4 polymorphisms, and clinical subtypes of JIA in the Japanese population.

Materials and methods

Study population

Patients were eligible if they met the ILAR classification criteria for JIA. A total of 188 JIA patients (39 oligoarthritis, 48 RF-positive polyarthritis, 19 RF-negative polyarthritis and 82 systemic), comprising 59 boys and 129 girls, was enrolled in this study and followed at the Yokohama City University Hospital between December 2006 and December 2009. This cohort included the 106 oligo- and poly-articular JIA patients who were described in our previous study [9]. Clinical data including age at onset, gender, RF and anti-cyclic citrullinated peptide antibody (ACPA) status were reviewed.

We conducted this study in accordance with the Declaration of Helsinki and with the approval of the Ethics Committee of the Yokohama City University School of Medicine. Written informed consent was obtained from each patient and/or their guardian. (Approval number: A090528002)

HLA genotyping

Genomic DNA was isolated from peripheral blood using the QIAamp DNA Mini kit (Qiagen K.K., Tokyo, Japan). JIA patients and healthy adult controls were genotyped for HLA-DRB1 using PCR sequence-specific oligonucleotide probes (SSOP) by the Luminex method with Genosearch HLA-A, -B and -DRB1 Ver. 2 (Medical & Biological Laboratories Co., Ltd. Nagoya, Japan), as described previously [9]. HLA-DRB1*01:01, *04:01, *04:04, *04:05, *04:10, *10:01, *14:02 and *14:06 were regarded as HLA-SE alleles [23].

PADI4 genotyping

Three single nucleotide polymorphisms (SNPs), rs2240340, rs2240337 and rs1748033 in the PADI4 gene were selected based on previous research [12,13]. Genotyping for these in 188 JIA patients and 188 healthy adult controls was performed using TaqMan SNP Genotyping Assays (AB assay ID: C__16176717_10 for rs2240340, C___3123009_1 for rs2240337 and C___7541083_1 for rs1748033). These SNPs were analyzed by real-time PCR using the AB7500 Real Time PCR system (Applied Biosystems, Foster City, CA, USA) under the conditions recommended by the manufacturer. Allele discrimination was accomplished using SDS software version 1.4 (Applied Biosystems).

Statistical analysis

The statistical significance of the differences in the frequencies of HLA-DRB1 alleles or PADI4 gene polymorphisms between JIA subtypes was evaluated by Fishers exact test. A corrected P-value (Pc) was calculated by multiplying the P-value by the number of HLA-DRB1 alleles tested at each locus. For the PADI4 gene polymorphisms, we examined 3 SNPs and used a total of 5 independent tests.

Results

Patients' characteristics

Characteristics of the patients studied are shown in Table 1. Patients comprised 39 children with oligoarthritis, 48 with RF-positive polyarthritis, 19 with RF-negative polyarthritis and 82 with systemic arthritis. The mean age at onset of oligoarthritis was 5.6 years, RF-positive polyarthritis was 8.2 years, RF-negative polyarthritis was 7.1 years and systemic arthritis 5.0 years.

Table 1. Clinical characteristics of JIA patients.

	Oligo articular JIA (n = 39)	RF positive, polyarticular JIA (n = 48)	RF negative, polyarticular JIA (n = 19)	Systemic JIA (n = 82)
Age at JIA onset (years, mean)	5.6	8.2	7.1	5
Gender (female,%)	35 (90%)	40(83%)	10(53%)	44 (53%)
ANA (>1:160,%)	16 (41%)	19(40%)	3(16%)	3/78 (4%)
RF (>14.0 (IU ml-1),%)	9 (23%)	48(100%)	0(0%)	-
Anti-CCP (>4.5(U ml-1),%)	8 (21%)	40(83%)	0(0%)	0/43 (0%)

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HLA-DRB1 and JIA subtypes

188 healthy controls was genotyped for HLA-DRB1 to determine associations of HLA-DRB1 and HLA-SE with JIA subtype susceptibility. According to ILAR classification criteria for JIA, RF-positive oligoarticular JIA is classified as "undifferentiated". Thus, such cases were excluded from the oligoarthritis group in HLA association studies. RF-positive polyarticular JIA was significantly associated with HLA-DRB1*04:05 and HLA-SE (OR = 5.1, 95% CI = 2.5–11, pc < 0.001; OR = 5.3, 95% CI = 2.5–11, Pc < 0.001, respectively) (Table 2). In contrast, frequencies of HLA-DRB1*04:05 and HLA-SE were not higher in the other types of JIA patients.

PADI4 polymorphisms and JIA subtypes

Frequencies of PADI4 gene polymorphisms studied in JIA patients and controls are shown in Table 3. There were no associations between clinical subtypes of JIA and PADI4 gene polymorphisms. Nonetheless, the PADI4 SNPs were significantly associated with ACPA positivity in JIA (Table 4). Because the ACPA status of all systemic JIA patients measured in this study was negative (0/43), systemic JIA was excluded from the data in Table 4. Hence, the A allele at rs2240337 is a significant risk factor for ACPA positivity in oligo- and poly-articular JIA (OR = 5.6, 95% CI = 1.7–24 Pc = 0.03). Finally, there were no associations between HLA-SE and PADI4 gene polymorphisms in oligo- and poly-articular JIA (Table 5).

Table 2. Association of HLA-DRB1*04:05 and HLA-SE with susceptibility to JIA subtypes.

HLA-DRB1*0405	Genotype (*0405/any)	OR	95% CI	P-value	Pc
control (n = 188)	40 (21.3%)	-	-	-	-
Oligoarticular JIA (n = 30)	1(3.3%)	0.1	0.01–0.82	0.02	NS
RF positive, polyarticular JIA (n = 48)	28 (58.3%)	5.1	2.50–10.7	<0.001	<0.001
RF negative,polyarticular(n = 19)	4(21.1%)	1	0.30–4.42	0.98	NS
RF negative(oligo+poly)(n = 49)	5(10.2%)	0.4	0.86–8.17	0.078	NS
Systemic JIA (n = 82)	21 (25.6%)	1.3	0.66–2.42	0.43	NS
HLA-SE	Genotype (SE/any)	OR	95% CI	P-value	Pc
control (n = 188)	68 (36.2%)	-	-	-	-
Oligoarticular JIA (n = 30)	6(20.0%)	0.4	0.14–1.18	0.082	NS
RF positive polyarticular JIA (n = 48)	36 (75.0%)	5.3	2.47–11.9	<0.001	<0.001
RF negative,polyarticular(n = 19)	5(26.3%)	0.6	0.17–1.96	0.39	NS
RF negative(oligo+poly)(n = 49)	15(30.6%)	0.8	0.37–1.60	0.47	NS
Systemic JIA (n = 82)	33 (40.2%)	1.8	0.67–2.09	0.59	NS

SE, shared epitope: HLA-DRB1*04:05,01:01,04:01,04:10,10:01,14:02,14:06

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Table 3. Association between PADI4 gene polymorphisms and susceptibility to JIA subtypes.

rs2240340	G allele	A allele	MAF	OR	95% CI	P	Pc
Control (n = 188)	223	153	0.41	-	-	-	-
Oligoarticular JIA (n = 30)	37	23	0.38	0.9	0.49–1.64	0.73	NS
RF positive, polyarticular JIA (n = 48)	49	47	0.49	1.4	0.87–2.25	0.17	NS
RF negative,polyarticular(n = 19)	24	14	0.37	0.9	0.39–1.78	0.64	NS
RF negative,oligo+poly articular(n = 49)	61	37	0.38	0.9	0.54–1.43	0.6	NS
Systemic JIA (n = 82)	92	72	0.44	1.1	0.77–1.68	0.51	NS
rs2240337	G allele	A allele	MAF	OR	95% CI	P	Pc
Control (n = 188)	350	26	0.07	-	-	-	-
Oligoarticular JIA (n = 30)	57	3	0.05	0.7	0.13–2.43	0.45	NS
RF positive, polyarticular JIA (n = 48)	85	11	0.12	1.7	0.75–3.82	0.14	NS
RF negative,polyarticular(n = 19)	37	1	0.03	0.4	0.01–2.36	0.25	NS
RF negative,oligo+poly articular(n = 49)	94	4	0.04	0.6	0.14–1.71	0.21	NS
Systemic JIA (n = 82)	149	15	0.18	1.4	0.65–2.74	0.38	NS
rs1748033	G allele	A allele	MAF	OR	95% CI	P	Pc
Control (n = 188)	239	137	0.36	-	-	-	-
Oligoarticular JIA (n = 30)	42	18	0.30	0,7	0.39–1.39	0.33	NS
RF positive, polyarticular JIA (n = 48)	55	41	0.43	1.3	0.80–2.10	0.29	NS
RF negative,polyarticular(n = 19)	26	12	0.32	0.8	0.36–1.72	0.55	NS
RF negative,oligo+poly articular(n = 49)	68	30	0.31	0.8	0.46–1.27	0.28	NS
Systemic JIA (n = 82)	120	44	0.27	0.6	0.42–0.97	0.03	NS

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Table 4. Association between PADI4 gene polymorphisms and ACPA positivity in oligo- and poly- articular JIA patients (n = 106).

		Anti-CCP(-) (<4.5U ml-1) (n = 58)	Anti-CCP (+) (>4.5U ml-1) (n = 48)	OR	95% CI	P	Pc
rs2240340	allele	75	46	2	1.1–3.6	0.018	NS
	recessive	26	11	2.7	1.1–7.1	0.024	NS
	dominant	49	35	2	0.70–6.0	0.158	NS
rs2240337	allele	112	80	5.6	1.7–24	0.002	0.03
	recessive	54	32	6.6	1.9–30	<0.001	<0.001
	dominant	-	-	-	-	-	-
rs1748033	allele	80	52	1.9	1.0–3.4	0.03	NS
	recessive	30	14	2.6	1.1–6.4	0.029	NS
	dominant	53	38	2.8	0.78–11	0.095	NS

Recessive: GG versus (GA/AA), dominant: (GG/GA) versus AA

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Table 5. Association between PADI4 gene polymorphisms and SE positivity in oligo- and poly- articular JIA (n = 106).

		GG	GA/AA	OR	95% CI	P-value
rs2240340	SE-	20	31	1.4	0.60–3.5	0.42
	SE+	17	38	-	-	-
rs2240337	SE-	42	9	1.2	0.39–3.5	0.81
	SE+	44	11	-	-	-
rs1748033	SE-	23	28	1.3	0.57–3.1	0.56
	SE+	21	34	-	-	-

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Discussion

Susceptibility to RA is influenced by both genetic and environmental factors such as smoking. Many studies have determined that the major RA disease susceptibility genes are the HLA class II alleles. The shared epitope (SE) hypothesis for risk of RA is well-established [7], indicating that multiple HLA-DRB1 alleles are the strongest known genetic risk factors for RA by virtue of encoding a shared amino acid sequence, known as a shared epitope, SE [6]. Several studies have also reported associations between the genetic background and JIA susceptibility [5], including associations with HLA alleles [24–29]. An association between HLA-SE and susceptibility to JIA has been confirmed in 204 RF- or ACPA-positive Caucasian JIA patients [8].

The contribution of HLA to RA susceptibility, however, accounts for only about 30% of incidence, implying that genes other than those in the HLA region are involved; some estimates suggest as many as 100. Other genes influencing RA susceptibility have now been identified, such as PADI4, PTPN22 and CTLA4. Numerous non-HLA JIA susceptibility genes have also been imputed using GWAS [11]. Variants at the PTPN22, STAT4, TNF-α, TNFAIP3, MIF, WISP3, SLC11A1 and IL2-Ra loci have been reported as risk factors for JIA by several investigators [5], although it was also reported that several of these are not necessarily shared between different ethnic groups [10,30]. Thus, there are likely to be different genetic risk factors for JIA in different ethnic groups. Therefore, here we sought an influence of HLA-SE and PADI4 on JIA susceptibility in Japanese, because both HLA-SE and PADI4 were reported as significant genetic risk factors for RA independent of ethnicity [14,15,31].

We previously reported an association of HLA-A*02:06 with JIA accompanied by uveitis and of HLA-DRB1*04:05 with polyarticular JIA [9]. In the present study, we confirmed the association between HLA-SE and RF-positive polyarticular JIA in Japanese. However, we found that HLA-SE was not associated with oligoarticular or systemic JIA in our cohort. Recently, it was reported that five amino acids in three HLA molecules, including three amino acid positions (11, 71 and 74) in HLA-DRB1, were associated with RF-seropositive RA by the HLA-imputation method [32]. It should therefore be evaluated whether these HLA amino acids are also associated with JIA susceptibility in future.

In addition to RF, ACPA is the most specific serologic marker in adult RA with a specificity of 95% and a sensitivity of 80%, similar to RF [33,34]. Considering all JIA subtypes together, ACPA was detected in 1.8–28.6% of patients, a low frequency compared to RA. However, ACPA was present in 70–90% of RF-positive polyarticular JIA patients [35]. Bone destruction is more severe in these ACPA-positive patients [36]. These results suggest that ACPA-positive polyarticular JIA may be similar to RA with regard to pathogenetic processes.

PADI4, a member of the PADI family, was first reported to be associated with RA in a Japanese population [12,13]. It encodes a peptidyl arginine deiminase responsible for the post-translational conversion of arginine residues into citrulline. We investigated associations between PADI4 gene polymorphisms and ACPA positivity in JIA in our Japanese population. The stability of PADI4 mRNA differs according to these gene polymorphisms, which may represent the mechanism by which it influences the production of ACPA [13]. To the best of our knowledge, there are no reports that PADI4 risk alleles are involved in JIA disease susceptibility. It is likely that PADI4 is also a JIA susceptibility gene in ethnic groups other than Japanese, especially in ACPA-positive JIA. This hypothesis needs further exploration.

We found no association between HLA-SE and PADI4 in JIA patients, implying that HLA-SE and PADI4 are independent JIA susceptibility genes. However, an association between HLA-SE and citrullination in the pathogenesis of RA has been noted [37]. The electropositive P4 pocket of HLA-DRB1*04:01/04 can accommodate citrulline-containing epitopes, and the $CD4^+$ T cell repertoire for citrullinated antigens is increased in RA patients

harboring HLA-DRB1*04:01/04. These potential pathogenetic mechanisms may also contribute to JIA. Further study is needed to determine whether this is the case.

In conclusion, we found an association of PADI4 gene polymorphisms with ACPA-positivity in JIA, as was already known for RA. We also confirmed the influence of HLA-SE on RF-positive polyarticular JIA in the Japanese population. Thus, JIA may be classified into clinical and genetic background-based subtypes using HLA-SE and PADI4 genotyping.

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Subtitle: Validation of classification criteria of MAS complicating s-JIA

Validation of classification criteria of macrophage activation syndrome in Japanese patients with systemic juvenile idiopathic arthritis

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Significance and Innovations:

1. Classification criteria for MAS complicating s-JIA had very high diagnostic performance for full-blown MAS.
2. Diagnostic sensitivity for MAS onset was relatively low.
3. Dynamics of laboratory values during the course of MAS should be further investigated.

Abstract

Objective. To validate whether the 2016 ACR/EULAR classification criteria of macrophage activation syndrome (MAS) complicating systemic juvenile idiopathic arthritis (s-JIA) is practical in the real world.

Methods. A combination of expert consensus and analysis of real patient data was conducted by a panel of 15 paediatric rheumatologists. Sixty five profiles comprised 18 patients with s-JIA–associated MAS and 47 patients with active s-JIA without evidence of MAS. From these profiles, 10 patient data points for full-blown MAS, 11 patient data points for MAS onset and 47 patient data points for acute s-JIA without MAS were evaluated.

Results. Evaluation of the classification criteria to discriminate full-blown MAS from acute s-JIA without MAS showed a sensitivity of 1.000 and specificity of 1.000 at the time of full-blown MAS. Sensitivity was 0.636 and specificity 1.000 at the time of MAS onset. The number of measurement items that fulfilled the criteria increased in full-blown MAS compared to that at MAS onset. At MAS onset, the positive rates of patients who met the criteria for platelet counts and triglycerides were low, whereas those for aspartate aminotransferase was relatively high. At full-blown MAS, the number of patients who met the criteria for each measurement item increased.

Conclusion. The classification criteria for MAS complicating s-JIA had a very high diagnostic performance. However, the diagnostic sensitivity for MAS onset was relatively low. For the early diagnosis of MAS in s-JIA, the dynamics of laboratory values during the course of MAS should be further investigated.

Key words: macrophage activation syndrome, systemic juvenile idiopathic arthritis, classification criteria

Introduction

Macrophage activation syndrome (MAS) is a severe complication of systemic juvenile idiopathic arthritis (s-JIA), which is clinically characterized by fever, hepatosplenomegaly, lymphadenopathy, depression of all 3 blood cell lines, deranged liver function, intravascular coagulation and central nervous system dysfunction [1]. MAS is a potentially life-threatening disease, and, thus, a timely and prompt diagnosis is essential to initiate life-saving treatment. However, it can be difficult to distinguish MAS from s-JIA flares, sepsis or other secondary haemophagocytic lymphohistiocytosis (HLH). Differentiation of MAS from these conditions is essential for the selection of an appropriate therapeutic intervention in a timely fashion. However, no definite clinical or laboratory parameter can establish

MAS diagnosis.

Researchers have recently developed the classification criteria for MAS complicating s-JIA [2,3]. These criteria define MAS complicating s-JIA as a febrile patient with known or suspected s-JIA who is classified as having MAS if the following criteria are met: ferritin > 684 ng/ml and any 2 of the following: platelet count ≤ 181 × 10^9/l, aspartate aminotransferase (AST) >48 units/l, triglycerides (TG) > 156 mg/dl and fibrinogen ≤ 360 mg/dl. These criteria include only laboratory data and no clinical manifestations except fever, because transition of MAS is commonly suspected by the detection of subtle laboratory alterations, and clinical symptoms are often delayed. Furthermore, these criteria do not include the detection of hemophagocytosis in bone marrow biopsy specimens or bone marrow aspirates as well.

Recent advances in understanding of the pathophysiology of MAS including underlying genetic defects and the treatment for MAS revealed clinical heterogeneity of MAS [1, 4-6]. Therefore, validation of new classification criteria for MAS complicating s-JIA in different patient cohort might have added value. In this report, we validated whether the classification criteria for MAS complicating s-JIA is practical in the real world in Japanese s-JIA patients with or without MAS.

Methods

Patients and Methods

We enrolled 96 Japanese s-JIA patients with or without MAS in this study, and performed validation of the classification criteria for MAS complicating s-JIA based on a combination of expert consensus and analysis of real patient data. The minimum required level of agreement among experts was set at 80%. Investigators were asked to include s-JIA patients with MAS seen after 2006. This timeframe was chosen because preliminary diagnostic guidelines for the diagnosis of MAS was reported in 2005 [7]. The patients who had been treated with any biological agents such as tocilizumab until the time of analysis were not included, because these agents might modify clinical presentation of MAS [8-10]. For patients with MAS, information on laboratory features from the acute s-JIA phase to MAS phase that included at least 3 time points (the last visit before MAS onset, the time of MAS onset and the period of full-blown MAS) were collected. MAS onset was defined as the time when the initial clinical and/or laboratory abnormalities suggesting the occurrence of MAS were detected [11]. Full-blown MAS was defined as the time at which MAS reached its most severe stage [11].

We reported 96 patients (30 with MAS and 66 with active s-JIA without MAS) from 13 institutes of pediatric rheumatology in Japan. A panel of 15 experts was first asked diagnose the 96 patient profiles with or without MAS based on clinical and laboratory features at the time of disease onset. Of the 96 patient profiles, 31 were excluded because of insufficient data or suspicion of systemic infection. Among the remaining 65 patient profiles, 18 patients were diagnosed with MAS and 47 without MAS by the experts. Of the 18 patients with MAS, 10 were diagnosed with full-blown MAS and 8 with MAS onset. Laboratory data was also available at MAS onset for 3 of the 10 patients with full-blown MAS. From these profiles, 15 experts evaluated the laboratory data based on MAS classification criteria that resulted in 10 patient data points for full-blown MAS, 11 (8+3) patient data points for MAS onset and 47 patient data points for acute s-JIA without MAS. The mean ages of 18 MAS patients and 47 s-JIA patients were both 6.5 year, and sex ratios were 10:8 and 23:24, respectively. Table 1 shows the clinical characteristics of patient data points in each phase. This study was approved by the institutional review boards of all participating centres.

Statistical analysis

The performance of the classification criteria was analyzed by assessing the sensitivity, specificity and likelihood ratio. Within-group comparison was analysed using Fisher's exact test. For the analysed measures, *p*-values less than 0.05 were considered statistically significant.

Results

Validation of the classification criteria of MAS complicating s-JIA at MAS onset and full-blown MAS

At the time of full-blown MAS, 10 of the 10 patients fulfilled the criteria, whereas no patient who did not have MAS fulfilled them. Evaluation of the ability of the new classification criteria to discriminate full-blown MAS from acute s-JIA without MAS showed a sensitivity of 1.000, a specificity of 1.000. At the time of MAS onset, 7 of the 11 patients with MAS fulfilled the criteria, whereas no patient who did not have MAS fulfilled them. Evaluation of the ability of the new classification criteria to discriminate MAS onset from acute s-JIA without MAS showed a sensitivity of 0.636, a specificity of 1.000. Negative likelihood ratio was 0.363.

Comparison of the number of measurement items that fulfilled the criteria between MAS onset and full-blown MAS

At the time of full-blown MAS, ferritin levels in 10 patients with MAS were all >684 ng/ml. At MAS onset, 10 of the 11 patients fulfilled it. As shown in Table 2, for full-blown MAS, in 9 out of 10 patients, over 3 items fulfilled the criteria. On the other hand, at the onset of MAS, in only 2 out of 11 patients, over 3 items fulfilled the criteria.

Comparison of the number of patients who met the criteria for each measurement item between MAS onset and full-blown MAS

As shown in Table 3, at MAS onset, the positive rates of patients who met the criteria for each measurement item were variable; rates for platelet count, TG and fibrinogen were low, whereas that for AST was relative high (about 70%). At full-blown MAS, the number of patients who met the criteria for each measurement item increased, and the positive rate for AST was 100%.

Discussion

We validated the classification criteria for MAS complicating s-JIA in 65 Japanese s-JIA patients with or without MAS. At the time of full-blown MAS, the classification criteria for MAS complicating s-JIA had a very high performance for the

diagnosis of full-blown MAS.

Ferritin levels exhibited the largest change over time during MAS, indicating that it plays a major role in MAS detection [12]. Furthermore, ferritin had the greatest influence on the experts' diagnosis of patients with or without MAS [2,3]. In this study, ferritin levels in patients with full-blown MAS were all >684 ng/ml. Furthermore, most of all patients during MAS onset fulfilled this. These findings support that ferritin is a key parameter in the classification of MAS.

The number of measurement items that fulfilled the criteria was increased in full-blown MAS compared to those at MAS onset. These findings indicate that an increased number of measurement items might be correlated with the severity of MAS. Serial observations of the time course of these measurement items might be useful for an evaluation of disease activity in MAS. At MAS onset, the positive rates of patients who met the criteria for platelet counts and TG were low, whereas that for AST was relative high. At full-blown MAS, the number of the patients who met the criteria for each measurement item increased. These findings indicate that the time course of these items during MAS might be different.

In this study, we observed the sensitivity of the MAS onset diagnosis to be relatively low. An early, timely and prompt diagnosis of MAS is essential to initiate life-saving treatment. The dynamics of laboratory values during the course of MAS should be further investigated and optimal variation cut-off values should also be established. Furthermore, recent studies showed clinical usefulness of serum cytokine measurement for assessing the transition to MAS from acute phase as well as the differentiation MAS from the other secondary hemophagocytic lymphohistiocytosis [13,14]. To establish the criteria for the timely diagnosis of the onset of MAS, further studies to determine the kinetics of laboratory tests and reliable biomarkers such as IL-18, sTNFRII/I ratio, IFN-γ, and IFN-γ-induced chemokines, and to clarify the usefulness of paneling them is desired.

The limitation of this study is a small sample size and a retrospective analysis of a multicenter gathered cohort. Further larger prospective study might bring high added value to this study.

In conclusion, the classification criteria for MAS complicating s-JIA had a very high performance for the diagnosis of full-blown MAS with high sensitivity and specificity. However, diagnosis sensitivity for MAS onset was relatively low. For an

early diagnosis of MAS in s-JIA, the dynamics of laboratory values during the course of MAS should be further investigated.

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Table 1: Clinical manifestations and laboratory findings in each group.

	Full-blown MAS		Onset of MAS		Non-MAS	
Number of patient data points	10		11		47	
Clinical manifestations						
	N		N		N	
Fever	10	10	11	11	47	43
Rash	10	9	11	9	47	30
Hepatomegaly	10	3	11	2	47	6
Splenomegaly	10	1	11	1	47	6
Lymphadenopathy	10	3	11	4	47	13
Central nervous system involvement	10	0	11	0	47	0
Hemorrhagic manifestations	10	0	11	0	47	0
Heart, Lung, Kidney failure	10	1	11	0	47	0
Laboratory results						
	N	mean	N	mean	N	mean
White blood cell count (/mm^3)	10	11897	11	12899	47	16738
Neutrophils count (/mm^3)	9	6935	11	10423	44	13643
Platelet count ($\times 10^4/mm^3$)	10	14.9	11	24.5	47	40.7
Hemoglobin (g/dL)	10	9.9	11	10.6	47	11.1
C-reactive protein (mg/dL)	10	8.3	11	9	47	9.9
Aspartate aminotransferase (IU/L)	10	519	11	88	47	36
Alanine aminotransferase (IU/L)	10	242	11	78	47	29
Lactate dehydrogenease (IU/L)	10	2153	11	632	47	338
Triglyceride (mg/dL)	10	246	11	164	47	98
Albumin (g/dL)	10	2.9	10	2.9	46	3.1
Serum sodium (mEq/L)	10	134	10	135	46	137
Fibrinogen (mg/dL)	10	267	11	366	47	608
Ferritin (ng/mL)	10	23797	11	11613	47	1508
Fibrin degradation product (μg/ml)	6	188.1	6	21.6	20	9.5
D-dimer (μg/ml)	7	78.8	11	26.4	41	4.2
Therapeutic interventions (at the time of analysis, treatment ever used before analysis)						
	N		N		N	
Any corticosteroids	10	6, 6	11	4, 4	49	11, 12
Cyclosporine	10	2, 2	11	1, 1	49	1, 1
Intravenous immunoglobulin	10	0, 1	11	0, 0	49	0, 0
Other immunosuppressants	10	0, 0	11	0, 1	49	4, 4
Outcome						

	N		N		N	
Death	10	0	11	0	49	0

Table 2: Comparison of the number of measurement items which fulfilled criteria between MAS onset and full-blown MAS

	Number of measurement items fulfilled criteria				
	4	3	2	1	Total
MAS onset	1	1	6	3	11
Full-Blown MAS	6	3	1	0	10

	MAS onset (n=11)	Full-blown MAS (n=10)
Platelet count	2 (18.2%)	8 (80%)
AST	8 (72.7%)	10 (100%)
TG	5 (45.5%)	8 (80%)
Fibrinogen	7 (63.6%)	9 (90%)

Table 3: Comparison of the number of the patients who met the criteria for each measurement item between MAS onset and full-blown MAS

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ORIGINAL ARTICLE

Safety and effectiveness of etanercept for treatment of juvenile idiopathic arthritis: Results from a postmarketing surveillance

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ABSTRACT

Objectives: The objectives of this surveillance were to determine safety and effectiveness of etanercept in patients with juvenile idiopathic arthritis (JIA).

Methods: In this postmarketing surveillance, patients aged 5–16 years with active polyarthritis JIA were treated with etanercept at the doses approved in the Japanese package insert. The occurrence and seriousness of adverse events (AEs) were assessed using the Japanese Medical Dictionary for Regulatory Activities version 15.1. Effectiveness was determined as the improvement from baseline in disease activity score in 28 joints (DAS28)–erythrocyte sedimentation rate (ESR), remission, and physician's assessment of overall improvement. The number of responders was expressed as a percentage. The last observation carried forward method was used to impute missing data.

Results: Safety analysis included 102 patients; 22 patients experienced 36 treatment-related AEs, three of which were unexpected. None of the AEs were deemed to need special safety warnings. Effectiveness analysis included 87 patients. At 24 weeks, 29/46 (63.0%) patients demonstrated either good or moderate response in DAS28-4/ESR and treatment was assessed to be markedly effective or effective by physicians in 79/83 (95.2%) patients.

Conclusions: These data are consistent with earlier reports showing that etanercept was effective and demonstrated no safety signals in patients with JIA.

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KEYWORDS
Etanercept; juvenile idiopathic arthritis; observational surveillance

Introduction

Juvenile idiopathic arthritis (JIA) is a chronic childhood inflammatory rheumatic disease of unknown etiology [1,2] and occurs in approximately 10–15 in 100,000 children [3]. JIA is characterized by persistent joint pain, swelling, inflammation, and limited range of mobility, with a high risk of disability [1,2] severely affecting the patient's physical abilities, psychology, and quality of life [4,5].

The lack of known etiology, the inherent heterogeneity of JIA, and the age of the patients make it difficult to develop a standard, targeted treatment regimen. Treatment paradigms are still evolving and vary widely depending on the treating physician [6]. Until recently, there were no guidelines for treatment of JIA. In general, the treatment goals include stopping active disease, relieving pain, restoring physical function and growth, and improving quality of life [7,8]. However, unlike strategies for treating adult rheumatic diseases, these guidelines do not specify treat-to-target or tight control strategies [6].

The choice of treatment strategy depends on the type of JIA [8]. For example, non-steroidal anti-inflammatory drugs (NSAIDs) along with physiotherapy are recommended for patients with oligoarthritis JIA. With increasing disease severity, glucocorticoids and disease-modifying anti-rheumatic drugs (DMARDs), including methotrexate, can be added. Tumor necrosis factor (TNF) inhibitors are the first choice for treating polyarthritis JIA [8,9]. Data from clinical trials [10–12] and open-label studies [13] demonstrate that etanercept is effective in 80% of patients, sustained over several years, and improves patient health-related quality of life, with an acceptable safety profile [14].

In this paper, we report on the results from an all-patient, postmarketing surveillance (NCT01145352) that evaluated the safety and effectiveness of etanercept for the treatment of polyarthritis JIA in daily medical practice.

Methods

Surveillance design and patients

In this multi-center, all-patient, postmarketing surveillance, patients 5–16 years of age with active polyarthritis JIA were targeted. Patients with other subtypes of JIA who enrolled in

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the study and treated with etanercept were included in the safety analysis. Patients were administered a single subcutaneous injection of etanercept 0.2–0.4 mg/kg twice weekly (pediatric etanercept dosage specified in Japanese package insert). The study was conducted in accordance with the good postmarketing surveillance practice.

Endpoints

The primary endpoint was to determine the safety of etanercept treatment in patients with JIA. All occurrences of adverse events (AEs), especially infection-associated AEs, after initiation of etanercept were recorded and assessed based on the Japanese Medical Dictionary for Regulatory Activities (MedDRA) version 15.1 [15]. Secondary endpoints included improvement in disease activity score in 28 joints-4 parameters/erythrocyte sedimentation rate (DAS28-4/ESR) [16] at 24 weeks and physician's assessment of patient overall improvement.

Data analysis

Disease activity was categorized as low (DAS28-4/ESR $\geq$2.6 to <3.2), moderate (DAS28-4/ESR $\geq$3.2 to $\leq$5.1), or high (DAS28-4/ESR >5.1). Remission was defined as DAS28-4/ESR <2.6. Patients were considered responders if they demonstrated either good (improvement >1.2 in DAS28-4/ESR) or moderate (improvement 0.6 to $\leq$1.2 DAS28-4/ESR) response to etanercept according to the European League Against Rheumatism (EULAR) response criteria [17]. Patients were also considered responders if etanercept was either markedly effective or effective as evaluated by the investigator. The number of responders was expressed as a percentage and the 95% confidence intervals (CI) were estimated. The paired *t*-test was used to compare DAS28 differences between baseline and weeks 4, 8, 12, 16, 20, and 24. Statistical significance was defined as $p < .05$ (two-tailed test). The last observation carried forward (LOCF) method [18] was used to impute missing data obtained after the administration of etanercept, except for baseline values which were not carried forward.

Results

Patient characteristics

A total of 113 patients were registered in the surveillance, of which six patients were found to have been duplicated due to change of treatment center (Figure 1); the duplicated assessments were excluded. Of the 107 patients, five patients were excluded from safety analysis: four patients discontinued treatment before it was approved and one patient used a different formulation of the drug. Of the 102 patients available for safety analysis (Table 1), 38 (37.3%) had a history of being treated with etanercept (these patients were involved in a clinical trial on etanercept or were receiving treatment prior to approval), and 7 (6.9%) had a history of being treated with tocilizumab. Of these 102 patients 24 (23.5%) were male and 78 (76.5%) female; 64 (62.8%) presented with rheumatoid factor-positive polyarthritis JIA, 21 (20.6%) with rheumatoid factor-negative polyarthritis JIA, 9 (8.8%) with oligoarthritis JIA, and 6 (5.9%) with systemic onset JIA. Thirty-nine patients were categorized to Steinbrocker functional class I, 58 patients to class II, and four patients to class III. The duration of disease was $\geq$5 years in 49 (48.0%) patients. Fifteen patients were excluded from effectiveness analysis including five patients with diseases not eligible for the surveillance, effectiveness data after treatment not being available for nine patients, and two patients receiving etanercept for less than two weeks; one

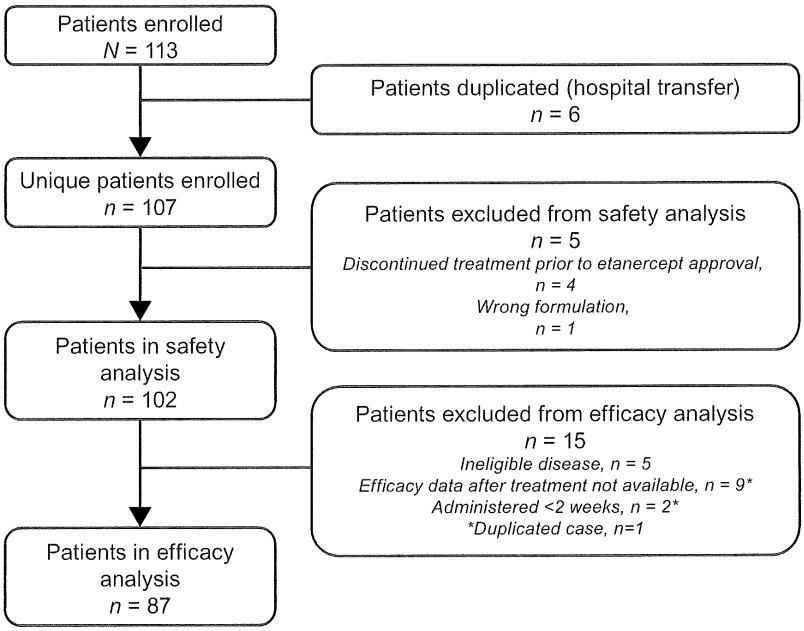

Figure 1. Patient disposition.

patient was excluded for multiple reasons. Of the remaining 87 patients available for effectiveness analysis, 34 had a history of etanercept treatment.

Safety

Of 102 patients evaluated for safety, 22 (21.6%) patients experienced 36 treatment-related AEs (Table 2). The most common treatment-related AEs reported were upper respiratory tract inflammation ($n=6$), injection site reaction ($n=5$), influenza ($n=3$), and pharyngitis, dermatitis, and alanine aminotransferase increase ($n=2$, each). There were three unexpected treatment-related AEs, based on the Japanese package insert for etanercept, which included one patient each of iritis, diarrhea, and acne. The case of diarrhea was considered serious, but it was unclear whether there was a causal relationship with treatment; the patient recovered when administration of the drug was stopped. Infections, including varicella, were reported for 7 (6.9%) patients (three with influenza, two with pharyngitis, and one each with gastroenteritis and gastroenteritis viral). None of these events were serious and all patients recovered. Four patients discontinued due to AEs. Three of these patients (one patient each of pyrexia, prolonged urticaria, and herpes zoster) experienced these AEs prior to receipt of market authorization for etanercept in Japan, were discontinued, and were excluded from the safety analysis. The fourth patient excluded was due to diarrhea. Serious AEs included one patient with diarrhea and the patient recovered when drug administration was stopped. However, it was not determined whether there was a causal relationship with the drug. None of the AEs were deemed to need special safety warnings.

Among patients with a history of etanercept treatment, 6/38 (15.8%) experienced 11 AEs, which included 2 (5.3%) patients with upper respiratory tract inflammation and influenza each, and 1 (2.6%) patient each with iritis, gastroenteritis, gastritis, dermatitis, pruritus, injection site reaction, and alanine aminotransferase increase; the iritis was not predicted by the Japanese package insert for etanercept. Among etanercept-naïve patients, 16/64 (25.0%) experienced 25 AEs, which included 4 (6.3%) with upper respiratory tract inflammation and injection site reaction each, 2 (3.1%) patients with pharyngitis, and 1 (1.6%) patient each with gastroenteritis viral, influenza, headache, epistaxis, diarrhea, acne, alopecia, dermatitis, urticaria, fatigue, injection site erythema, malaise, alanine aminotransferase increase, aspartate

Table 1. Patient demographics.

Parameter	Details	Number of patients (%)
Total		102 (100)
Age (years)	Mean (SD)	13.3 (4.5)
	<5	1 (1.0)
	≥5 to <10	24 (23.5)
	≥10 to <15	37 (36.3)
	≥15 to <20	30 (29.4)
	≥20 to <30	10 (9.8)
Gender	Male	24 (23.5)
	Female	78 (76.5)
Body weight (kg)	Mean (SD)	40.6 (15.7)
	<30	26 (25.4)
	30 to <40	20 (19.6)
	40 to <50	31 (30.4)
	50 to < 60	18 (17.7)
	≥60	7 (6.9)
BMI (kg/m^2)	Mean (SD)	18.9 (4.0)
	<18.5	56 (54.9)
	18.5 to <25.0	37 (36.3)
	25.0 to <30.0	6 (5.9)
	≥30.0	1 (1.0)
JIA onset type	Polyarthritis RF$^+$ JIA	64 (62.8)
	Polyarthritis RF$^-$ JIA	21 (20.6)
	Oligoarthritis JIA	9 (8.8%)
	Systemic onset JIA	6 (5.9)
	Unknown	2 (2.0)
Steinbrocker functional class	I	39 (38.2)
	II	58 (56.9)
	III	4 (3.9)
	Unknown	1 (1.0)
Disease duration	<2 years	29 (28.4)
	≥2 to <5 years	20 (19.6)
	≥5 years	49 (48.0)
	Unknown	4 (3.9)
Varicella vaccination	Yes	28 (27.5)
	No	61 (59.8)
	Unknown	13 (12.8)
Comorbidities	Hepatic dysfunction	1 (1.0)
	Renal dysfunction	0 (0)
Prior drug use	Methotrexate	90 (88.2)
	Etanercept	38 (37.3)
	Tocilizumab	7 (6.9)
Concomitant drug use	DMARDs	89 (87.3)
	Methotrexate	87 (85.3)
	Glucocorticoids	68 (66.7)

DMARD: disease-modifying anti-rheumatic drug; JIA: juvenile idiopathic arthritis; RF: rheumatoid factor.

Table 2. Treatment-related adverse events observed by system organ class preferred term.

	Number (%)
Number of patients analyzed	102 (100)
Number of patients with AEs	22 (21.6)
Number of AEs	36
Number of patients with serious AEs	1
Number of serious AEs	1
System organ class	
Preferred term	
Infections and infestations	7 (6.9)
Gastroenteritis	1 (1.0)
Gastroenteritis viral	1 (1.0)
Influenza	3 (2.9)
Pharyngitis	2 (2.0)
Nervous system disorders	1 (1.0)
Headache	1 (1.0)
Eye disorders	1 (1.0)
Iritis[a]	1 (1.0)
Respiratory, thoracic, and mediastinial disorders	
Epistaxis	1 (1.0)
Upper respiratory tract inflammation	6 (5.9)
Gastrointestinal disorders	2 (2.0)
Diarrhea[a]	1 (1.0)
Gastritis	1 (1.0)
Skin and subcutaneous tissue disorders	5 (4.9)
Acne[a]	1 (1.0)
Alopecia	1 (1.0)
Dermatitis	2 (2.0)
Pruritus	1 (1.0)
Urticaria	1 (1.0)
General disorders and administrative site conditions	7 (6.9)
Fatigue	1 (1.0)
Injection site erythema	1 (1.0)
Injection site reaction	5 (4.9)
Malaise	1 (1.0)
Investigations	3 (2.9)
Alanine aminotransferase increased	2 (2.0)
Aspartate aminotransferase increased	1 (1.0)
Blood β-D-glucan increased	1 (1.0)

[a]AE: adverse event. Unexpected based on Japanese package insert for etanercept.

aminotransferase increase, and blood β-D-glucan increase; the diarrhea and acne were not predicted by the Japanese package insert for etanercept. There were no safety warnings deemed necessary for either group.

Effectiveness

Data to calculate EULAR response rates based on DAS28-4/ESR were available for 46 patients, of whom 29 (63.0%) demonstrated good or moderate DAS28 response (Table 3). There was a steady increase over time in the number of patients who experienced good response.

Overall, the mean ± standard deviation (SD) in DAS28-4/ESR decreased from 3.5 ± 1.6 prior to administration of etanercept to 1.9 ± 1.0 at the end of 24 weeks of treatment (Figure 2); the decrease was statistically significant ($p < .001$) for all observation points through the surveillance period. In patients with a history of treatment with etanercept, the mean ± SD DAS28-4/ESR was 2.7 ± 2.2 and decreased to 2.0 ± 1.2 at 24 weeks, but the change was not statistically significant. However, the median DAS28-4/ESR score for this subgroup was 1.9 at baseline, indicating that the majority of patients were already in remission (DAS28-4/ESR <2.6). Among patients naïve for drug treatment, i.e. had not been treated with etanercept prior to participation in this surveillance, the mean ± SD pre-administration DAS28-4/ESR was 3.7 ± 1.4 and decreased to 1.9 ± 0.9 after 24 weeks of treatment; the decrease was statistically significant ($p < .001$) for all observation points through the surveillance period.

At the beginning of the surveillance period, 10/52 (19.2%) patients had high, 17/52 (32.7%) had moderate, 8/52 (15.4%) had low disease activity, and 17/52 (32.7%) were in remission (Figure 3(A)). Over the 24 weeks of treatment with etanercept, the number of patients in the high and moderate disease activity groups decreased steadily to only 1 (1.6%) patient still having high and 5 (7.9%) having moderate disease activity; there was almost no change in the proportion of patients with low disease activity (Figure 3(A)). Throughout the surveillance period the proportion of patients in remission increased steadily from 17/52 (32.7%) to 48/63 (76.2%) indicating the shift towards remission with treatment (Figure 3(A)). Among patients with a history of etanercept treatment, at the beginning of the surveillance period only 2/12 (16.7%) had high, 1/12 (8.3%) each had moderate or low disease activity, with 8/12 (66.7%) patients already being in remission (Figure 3(B)). Nevertheless, over the course of the surveillance period, the number of patients

Table 3. Assessment of EULAR response over time using LOCF imputation of data.

Response[a]	Week 4 ($n = 25$)	Week 8 ($n = 30$)	Week 12 ($n = 31$)	Week 16 ($n = 32$)	Week 20 ($n = 34$)	Week 24 ($n = 46$)
Good response, *n* (%)	6 (24.0)	11 (36.7)	11 (35.5)	15 (46.9)	18 (52.9)	25 (54.4)
Moderate response, *n* (%)	12 (48.0)	11 (36.7)	11 (35.5)	9 (28.1)	7 (20.6)	4 (8.7)
No response, *n* (%)	7 (28.0)	8 (26.7)	9 (29.0)	8 (25.0)	9 (26.5)	17 (37.0)
Response rate, % (95% CI)[b]	72.0 (50.6, 87.9)	73.3 (54.1, 87.7)	70.9 (52.0, 85.8)	75.0 (56.6, 88.5)	73.5 (55.6. 87.1)	63.0 (47.6, 76.8)

[a]Good response: improvement of >1.2 in DAS28 score; moderate response: improvement of 0.6 to ≤1.2 in DAS28 score; no response: improvement of ≤.6 in DAS28 score.
[b]Response rate = (good response + moderate response) × 100/number of patients at respective week.
CI: confidence interval; DAS28: disease activity score based in 28 joints; EULAR: European League Against Rheumatism; LOCF: last observation carried forward.

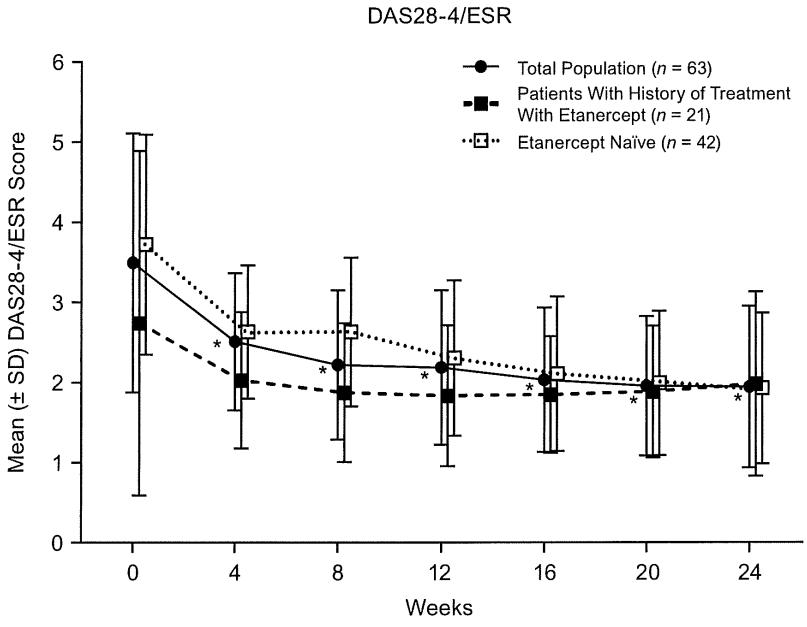

Figure 2. Change in DAS28-4/ESR score over time. The paired *t*-test was used to compare DAS28 differences between week 0 and week 4, 8, 12, 16, 20, and 24. *$p < .001$. DAS28-4ESR: disease activity score in 28 joints-4 parameters/erythrocyte sedimentation rate; SD: standard deviation.

in remission increased to 17/21 (81.0%) in response to etanercept treatment. By comparison, among etanercept-naïve patients there was a greater proportion of patients with high (8/40; 20.0%), moderate (16/40; 40.0%), and low (7/40; 17.5%) disease activity with a corresponding lower proportion of patients in remission (9/40; 22.5%) at the onset of the surveillance period (Figure 3(C)). By 24 weeks, there were no patients with high disease activity in this group and those with moderate disease activity had decreased to 4/42 (9.5%); there was no change in the number of patients with low disease activity (7/42; 16.7%). The proportion of patients in remission increased throughout the surveillance period to 31/42 (78.8%), indicating a steady shift towards more remission in response to treatment (Figure 3(C)).

Of the 83 patients evaluated for improvement at week 24 by the treating physician, etanercept was found to be markedly effective in 39 (47.0%) patients and effective in 40 (48.2%) patients for an overall efficacy rate of 95.2% (Table 4).

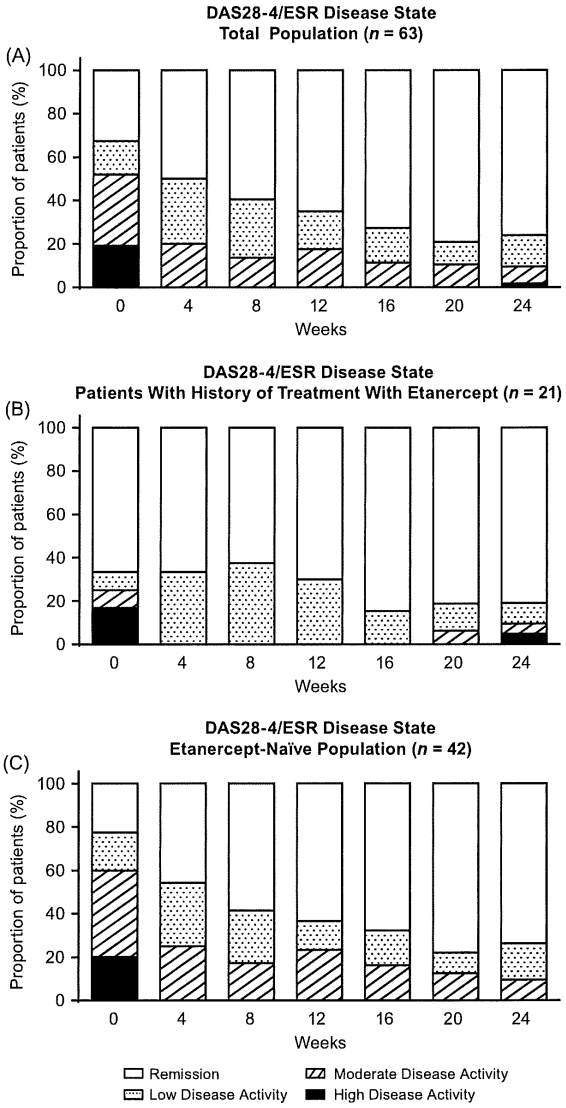

Figure 3. Proportion of patients by disease state over time.

Discussion and conclusions

The unknown etiology and the heterogeneity of symptoms make it difficult to diagnose and treat JIA. Treatment is further complicated by the age of the patients – under 16 years. Despite these hurdles, an increasing number of drugs have been shown to be effective, including NSAIDs, DMARDs (methotrexate, interleukin-6 inhibitors, and TNF inhibitors) [6–8]. Although patients often achieve long-term clinical remission when on treatment, achieving sustained remission when not on an active treatment regimen is still not very common [6]. Current treatment guidelines focus on elimination of active disease, management of pain, restoring physical activity, and improving quality of life [6–8]. However, it is unclear how these guidelines are implemented in a clinical situation.

In this paper, we report data derived from an all-patient, postmarketing surveillance of real-world practice undertaken at several clinics in Japan. Our data, although limited, demonstrate that etanercept was well tolerated by Japanese patients with JIA with only one serious AE (1.0%) being reported. Our data also show that etanercept was effective in substantially reducing disease activity as demonstrated by the increase in the proportion of patients in remission by the end of the 24 weeks. In addition, assessments by the treating physicians showed improvement in 95.2% of the patients in response to etanercept treatment. These data are consistent with earlier reports showing that etanercept was effective and demonstrated no safety signals in patients with JIA [11–14,19–21].

In our surveillance, there was a distinct difference with respect to effectiveness between patients who were etanercept-naïve and those with a history of etanercept treatment. Etanercept-naïve patients demonstrated significant improvements from baseline throughout the surveillance period indicating a beneficial response to etanercept treatment. In contrast, patients with a history of etanercept treatment demonstrated numerical, but not statistically significant, improvements over the same period. This is very likely due

Table 4. Physician's assessment of overall improvement over time using LOCF imputation of data.

Response	Week 4 ($n = 51$)	Week 8 ($n = 63$)	Week 12 ($n = 64$)	Week 16 ($n = 65$)	Week 20 ($n = 67$)	Week 24 ($n = 83$)
Markedly effective, *n* (%)	16 (31.4)	25 (39.7)	23 (35.9)	26 (40.0)	27 (40.3)	39 (47.0)
Effective, *n* (%)	32 (62.8)	34 (54.0)	37 (57.8)	35 (53.9)	34 (50.8)	40 (48.2)
Not effective, *n* (%)	1 (2.0)	2 (3.2)	2 (3.1)	3 (4.6)	4 (6.0)	2 (2.4)
Not assessable, *n* (%)	2 (3.9)	2 (3.2)	2 (3.1)	1 (1.5)	2 (3.0)	2 (2.4)
Efficacy rate, % (95% CI)[a]	94.1 (83.8, 98.8)	93.7 (84.5, 98.2)	93.8 (84.8, 98.3)	93.9 (85.0, 98.3)	91.0 (84.5. 96.6)	95.2 (88.1, 98.7)

[a]Efficacy rate = (markedly effective + effective) × 100/number of patients at respective week.
CI: confidence interval; LOCF: last observation carried forward.

to the very high proportion of patients (66.7%) in this group who were already in remission prior to initiation of treatment (Figure 3(B)) during this surveillance, leaving very little room for further improvement. Nevertheless, there was an increase in number and proportion of patients responding to etanercept treatment in this group. The high proportion of patients in remission in the group with prior history of treatment also resulted in the relatively high remission rate at baseline of 32.7% for the whole patient population.

The EULAR response could not be calculated for 41 patients as DAS28-4/ESR data either at baseline or after administration of etanercept were not available for these patients. The overall response rate after 24 weeks of treatment with etanercept was 63.0% based on patients exhibiting moderate or good response per EULAR criteria.

The main limitation of this surveillance is the small number of patients enrolled. This limitation was compounded due to unavailability of DAS28-4/ESR data in almost half of the patients. As this was an observational survey, there was no comparator arm. As such, the relative efficacy and comparative effectiveness of etanercept treatment in JIA could not be determined. Despite these limitations, the data reported herein are encouraging.

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Conflict of interest

Masaaki Mori has received grants from Chugai Pharmaceutical, Ono Pharmaceutical Co. Ltd., Mitsubishi Tanabe Pharma, UCB, CSL Behring, Towa Pharmaceutical Co. Ltd., AbbVie, Japan Blood Products Organization, Ayumi Pharmaceutical Co., Takeda Pharmaceutical Company, and Nippon Kayaku Co. Ltd.; lecture fees from MSD K.K.; and consulting fees from Daiichi Sankyo Company and Taisho Pharmaceutical Co. Ltd.

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Shumpei Yokota has received lecture fees from AbbVie, Bristol-Myers Squibb, Taisho Toyama Pharmaceutical, and Chugai Pharmaceutical; has patents, royalties, or other intellectual property with Chugai Pharmaceutical; and has been a consultant for Asahi Kasei Pharma, Japan Blood Products Organization, Novartis, Santen Pharmaceutical, and Chugai Pharmaceutical.

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ORIGINAL ARTICLE

Survey of attitudes of non-pediatric rheumatologists among councilors of the Japan College of Rheumatology regarding transitional care

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ABSTRACT

Objectives: The transition from pediatric to adult healthcare systems has recently received worldwide attention. Surveys of the attitudes of Japanese non-pediatric rheumatologists regarding transitional care were conducted.

Methods: Non-pediatric rheumatologists among councilors of the Japan College of Rheumatology were enrolled in the surveys. Experiences of adult patients with childhood-onset rheumatic diseases, ideal medical care for these patients, and factors that made the transition to adult care difficult were examined via e-mail.

Results: Overall, 201 non-pediatric rheumatologists (21.2%) responded to the surveys. Ninety-one percent had previous experience with patients with childhood-onset rheumatic disorders. Transition to non-pediatric institutes was supported by about 90% of respondents. However, only 32% of non-pediatric rheumatologists had no hesitation about caring for adults with childhood-onset rheumatology disorders. Two main factors prevented smooth transitions to non-pediatric care: inadequacy of non-pediatric care (57%) and lack of independence from parents/family (53%). The majority of non-pediatric rheumatologists hesitated about medical care for patients with autoinflammatory syndromes, whereas they became familiar with articular juvenile idiopathic arthritis without hesitation (86.6%); 93% of respondents requested more opportunities to learn about pediatric rheumatology disorders.

Conclusions: Sharing additional knowledge about pediatric rheumatology within the non-pediatric rheumatology field is required.

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Attitude survey; autoinflammatory syndrome; childhood-onset rheumatic disease; pediatrics; rheumatology; transition

Introduction

Advances in medicine have dramatically improved the prognosis of childhood-onset chronic diseases, allowing patients to survive and reach adulthood [1,2]. In Japan, 1000 patients with childhood-onset chronic disease reach adulthood every year, and many of them survive without serious sequelae or disabilities [3]. The transition from pediatric to adult healthcare systems has recently received worldwide attention, and transitional care, as defined by the Society for Adolescent Medicine, is 'the purposeful, planned movement of adolescents and young adults with chronic physical and medical conditions from child-centered to adult-oriented healthcare systems' [4].

Pediatric patients with rheumatic disorders also frequently survive into adulthood [5–7]. Among pediatric rheumatic disorder, juvenile idiopathic arthritis (JIA) is the most common in Japan and develops in about one out of 100,000 children per year in Japan, with a prevalence of 9.74 out of 100,000 children less than 16 years of age according to a nationwide survey [8,9]. Some long-term studies of the outcomes of childhood-onset chronic rheumatic diseases have been conducted; however, most reports are retrospective, cross-sectional, or selective. Thus far, very few reports that followed patients beyond adolescence have been published, and studies of adult outcomes in other childhood-onset rheumatic diseases have shown high rates of active disease [10]. Moreover, most reports of transition were authored by pediatric health professionals.

Non-pediatric rheumatologists are expected to be primarily responsible for the transitional care of patients with childhood-onset rheumatic disorders. Prior to the establishment of supportive guidelines for the transition of patients to adult medical care in Japan, surveys of the attitudes of non-pediatric rheumatologists regarding transitional care were conducted.

Methods

Non-pediatric rheumatologists among councilors in the Japan College of Rheumatology were enrolled in the surveys. Experiences of adult patients with childhood-onset rheumatic diseases, ideal medical care for these patients, and factors that made the transition to adult care difficult were examined via e-mail. Regarding issues preventing a smooth

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transition to non-pediatric care, multiple choices were allowed from checking the preset-questions.

Results

Of 947 councilors, 205 (21.6%) responded to the surveys; of these, 201 were non-pediatric rheumatologists (21.2%). The profiles of the councilors who responded are shown in Table 1. The most common specialty was rheumatology (77.1%), and 46.2% and 42.8% of them belong to university hospitals and community hospitals, respectively. Their experience in clinical practice was: 10–20 years, $n = 58$ (28.9%); 20–30 years, $n = 66$ (32.8%); and >30 years, $n = 77$ (38.3%). Of the 201 councilors, 182 (90.5%) had already cared for patients with childhood-onset rheumatic diseases. As the ideal medical care for adult patients with disorders in pediatric rheumatology, transition to a non-pediatric institute was supported by 88% of respondents (complete switch to non-pediatric care, 41.2%; gradual (stepwise) transition to non-pediatric care, 46.8%; Figure 1). However, non-pediatric councilors with no hesitation about caring for patients with any childhood-onset rheumatic disorder accounted for only 31.8% of all respondents (Figure 2). Attitudes such as 'No hesitation about medical care of the diseases with enough experience' and 'Want to become familiar with the medical care of the diseases if necessary information is provided' were observed in 45.8% and 16.9% of respondents, respectively, suggesting inexperience to a greater or lesser extent. With regard to issues preventing a smooth transition to non-pediatric care, two main observations were noted: inadequacy of non-pediatric care (57.2%) and patients lacking independence from their parents/family (52.7%) following attachment to pediatric rheumatologists by their patients (44.8%) (Figure 3). Among childhood-onset diseases, most of the councilors became familiar with articular JIA without hesitation (86.6%), whereas the majority hesitated regarding the medical care of patients with autoinflammatory syndromes (Figure 4). Even for patients with familial Mediterranean fever, the most frequent autoinflammatory syndrome in Japan, less than half (43.8%) did not hesitate about their medical care. Ninety-three percent of the councilors answered that more opportunities to learn about pediatric rheumatologic disorders are needed.

Table 1. Profiles of responding non-pediatric councilors of the Japan College of Rheumatology ($n = 201$).

	n (%)
Clinical practice experience	
<10 years	0 (0%)
10–20 years	58 (28.9%)
20–30 years	66 (32.8%)
>30 years	77 (38.3%)
Affiliation	
University hospitals	93 (46.2%)
Community hospitals	86 (42.8%)
Clinics	17 (8.5%)
Others	5 (2.5%)
Specialty	
Rheumatology	115 (77.1%)
Orthopedics	35 (17.4%)
Nephrology	3 (1.5%)
Respiratory medicine	1 (0.5%)

Discussion

Surveys of the attitudes of non-pediatric rheumatologists regarding transitional care were conducted in this study. The subjects of the survey were councilors of the Japan College of Rheumatology; therefore, they were relatively elderly compared to the actual population of non-pediatric rheumatologists, which might have biased the results. However, a preliminary survey of the attitudes of 37 relatively young non-pediatric rheumatologists who practiced at the Institute of Rheumatology, Tokyo Women's Medical University yielded quite similar results (data not shown), suggesting that the results of this study likely reflect the attitudes of more experienced non-pediatric rheumatologists in Japan.

Ideal transitional care for patients and physicians should be developed by both pediatricians and non-pediatricians. Of all 58 medical institutes represented at the 2015

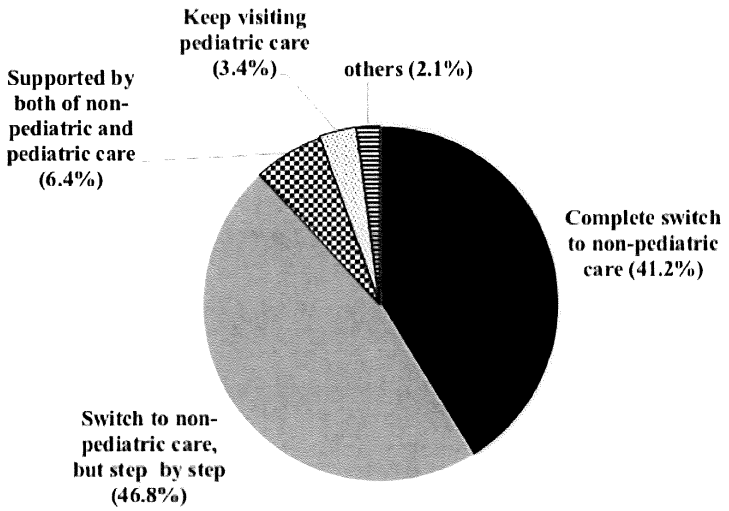

Figure 1. Ideal medical care for adult patients with pediatric rheumatology disorders (total of 233, multiple answers allowed).

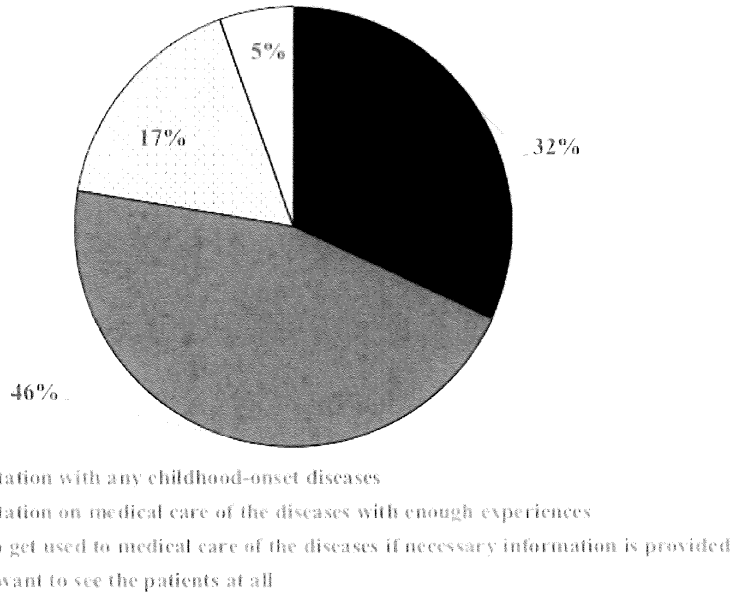

Figure 2. Attitudes of non-pediatric rheumatologists regarding the medical care of adult patients with pediatric rheumatology disorders ($n = 201$).

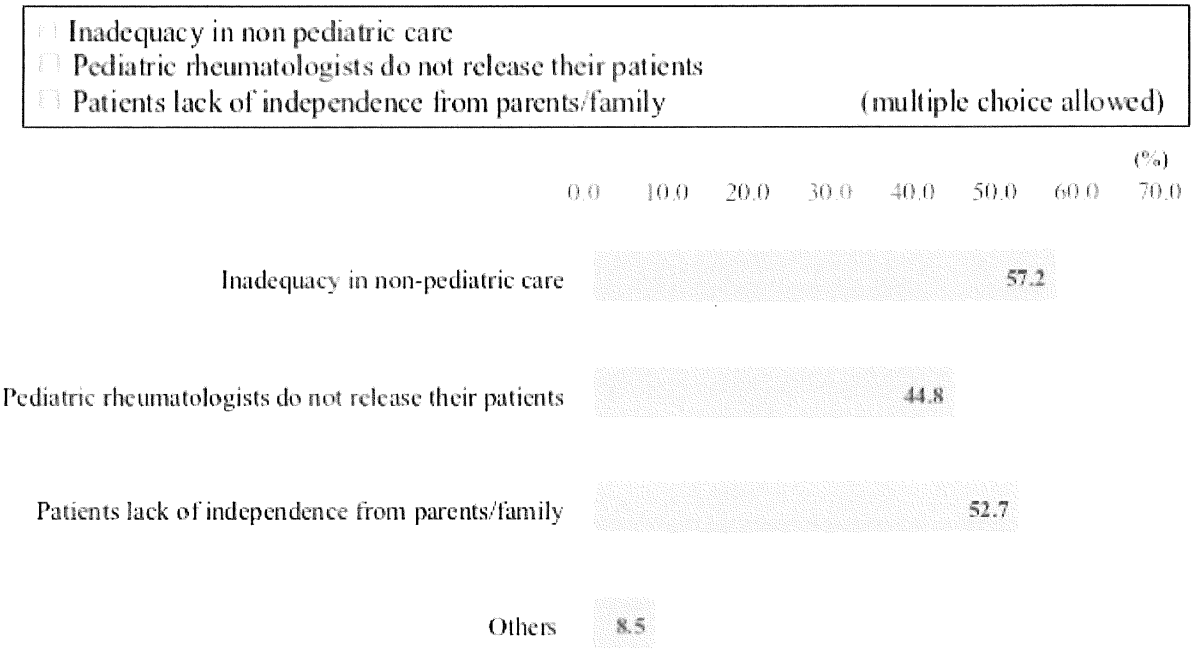

Figure 3. What factors prevent an ideal transition to non-pediatric care in pediatric rheumatology? ($n = 201$).

25th Annual Meeting of the Association of Pediatric Rheumatology Association of Japan, only two institutes were universal medical centers taking care of all generation of rheumatology disorders through childhood to adulthood, whereas 10 children's hospitals and 46 pediatric departments in general hospitals (27 universities and 19 community hospitals) were represented. These results suggest that not all pediatric rheumatologists have sufficient opportunities to understand the attitudes and thoughts of non-pediatric rheumatologists regarding the medical care of patients

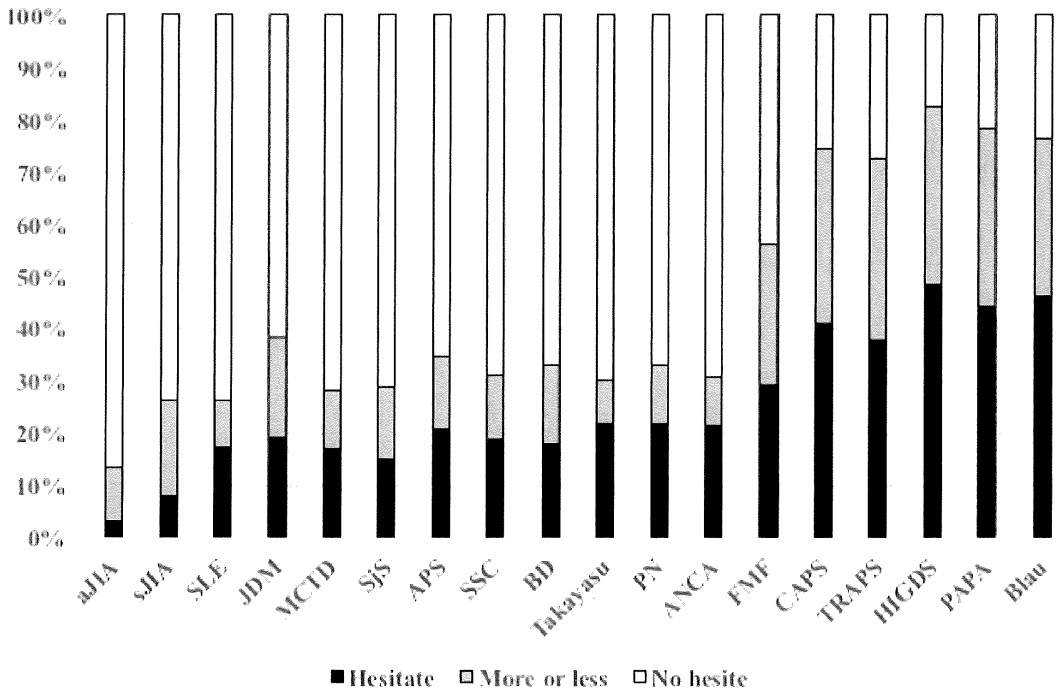

Figure 4. Do you hesitate to care for patients with the following adult childhood-onset rheumatology disorders? ($n = 201$).

with childhood-onset pediatric rheumatic disorders. The results of this study concluded that most non-pediatric rheumatologists are currently in favor of medical care of patients with childhood-onset rheumatic diseases; however, two essential factors must be improved: inadequacy of non-pediatric care and patients lacking independence from their parents/family. To address the latter issue, pediatric rheumatologists must initiate programs and/or make recommendations regarding patient transition as well as promote individual independence at regular clinic visits. The former issue underscores the importance of opportunities to learn about pediatric rheumatologic disorders, particularly autoinflammatory syndromes. Autoinflammatory syndromes, also known as autoinflammatory diseases or periodic fever syndromes, are a group of disorders characterized by recurrent episodes of systemic and organ-specific inflammation caused by abnormalities of the innate immune system [11]. Most autoinflammatory diseases are genetic, are present during childhood, and last throughout life. Non-pediatric rheumatologists should at least become familiar with the most common genetic autoinflammatory syndrome, familial Mediterranean fever. Otherwise, pediatricians should prepare the opportunities to learn autoinflammatory diseases for non-pediatric rheumatologists.

Conflict of interest

None.

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ORIGINAL ARTICLE

National survey of Japanese patients with mevalonate kinase deficiency reveals distinctive genetic and clinical characteristics

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ABSTRACT

Objectives: Mevalonate kinase deficiency (MKD), a rare autosomal recessive autoinflammatory syndrome, is caused by disease-causing variants of the mevalonate kinase (MVK) gene. A national survey was undertaken to investigate clinical and genetic features of MKD patients in Japan.

Methods: The survey identified ten patients with MKD. Clinical information and laboratory data were collected from medical records and by direct interviews with patients, their families, and their attending physicians. Genetic analysis and measurement of MVK activity and urinary excretion of mevalonic acid were performed.

Results: None of the 10 patients harbored *MVK* disease-causing variants that are common in European patients. However, overall symptoms were in line with previous European reports. Continuous fever was observed in half of the patients. Elevated transaminase was observed in four of the 10 patients, two of whom fulfilled the diagnostic criteria for hemophagocytic lymphohistiocytosis. About half of the patients responded to temporary administration of glucocorticoids and NSAIDs; the others required biologics such as anti-IL-1 drugs.

Conclusion: This is the first national survey of MKD patients in a non-European country. Although clinical symptoms were similar to those reported in Europe, the incidence of continuous fever and elevated transaminase was higher, probably due to differences in disease-causing variants.

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Canakinumab; genotype-phenotype relationship; mevalonate kinase deficiency; national survey

Introduction

Mevalonate kinase deficiency (MKD) is a rare autosomal recessive autoinflammatory syndrome caused by disease-causing variants of the gene encoding mevalonate kinase (*MVK*), an enzyme involved in biosynthesis of cholesterol and isoprenoids [1]. The disease manifests as a continuous spectrum of clinical signs ranging from recurrent febrile attacks, known as hyperimmunoglobulinemia D syndrome (HIDS, MIM no. 260920), to a more severe form known as mevalonic aciduria (MA, MIM no. 610377), which is also associated with psychomotor retardation, facial dysmorphia, cataract, and failure to thrive [2]. Low MVK activity reduces production of cholesterol and non-sterol isoprenoids. A shortage of isoprenoids, mainly geranyl-geranyl groups, leads to decreased geranylgeranylation of RhoA and increased production of IL-1β [3,4]. Currently, treatment of MKD is based on the severity of the symptoms shown by each patient; mild cases require NSAIDs or glucocorticoids, whereas more severe cases require biologics or hematopoietic stem cell transplantation [5,6].

Large international surveys, mainly conducted in Europe, provide useful information about the clinical, genetic, and therapeutic characteristics of MKD [6,7]; however, no national surveys have been undertaken in non-European countries. Several MKD patients have been identified in Japan since 2009 [8], some of whom harbor novel disease-causing variants [9,10]. Therefore, we performed a national survey to investigate the clinical and genetic characteristics of Japanese pediatric MKD patients. We identified 10 patients who presented with disease of varying severity, experienced different complications, and showed different responses to treatment. The information reported herein provides clinical and genetic profiles of Asian patients with

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MKD and increases our understanding of MKD from a global perspective.

Subjects and methods

Patients and clinical information

Questionnaires were sent to 705 hospitals in Japan that admit children, and 282 replies (40%) were received. More detailed questionnaires were then sent to these hospitals and clinical information regarding suspected MKD cases was obtained. Three of the patients enrolled in the study were referred directly to our institution by pediatricians. Clinical information, patient history, and laboratory data were collected from medical records and via direct interviews with patients, their families, and their attending physicians. MKD was suspected if, prior to the age of 6 years, patients began to experience recurrent attacks of fever accompanied by lymphadenopathy, vomiting, or diarrhea [7]. Patients harboring two known *MVK* variants, or less than two known variants combined with an abnormal metabolic result, were considered to be true MKD patients. The metabolic criteria were increased urinary mevalonic acid levels or reduced MVK enzyme activity.

The disease pattern was defined as recurrent or continuous according to whether the patient recovered completely between attacks. Responses to different treatments were classified as follows: complete response (complete control of the clinical manifestations and normalization of laboratory parameters); partial response (persistence of some clinical manifestations and/or abnormal laboratory findings); or no response (absence of any substantial impact on disease activity) [6].

Genetic analysis and measurement of mevalonate kinase activity and mevalonic acid levels

Both the nationwide survey and the subsequent genetic analyses were approved by the ethics committee of Kyoto University, and the study was undertaken in accordance with the Declaration of Helsinki. After obtaining written informed consent from all study subjects (or their parents or guardians), blood samples were taken and peripheral blood mononuclear cells (PBMCs) were isolated, followed by genetic analysis of *MVK*. MVK activity was measured as previously described [11]. Briefly, PHA-stimulated lymphoblasts derived from PBMCs isolated from patients were lysed by three cycles of freezing and thawing and then incubated for 25 min at 37 °C with 100 mmol/L potassium phosphate (pH 7.0), 4 mmol/L ATP, 6 mmol/L $MgCl_2$, and 0.4 mmol/L mevalonic acid (hydrolyzed mevalonolactone). Mevalonolactone solution contained R,S-[5-^{3}H]-mevalonolactone (PerkinElmer; Waltham, MA). Next, mevalonate 5-phosphate and 5-pyrophosphate were separated from the reaction mixture by thin layer chromatography. MVK activity was calculated by subjecting each chromatography strip to liquid scintillation counting. In some cases, MK activity was also measured in PBMCs at the Laboratory of Genetic Metabolic Diseases (Department of Clinical Chemistry and

Table 1. Clinical characteristics of MKD patients in Japan.

Patient number	Diagnosis	Onset (month)	Current age (year)	Gender	Continuous disease	Lymphadenopathy	Mucocutaneous involvement	Gastrointestinal symptoms			Musculoskeletal involvement	Hepatomegaly	Spleno megaly
								Abdominal pain	Vomiting	Diarrhea			
1	HIDS	0	13.8	F	+	+	+	+	+	+	−	+	−
2	HIDS	0	7.3	F	−	−	−	+	−	+	−	−	−
3	MA	0	Dead	M	+	−	+	−	+	+	−	+	+
4	MA	1	2.8	M	+	+	+	−	+	−	−	−	+
5	HIDS	3	10.3	M	−	+	+	−	+	−	−	−	−
6	HIDS	1	8.2	F	−	+	+	−	−	−	+	−	−
7	HIDS	1	8.2	F	−	+	+	−	+	−	+	−	−
8	HIDS	0	15.3	M	+	+	+	−	+	+	+	+	+
9	HIDS	1	0.6	F	+	−	+	+	+	+	−	−	−
10	HIDS	2	Dead	F	−	+	+	+	−	+	+	−	−
Summary	−	−	−	−	50%	70%	90%	40%	70%	60%	40%	30%	30%
*European cohort	−	−	−	−	13%	85%	87%	88%	69%	84%	79%	21.6%	32.4%

*European cohort; percentage of patients with each symptom 6 and 7.
HIDS: hyper-IgD syndrome; MA: mevalonic aciduria; +: present; −: absent.

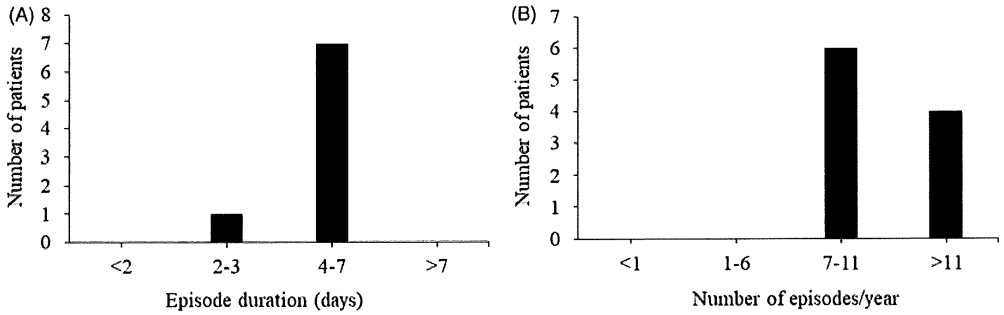

Figure 1. Characteristics of inflammatory attacks in MKD patients. (A) Duration of episode treatment (day). (B) Number of episodes per year.

Pediatrics, Academic Medical Centre, Amsterdam), mevalonic acid concentrations in urine were determined using the stable-isotope dilution method, as previously described [12]. Briefly, the extract from an aliquot of urine (pH 2.0) spiked with $^{13}C_2$-mevalonolactone was derivatized using *N,O*-bis(trimethylsilyl)trifluoroacetamide and analyzed by gas chromatography-mass spectrometry (selected ion monitoring mode) using a Thermo-Fisher Scientific DSQ mass spectrometer (Thermo-Fisher Scientific, Waltham, MA).

Measurement of cytokine secretion

PBMCs (1×10^5 per well) were seeded in 96-well flat-bottomed microtiter plates in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum and 1% penicillin/streptomycin. Cells were then stimulated with LPS (100 ng/mL; InvivoGen, San Diego, CA) for another 24 h and supernatants were collected. IL-1β concentrations were measured in triplicate using Bio-plex Pro Human Cytokine Assay (Bio-Rad Laboratories Inc., Hercules, CA).

Statistical analysis

The statistical significance of differences was determined by Student's t-test using GraphPad Prism version 5 (La Jolla, CA). Differences were considered to be significant at $p < .05$.

Results

Demographic features

The study population comprised three male and seven female patients (Table 1). The median age at the time of symptom onset was 1 month (range, 1 week to 3 months). The median follow-up period since age at onset was 8.1 years. Patients 5, 6, and 7 were siblings. No consanguinity was observed.

Fever

The clinical characteristics of the patients are summarized in Table 1. Seven patients (70%) showed lymphadenopathy, accompanied by attacks of fever. When the patients were categorized into four groups based on flare duration or flare frequency per year, most patients had a flare duration of between 4 and 7 d (Figure 1(A)); the flare frequency was between 7 and 11 episodes for six patients and more than 11 episodes for four patients (Figure 1(B)). Prior to anti-inflammatory treatment, five of the ten patients (50%) had continuous disease between flares.

Mucocutaneous symptoms

Nine patients (90%) had mucocutaneous symptoms, including maculopapular rash ($n = 8$) and aphthous stomatitis ($n = 4$). Two patients experienced purpura on the legs, leading to a diagnosis of IgA vasculitis. At the age of 8, Patient 5 had abdominal pain, purpura on the legs, temporal microscopic hematuria, and proteinuria. At the age of 8, Patient 6 had abdominal pain and purpura on the legs, with no urine abnormalities.

Gastrointestinal symptoms

Most patients ($n = 9$) had gastrointestinal symptoms, including abdominal pain ($n = 4$), vomiting ($n = 7$), and diarrhea ($n = 6$). One patient (Patient 9) presented with abdominal symptoms after formula feeding as a newborn [13]. Her symptoms included fever, rash, vomiting, and diarrhea. Laboratory examination revealed sensitization to milk allergens; feeding with low-allergy milk ameliorated the gastrointestinal symptoms temporarily. Patient 9 also suffered from intestinal occlusion, probably due to peritonitis caused by MKD attacks.

Musculoskeletal involvement

Musculoskeletal symptoms were noted in four patients. Two had arthralgia and two had arthritis. These particular symptoms not severe in three cases but were severe in one case (Patient 8). Patient 8 presented with recurrent fever, abdominal distention, feeding difficulty, and diarrhea soon after birth. The patient had arthritis at left wrist and right elbow from the age of 4 months. Prednisolone was administered first, and tocilizumab was added at the age of 8. This patient was diagnosed with MKD 2 years later; therefore, tocilizumab was switched to anakinra and then to canakinumab, which led to complete remission of inflammatory symptoms such as fever, headache, and arthritis. The patient does not have any joint contractures.

Transaminase elevation

Four of the ten patients had elevated transaminases (Table 2). Of note, in two patients, transaminase elevation preceded onset of periodic fever (Patients 1 and 2). Patient 1 presented with neonatal-onset chronic hepatitis and increased CRP levels [9]. Persistent elevation of serum transaminase levels, serum IgG, and anti-smooth muscle antibodies led to a diagnosis of autoimmune hepatitis at the age of 14 months. Treatment with prednisolone and azathioprine normalized serum transaminase levels. From the age of 32 months, the patient experienced periodic fever accompanied by maculopapular rashes. Based on the genetic and laboratory results, a diagnosis of MKD was established at the age of 6 years. At birth, Patient 2 presented with hydrops fetalis, respiratory distress syndrome, anemia, and pulmonary artery stenosis. Serum transaminase levels fell gradually without specific treatment. The patient was admitted to hospital with fever of unknown origin aged 1 year. The fever was accompanied by elevated serum transaminase levels (AST, 1321 U/L; ALT, 1602 U/L). Transaminase levels normalized again without specific treatment and remained within the normal range thereafter. The patient started to experience recurrent febrile attacks aged 6 years and was diagnosed with MKD.

In the cohort described herein, the other two patients (Patients 3 and 4) showing elevated transaminase fulfilled the diagnostic criteria for lymphohistiocytosis (HLH) [14]. Patient 3 presented with low body weight, generalized edema, generalized skin eruptions, and hepatosplenomegaly [15]. The patient was treated with a chemotherapy regimen according to the HLH-94 protocol. Due to the high etoposide dose (75 mg/m^2 every 4 d) needed to control inflammatory symptoms, the patient received hematopoietic stem cell transplantation with cord blood but died of complications associated with the transplant. Subsequent whole-exome sequencing analysis revealed the presence of two *MVK* genetic variants. Patient 4 was diagnosed with MA at the age of 1 year; the diagnosis was based on inflammatory symptoms and developmental delay. Four weeks after the first administration of canakinumab, the patient presented with acute liver failure. Liver metabolism capacity deteriorated rapidly (total bilirubin, 15 mg/dL; direct bilirubin, 11.5 mg/dL; prothrombin activity, 19%; ammonia, 113 μg/dL) and the patient became lethargic. The patient recovered after repeated plasma exchange and supportive care, without the need for a liver transplant.

Other laboratory results

All patients showed vigorous acute-phase responses during attacks, with elevated leukocyte counts (median, 19,250/μL) and CRP levels (median, 14.5 mg/dL) (Table 2). Increased serum IgD or IgA was observed in 56 and 60% of patients, respectively. Urinary mevalonic acid excretion was elevated in all patients examined (Table 2). All patients tested showed MVK activity ≤2% of that in healthy controls (data not shown). Stimulation of IL-1β secretion by LPS was significantly elevated in MKD PBMCs (Figure 2). All patients

Table 2. Laboratory data, treatment responses, and complications in MKD patients in Japan.

Patient number	Diagnosis	WBC count (/μL)	CRP (mg/dL)	ALT (IU/L)	IgD (mg/dL)	IgA (mg/dL)	*MVK* variation	Urinary mevalonate (μmol/mmolCr)	Prednisolone	Anakinra	Canakinumab	Complications
1	HIDS	40,000	24	1300	19	1335	A262P[a], H380R	37.5	PR	PR	CR	Transaminase elevation
2	HIDS	18,000	12.5	1602	73	535	L51F, M282T[a]	42.3	CR	NA	NA	Transaminase elevation
3	MA	35,900	19	100	ND	9	A147T, c.227−1 G > A[a]	ND	PR	NA	NA	HLH, death
4	MA	40,000	7.0	900	<0.6	399	c.227−1 G > A[a] homozygous	355.3	PR	NA	NR	Liver failure, HLH
5	HIDS	15,000	16.5	9	6.5	584	V278A[a], c.227−1 G > A[a]	51.9	CR	NA	NA	IgA vasculitis
6	HIDS	20,500	9.0	9	93	2223	V278A[a], c.227−1 G > A[a]	42.5	CR	NA	NA	IgA vasculitis
7	HIDS	15,000	6.5	8	118	1120	V278A[a], c.227−1 G > A[a]	44.9	CR	NA	NA	–
8	HIDS	22,500	24	14	<1.0	225	G326R, homozygous	36.2	PR	PR	CR	Arthritis
9	HIDS	17,600	24	4	<0.6	47	N205D, c.382_383 del AG[a]	27.4	PR	NA	NA	Milk allergy, ileus
10	HIDS	17,500	6.5	18	38.6	ND	G326R, homozygous	185 (μmol/day)	PR	NA	NA	Death
Summary	–	–	–	–	56%	60%	–	–	–	–	–	–
[b]European cohort	–[c]	–	–	–	78%	64%	–	–	–	–	–	–

[a]Specific genetic variants.
[b]European cohort, percentage of patients with an abnormal value 6 and 7.
WNL: within normal limits; ND: not determined; CR: complete response; PR: partial response; NR: no response; NA: not administered; HLH: hemophagocytic lymphohistiocytosis.
Normal ranges for each measurement (WBC <13,000/μL; CRP <0.2 mg/dL; ALT <30 IU/L; IgD <9.0 mg/dL; IgA< 363 mg/dL; urinary mevalonate <0.40 μmol/mmol Cr; MK activity >10%).

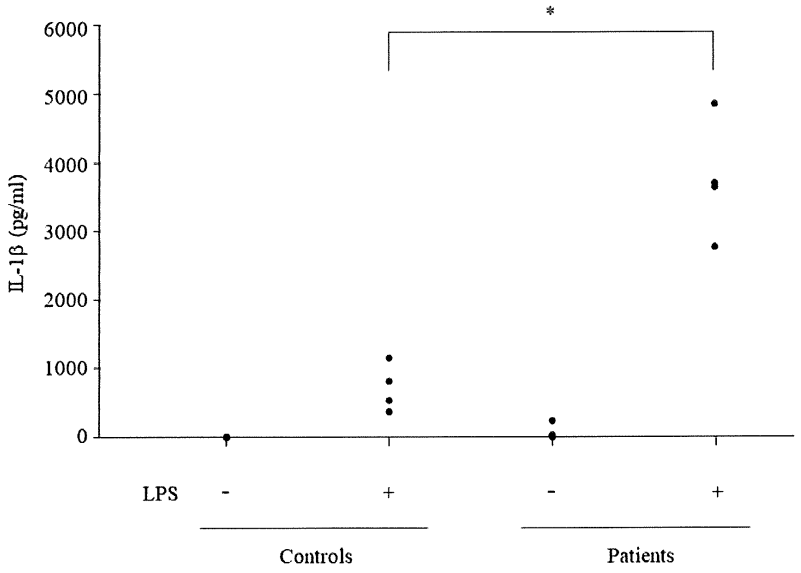

Figure 2. IL-1β secretion by PBMCs isolated from four MKD patients and four controls. After incubation for 24 h, cells were cultured for a further 24 h in the absence or presence of LPS. Each dot represents the mean of the triplicate data from each individual. *$p < .001$.

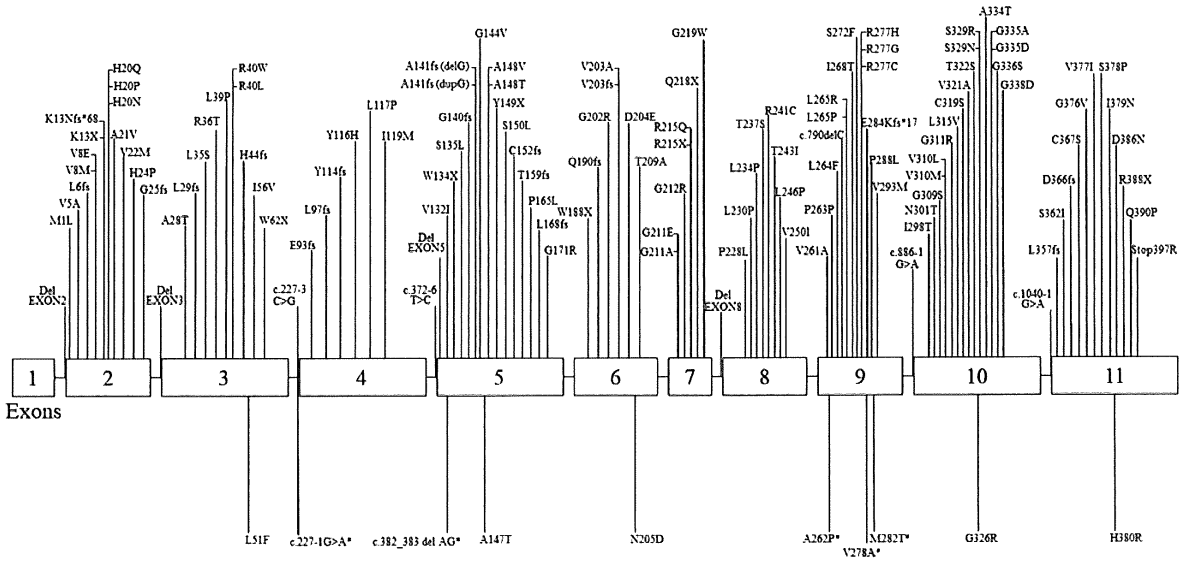

Figure 3. Schematic representation of genetic variants of *MVK* identified in MKD patients. Previously reported variants are depicted above the exons and the variants observed in the current cohort are depicted below. '*' indicates specific genetic variants.

harbored homozygous or compound heterozygous disease-causing variants of the *MVK* gene. All parents examined harbored one of the heterozygous variants shared by the patients (data not shown). Notably, five of the ten genetic variants were specific to this cohort (Figure 3) and were considered pathogenic based on their absence from the Exome Aggregation Consortium database, and from at least one of two function prediction programs: Polyphen 2 or Mutation Taster (data not shown). Disease-causing variants commonly found in Europe (i.e. V377I, I268T, H20P/N, and P167L) [6,7] were not detected in our patients.

Treatment

Febrile attacks experienced by Patients 2, 5, 6, and 7 were well controlled by oral prednisolone (taken as required) (Table 2). Three patients (Patients 1, 4, and 8) required treatment with biologics, which led to a marked reduction in inflammation in two patients (Patients 1 and 8). The efficacy of canakinumab was difficult to evaluate in Patient 4 because he received only one dose of 2 mg/kg canakinumab. One patient (Patient 3) received hematopoietic stem cell transplantation. Patient 3 died of interstitial pneumonia due to transplantation-related complications soon after

transplantation; therefore, it is difficult to estimate the efficacy of transplantation in this case.

Discussion

This study describes the clinical and genetic profiles and responses to treatment of MKD patients in Japan. Overall, the symptoms in the Japanese cohort were similar to those reported previously [6,7]. Specifically, most patients had attacks lasting between 4 and 7 d; these are comparable with the median flare duration of 4 d observed in the European cohort. In addition, 60% of patients in our cohort had 7–11 episodes per year (the others had more than 11 episodes), and the frequency of attacks is similar to that in European patients (median, 12 episodes per year). The percentage of patients with lymphadenopathy, mucocutaneous involvement, or gastrointestinal symptoms was comparable between the current and European cohorts (70 and 87%, 90 and 87%, and 90 and 95%, respectively) (Table 2). However, whereas 79% of the European patients experienced musculoskeletal involvement, only 40% of the current cohort did. Since all patients without musculoskeletal involvement harbored a genetic variant specific to the current cohort, differences in genetic variants might be related to differences in clinical manifestations (e.g., musculoskeletal symptoms).

The results of this study confirm previous findings that measurement of IgD is not a reliable and sensitive method for diagnosing MKD [16]; 44% of the patients tested in this cohort did not have elevated IgD. Elevated urinary mevalonic acid excretion and reduced MVK activity were similarly observed in Japanese and European MKD patients, despite these cohorts harboring different *MVK* variants [6]. Recently, Munoz et al. reported a novel diagnostic test for MKD [17] called the *in vitro* prenylation assay. The assay involves incorporation of a biotinylated isoprenoid lipid into unprenylated proteins in cell lysates and subsequent detection of biotinylated isoprenoids using streptavidin. The *in vitro* prenylation assay shows high sensitivity and specificity for discriminating other autoinflammatory diseases. This promising assay should help establish a clear diagnosis of MKD, particularly in patients harboring less than two disease-causing variants (as assessed by genetic analysis).

MKD patients sometimes experience liver complications [18]; indeed, one patient with severe liver failure required liver transplantation [19]. Four out of ten patients (40%) in this study had liver complications. In two patients, elevated transaminase levels preceded onset of periodic fever, which made it difficult to establish a diagnosis of MKD at the time of initial presentation. Since elevated transaminase levels were not mentioned in studies of European cohorts [2,6,7], no direct comparison is possible; however, the percentage of Japanese patients with liver complications seems to be higher. Patient 4 presented with acute liver failure but recovered after repeated plasma exchange and supportive care. Since MKD itself can increase transaminase levels, we suspected that this complication might not be caused by canakinumab; however, we decided not to administer canakinumab again. In addition, macrophage activation syndrome or HLH may occur in association with MKD [20,21]; two patients fulfilled the diagnostic criteria for HLH, more often than in the study by Haar et al. [6].

A continuous disease pattern was observed in 50% of Japanese patients, higher than the 13% observed in European patients [6]. Genotype-phenotype analysis in the European cohort revealed that fewer patients harboring the V377I mutation had a continuous disease course when compared with those without V377I: 4% versus 28%, respectively. Given that none of the patients in our cohort harbored V377I, the high percentage of patients with persistent inflammation could be attributed to differences in disease-causing variants.

Most MKD patients have their first attack within the first year of life [7]. Hence, we contacted all hospitals in Japan that have pediatric departments and performed the national survey of MKD patients by recruiting patients with recurrent or persistent fever. One of the limitations of the survey is that we might have missed adult MKD patients, as adults tend to have less severe clinical manifestations. A recent report shows that some MKD patients lack fever as a clinical manifestation; therefore, we may have overlooked some MKD patients without fever [6]. These caveats need to be addressed in the future if we are to gain a comprehensive understanding of the clinical picture of MKD in Japan.

Both anakinra and canakinumab are effective biologics for MKD patients [6,22]. Two patients (Patients 1 and 8) received anakinra followed by canakinumab, and both reported that canakinumab was more effective at controlling inflammation. In agreement with the clinical course observed herein, four out of five European patients treated with canakinumab achieved complete remission; however, two of the four showed only a partial response to anakinra [6]. The low incidence of MKD makes it difficult to undertake a clinical trial to compare anakinra and canakinumab; therefore, accumulating knowledge about treatment responses to these biologics is important.

In conclusion, we have described the clinical and genetic characteristics of Japanese patients with MKD. This is the first national survey undertaken in a non-European country. Although clinical symptoms were similar to those reported in European studies, the incidence of continuous fever and elevated transaminase was higher, probably due to the differences in disease-causing variants.

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Conflict of interest

RN received consultancy and speaker fees from Novartis. TK, TY, and TH received speaker fees from Novartis. The other authors have no conflicts of interest to declare.

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ORIGINAL ARTICLE

Survey of the awareness of adult rheumatologists regarding transitional care for patients with juvenile idiopathic arthritis in Japan

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ABSTRACT

Objectives: To understand the current status of adult rheumatology care for patients who had previously had juvenile idiopathic arthritis (JIA) (excluding systemic JIA), and to identify issues interfering with the transition from pediatric to adult care in Japan.

Methods: Questionnaire-based survey among 30 adult rheumatologists.

Results: Eighty-seven percent of adult rheumatologists responded that they had provided medical care to adults who had had JIA; 44% of them had felt hesitation or anxiety when providing such care. The reasons for this included lack of independence of the patients, lack of knowledge and experience among adult rheumatologists, and lack of preparation for accepting such patients. Many adult rheumatologists believed that the timing of transition from pediatric to adult rheumatology care must be considered based on therapeutic regimens or clinical conditions/disease states, not solely chronological age. A majority of adult rheumatologists showed great interest in transitional care for JIA patients and desired to communicate better with pediatric rheumatologists.

Conclusion: Transitional care for JIA patients is not sufficiently developed in Japan. Education and advocate campaign of transitional care is required for adult rheumatologists as well as patients and their parents.

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Juvenile idiopathic arthritis; transitional care; rheumatologist; questionnaire

Introduction

Progress in pediatric medical care has made it possible for many children suffering from intractable diseases to survive into adulthood [1,2]. At the same time, the number of children is increasing who have grown up to adulthood with a primary disease and its complications [3]. The transition from pediatric to adult health care systems has recently received significant attention worldwide [3–5]; however, Japan lags behind the United States and Europe.

Medical care for juvenile idiopathic arthritis (JIA) has also made significant progress with the increased use of MTX and biological anti-inflammatory agents. This has helped to delay or inhibit joint destruction in childhood and to improve their activity of daily life. Nonetheless, treatments must be continued for the majority of JIA patients even after transition to adult rheumatology care [6]. Although transitional care program for JIA have been developed and operated in other countries, it has rarely been applied in Japan [7]. In a recent survey among Japanese non-pediatric rheumatologists, 'inadequacy of adult rheumatology care' and 'lack of independence from parents' were extracted as the main factors to prevent smooth transitions of patients with pediatric rheumatic diseases [8]. That survey revealed the current status and issues regarding adult rheumatology care for pediatric rheumatic diseases in general, but not regarding specialized for JIA.

Therefore, we conducted this survey to understand the current status of adult rheumatology care for patients who had previously had JIA (excluding systemic JIA), and to identify issues interfering with the transition from pediatric to adult care in Japan.

Subjects and methods

A questionnaire-based survey was conducted anonymously among 30 adult rheumatologists working in 23 hospitals across Japan. All these hospitals had contributed to a nationwide Japanese database of rheumatic diseases (*NinJa*: National Database of Rheumatic diseases in Japan) [9]. The questionnaire asked adult rheumatologists for the

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following: their specialization (internal medicine or orthopedics); their experience in providing medical care to JIA patients; current status and issues of adult rheumatology care for JIA patients who were transferred from pediatric rheumatology care; the current situation and challenges of transition from pediatric to adult rheumatology care among JIA patients; and their interest in transitional care for JIA patients. Systemic JIA was excluded from this survey. Single-choice questions (multiple answers are partially allowed) were used in assessments. This survey was conducted in November 2016.

Results

Specialization and experience of participants in providing medical care to JIA patients

The participants' specialties were internal medicine ($n = 25$) and orthopedics ($n = 5$). Many had long-term clinical experience as a doctor (<10 years, 10%; 10–19 years, 13%; 20–29 years, 53%; ≥30 years, 23%). Of them, 90% belonged to community hospitals and 10% to university hospitals, respectively. Fifty percent of the participants had been involved in treating JIA patients under the age of 16, and 50% had clinical experience of treating JIA patients 16–19 years old. Eighty-seven percent of the participants had examined adults (≥20 years) who had had JIA and were transferred from pediatric rheumatology care. Therefore, one half of the participating adult rheumatologists had experience examining children with JIA.

Current status and issues of adult rheumatology care for JIA patients transferred from pediatric rheumatology care

Forty-four percent of the participants 'felt hesitation or anxiety in providing adult rheumatology care for JIA patients who were transferred from pediatric rheumatology care' (Figure 1). The reasons for hesitation or anxiety (multiple answers are allowed) were: 'sense of difficulty responding to parents of patients' (35%), 'lack of independence of patients' (24%), 'lack of overall understanding of JIA among adult rheumatologists' (24%), 'lack of knowledge about JIA treatments' (24%), 'ignorance of drugs that are approved or not approved for JIA treatments' (21%), 'sense of ambiguity about differences from adult patients with RA' (21%), and 'no pediatric rheumatologists available to consult' (10%) (Figure 2).

With regard to the relationship with patients who were transferred from pediatric to adult rheumatology care, 79% of participants responded, 'no problem occurred,' but 8% answered that 'transition sometimes did not work well although patients were transferred to adult rheumatology care after agreeing to the needs of transition.'

In terms of outpatient treatment of adults with JIA who were transferred from pediatric rheumatology care, 13% of the participants responded, 'there were some cases where outpatient treatment was discontinued according to the patient's own wishes,' but another 13% responded, 'there were some cases where outpatient treatment was completed such that patients achieved a drug-free state.' There were also some cases where patients returned to pediatric rheumatology care due to 'the will of patients' (4%) and 'the decision by adult rheumatologist' (4%).

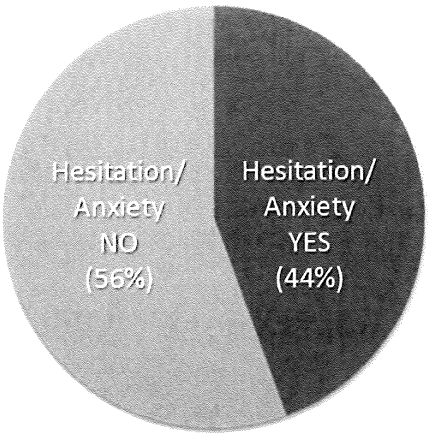

Figure 1. The presence or absence of feeling hesitation or anxiety about adult rheumatology care for JIA patients who were transferred from pediatric rheumatology care.

Current status and challenges in transitioning from pediatric to adult rheumatology care

Regarding JIA patients who had reached adulthood, all participants responded that 'a complete transition to adult rheumatology care' is beneficial for the patients. Although 62% of the participants agreed that 'a combined care of both pediatric and adult rheumatology before a complete transition to adult rheumatology care' is ideal, this had been practiced by only 3% of them.

Reasons why the transition to adult rheumatology care did not proceed smoothly were because 'lack of knowledge and experience among adult rheumatologists and lack of preparation for the acceptance of the patients' (61%) as well as 'inadequacy of education about transitional care to adult rheumatologists' (43%); therefore, many participants expressed their opinion that main problems concerning transition could be attributed to adult rheumatology care or adult rheumatologists (68%). They also pointed out 'excessive parental involvement' (29%), 'incomplete educational campaigns to address transitional care for patients and their parents' (29%), and 'lack of independence of patients' (25%). In this regard, 54% of the participants agreed that patients or their parents are responsible for the transitioning problems. Additionally, the participants pointed out that 'awareness of transitional care is insufficient among pediatric rheumatologists' (29%), 'pediatric rheumatologists are overly indulgent with patients' (7%) and 'pediatric rheumatologists do not want to end the relationship with their patients' (7%). In this respect, only 39% of the participants were of the opinion that the problem is attributable to pediatric rheumatology care.

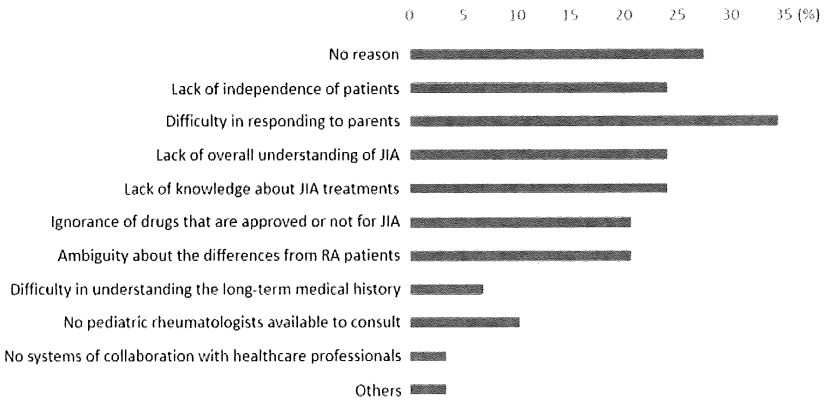

Figure 2. Reasons why adult rheumatologists hesitated to deliver adult rheumatology care for JIA patients who were transferred from pediatric rheumatology care (check all that apply).

'Offering training and educational campaigns about JIA for adult rheumatologists' (75%) was most needed to proceed smoothly with transitional care for JIA patients. Other requirements included 'training and educational campaigns to foster patients' independence for the patients themselves and their parents' (50%), 'training and educational campaigns related to adults with JIA for pediatric rheumatologists' (43%), 'creation of textbooks and materials regarding transitional care for JIA patients' (29%), 'communication/information exchange between pediatric and adult rheumatologists' (25%) and others.

As for the appropriate timing of the transition to adult rheumatology care, the number of participants who answered that the timing of the transition 'must be considered based on therapeutic regimens or clinical conditions/diseases, regardless of age' (48%) or must be considered based on 'school attendance/getting employed/moving to another place/marriage, etc.' (7%) was greater than the number who answered that JIA patients 'must in principle be transferred to adult rheumatology care based on chronological age (34% indicated 18 years of age and 7% suggested 20 years)' (Figure 3).

Answers to the question of which specialty should play the principal role in the treatment of adults with JIA who were transferred from pediatric rheumatology care were 'internal medicine' (38%), 'both internal medicine and orthopedics' (34%), and 'either internal medicine or orthopedics' (28%). Many participants agreed that there is a great need for orthopedic surgeons managing patients with joint deformities caused by long-term disease, as well as for internists who are familiar with drug treatment.

The interest in transitional care for JIA patients

Regarding transitional care for JIA patients, 30% of the participants answered that they 'think that this is an important issue' and 47% 'have thought that this is an important issue'. Furthermore, 17% of the participants showed 'great interest' (17%) and 60% showed 'interest' in pediatric rheumatic diseases in addition to JIA, indicating that adult rheumatologists had a great interest in pediatric rheumatic diseases.

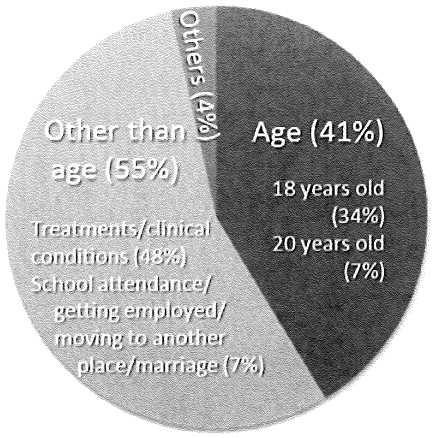

Figure 3. The adequate timing of transition to adult rheumatology care thought by adult rheumatologist.

However, our results also demonstrated that only 42% of the participants had attended seminars or conferences on pediatric rheumatic diseases and 47% confessed that they did not know about childcare subsidies (Medical fee is free for patients with chronic pediatric diseases of specified categories and with specified intractable diseases in Japan).

Answers to the question of where pediatric rheumatologists who can give advice on pediatric rheumatology patients were available 'in the hospital' (7%), 'in the neighborhood' (10%), and 'somewhere else' (30%), but 43% answered that 'nobody' is around. In addition, although only 17% of the participants answered that there is an opportunity to hold study sessions for communication/exchange information between pediatric and adult rheumatologists in neighborhood areas, they 'desired to attend' (33%) or 'desired to attend depending on the content' (60%).

Discussion

Our survey showed that 44% of the participants felt hesitation or anxiety in providing adult rheumatology care for adult patients with JIA. That rate was higher in this study than in the previous similar one on adult patients with

childhood-onset rheumatic diseases [8], but the reasons of hesitation were similar between them. Higher rate of hesitation in this study may partly depend on the smaller number of the participants belonging to university hospital and/or on their shorter clinical experiences. However, it remains unclear whether it is characteristic for JIA among childhood-onset rheumatic diseases. Similar to the previous study [8], independence of patients from their parents was extracted as one of the issues for transitional care. Children with JIA are often less mature and more dependent than healthy children of similar age [7]. For encourage the development of emotional and physical independence in children with JIA, it would be desirable to establish a total transitional support team composed of pediatric and adult rheumatologists, multidisciplinary medical workers and patients themselves [10]. Furthermore, our results showed that adult rheumatologists were highly motivated to learn and wished to communicate with pediatric rheumatologists. Thus, promotion of conference-level training, educational campaigns and creating textbooks related to transitional care would grow their interest in transitional care and solve the issues at the time of transitions.

As for the timing of transition, the most common answer was that it is preferable to decide the timing according to therapeutic regimens or clinical conditions/disease states rather than simply to a chronological age. This agrees with 'Proposal on transitional care for patients with childhood onset disorder' by the Japan Pediatric Society [11]. According to transitional care programs for pediatric rheumatic diseases in other countries, the timing of transition is determined just by age (range between 16 and 18) in some programs, whereas it is determined by setting 'criteria for transition' based on the ability of understanding the disease, therapeutic regimens, disease activity, etc. in others [10]. In our study, many participants expressed their opinion that 'a combined care of both pediatric and adult rheumatology before a complete transition to adult rheumatology care' is ideal for JIA patients. Hence, transitional program should be prepared which patients and their parents can join at each appropriate timing and bridge the gap between pediatric and adult rheumatology care. For seamless acceptance, adult rheumatologists must learn about the medical system for children including the childcare subsidies and get the license of chronic pediatric diseases of specified categories.

In our study, 87% of adult rheumatologists had clinical experience treating JIA patients, and 50% had been involved in treating JIA patients under 16 years old. There are only approximately 70 pediatric rheumatologists in Japan [12] and they are unevenly distributed across the country. Therefore, children with JIA do not always receive treatment from them [13]. In contrast, there are over 4600 rheumatologists across the country, so that, it seems that they are partially involved in pediatric rheumatology care in some local areas. However, because nearly half of adult rheumatologists do not have pediatric rheumatologists available to consult, they hesitate to engage in pediatric rheumatology services, and therefore, most of them wish to communicate better with pediatric rheumatologists. Building a local network of pediatric and adult rheumatologists will be essential.

The main limitation of this survey is a small number of the participants. In addition, participants included much more internists than orthopedic surgeons and many of them had longer clinical experience. Therefore, it is undeniable that these biases may affect the results of this survey. Furthermore, to understand the issues concerning transitional care for JIA more, similar surveys will be necessary for pediatric rheumatologists, patients and their parents besides adult rheumatologists.

In conclusion, this survey showed that transitional care for JIA patients is not sufficiently developed in Japan. For smooth transition, education and enlightenment campaign of transitional care is required for adult rheumatologists as well as patients and their parents.

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Conflict of interest

Tokyo Medical and Dental University (TMDU) received unrestricted research grants for Department of Lifetime Clinical Immunology from AbbVie GK, Ayumi Pharmaceutical Corporation, Chugai Pharmaceutical Co., Ltd., CSL Behring K.K., Japan Blood Products Organization, Mitsubishi Tanabe Pharma Corporation, Nippon Kayaku Co., Ltd., Ono Pharmaceutical Co., Ltd., Towa Pharmaceutical Co., Ltd., UCB Japan Co. Ltd., with which TMDU paid the salary of Toshihiro Matsui, Fumio Hirano, and Masaaki Mori. The other authors declare no conflicts of interest associated with this manuscript.

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RAPID COMMUNICATION

Elevated soluble CD14-subtype (PRESEPSIN; P-SEP) levels in rheumatoid arthritis (RA) patients with bacterial infection

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Infection is a serious complication observed in the management of rheumatoid arthritis (RA) patients. The acute inflammatory marker C-reactive protein (CRP) is elevated both during infection and during high disease activity of RA, and this often poses a problem when distinguishing the two. While infection markers should be measured quickly and inexpensively in a simple matter, there also is a need to distinguish them from disease activity markers of RA in clinical practice. Recently, the usefulness of a subtype of soluble CD14, presepsin (P-SEP), has been reported in critical care and pediatric areas. P-SEP is a novel effective marker for the diagnosis, severity, and treatment response of sepsis [1–3]. Phagocyte involvement has already been reported in the production of P-SEP; therefore, we took into consideration that P-SEP may function as a specialized inflammatory maker for bacterial infection [4].

P-SEP has not been evaluated in RA patients. To evaluate whether P-SEP production is specific to bacterial infections rather than inflammation due to RA, we investigated the serum levels of P-SEP in RA patients with infection.

Data were collected from June 2010 to March 2014 at the Department of Rheumatology the National Hospital Organization Osaka Minami Medical Center, Japan. Subjects were divided into 48 RA patients identified with a definite pathogen for bacterial infection (inf RA), 103 RA patients without infection (non-inf RA) and 34 healthy control (HC) (Table 1). All RA patients were required to meet both the 1987 American Rheumatism Association criteria and 2010 ACR/EULAR criteria for RA.

Inf RA patients were strictly identified as having infection with positive pathogen identification through cultures, presence of fever (>38.0°C) and/or C reactive protein (CRP) elevation and/or increase in white blood cell count (WBC), and improvements of these manifestations with antibiotics. RA patients with suspected bacterial but in whom no pathogen could be identified were excluded from this study.

Concentration of P-SEP was measured with an immunoassay analyzer (PATHFAST^{R}, LSI Medience Corporation, Tokyo, Japan). Measurements were taken before and after treatment in the inf RA group. Clinical disease activity index (CDAI) was measured in the non-inf RA group (mean CDAI ± S.D. 11.86 ± 9.32; disease activity severity; high 7, moderate 41, low 33, clinical remission 12) ($n = 93$; not all patients have physician VAS score measured).

The breakdown of bacterial infections in the inf RA group was as follows: 58.3% ($n = 28$) were gram-negative, and 31.3% ($n = 15$) were gram positive, and the others were mixed infections. There were 12 cases of blood culture positive sepsis. Of local bacterial infections, respiratory infection was most frequent ($n = 20$), followed by urinary tract infections ($n = 7$), muscular skeletal infections ($n = 4$), and skin/soft tissue infections ($n = 3$).

The serum levels of P-SEP (mean ± SD) were 1514 ± 3475 pg/ml (range: 168–16,947 pg/ml), 268 ± 397 pg/ml (range: 75–2648 pg/ml), and 136 ± 60 pg/ml (range: 62–327 pg/ml) for inf RA (pre-antibacterial treatment), non-inf RA, and the HC group, respectively.

In the inf RA group, P-SEP levels were correlated with CRP ($r = 0.30$, $p < 0.05$). P-SEP levels of the inf RA were significantly higher than those of non-inf RA and also significantly higher than those of the HC group. Furthermore, P-SEP levels were significantly reduced after anti-bacterial treatment (372 ± 399 pg/ml) (Figure 1).

As for individual P-SEP level in inf RA patients, the mean P-SEP level for the sepsis cases was 2938 ± 4899 pg/ml and it was significantly higher than local infections, 1040 ± 2784 pg/ml ($p < 0.01$). Moreover, no significant differences were observed for P-SEP levels between the gram negative and gram positive bacterial infections ($p = 0.10$). In evaluation concomitant medications in the RA groups according to whether patients were taking low-dose corticosteroids or methotrexate. P-SEP levels were significantly higher in the non-inf RA compared with the HC group. CDAI in the non-inf RA group correlated with CRP ($r = 0.28$, $p < 0.01$), but not P-SEP ($r = 0.17$, $p = 0.10$) (Figure 2a and b). In ROC analysis of P-SEP levels for bacterial infection, the cutoff value was 278pg/ml and the area under curve (AUC) was 0.82, sensitivity and specificity were 79.2% and 80.6%, respectively.

CRP is a useful marker for infection, while also being a useful marker for RA disease activity. There are numerous cases in clinical practice in which the reason for the state of increased CRP cannot be determined. Therefore, as P-SEP is not affected by RA disease activity, it is an effective marker for bacterial infection.

One of the limitations of this study is that only bacterial infections were included as the source of infection. We detected

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Table 1. Patients of demographic and disease characteristics.

	Bacterial infection RA ($n = 48$)	Non-infecion RA ($n = 103$)	Healthy control ($n = 34$)
Female (%)	35 (72.9)	80 (77.7)	27 (79.4)
Age (years)	67.6 ± 9.3	59.7 ± 15.5	57.0 ± 16.2
RA duration (years)	13.8 ± 9.9	11.5 ± 10.6	-
RA Stage (I/II/III/IV)	2/12/15/17	6/41/28/28	-
RA Class (1/2/3/4)	5/33/7/2	15/79/7/0	-
CRP (mg/dl)	10.58 ± 7.72	0.80 ± 1.41	-
CDAI (*n=93) (HDA/MDA/LDA/CR)	-	11.86 ± 9.32 (7/41/33/12)	-
DAS28-ESR (HDA/MDA/LDA/CR)	-	3.34 ± 1.58 (7/37/20/25)	-
Concurrent PSL use rate (%)	79.2	59.2	-
Concurrent PSL use dose (mg/day)	5.64 ± 3.75	4.26 ± 3.09	-
Concurrent MTX use rate (%)	37.5	58.3	-
Concurrent MTX use dose (mg/week)	8.21 ± 2.74	8.53 ± 2.20	-
Other DMARDs	SASP7, Buc8, TAC5, MMF1, AZA1, CyA1	SASP5, TAC6, Buc9, Aur1, LEF1, GST1	-
Biologic agents	10 (ETN2, ADA2, TCZ5, ABT1)	58 (TCZ25, ABT12,IFX 8, ETN3, ADA1, GLM9)	-

*Physician VAS score were not measured for all cases.

CDAI: clinical disease activity index; DAS: disease sctivity score; HDA: High disease activity; MDA: moderate disease activity; LDA: Low disease activity; CR: clinical remission; PSL: Prednisolone; MTX: Methotrexate; SASP: Salazosulfapyridine; Buc: Bucillamine; TAC: Tacrolimus hydrate; Aur: Auranofin; LEF: Leflunomide; GST: Gold Sodium Thiomalate; MMF: Mycophenolate mofetil; CyA: cyclosporine; IFX: Infliximab; ETN: Etanercept; ADA: Adalimumab; GLM: Golimumab; TCZ: Tocilizumab; ABT: Abatacept.

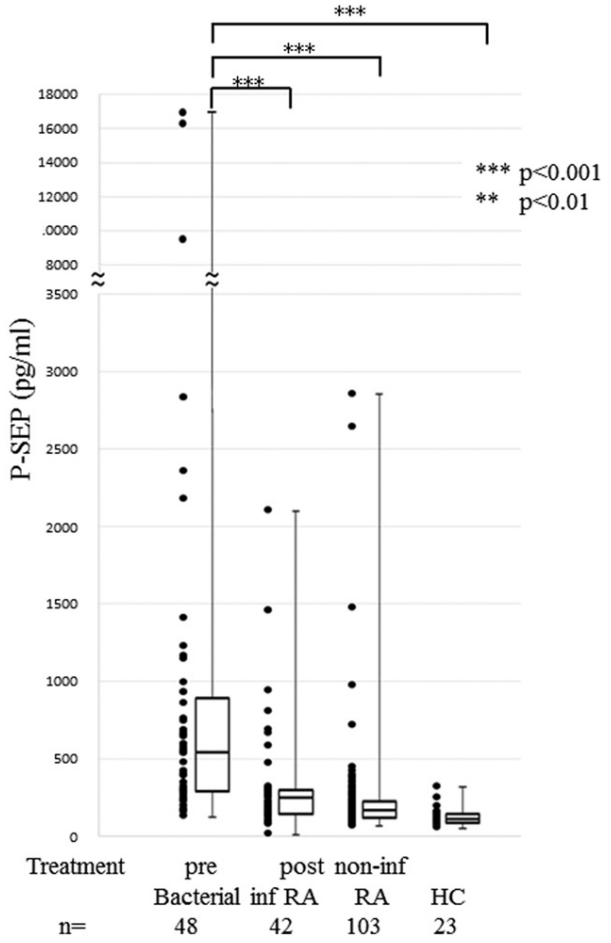

Figure 1. The level of P-SEP in the bacterial infection RA, non-inf RA and the HC group.

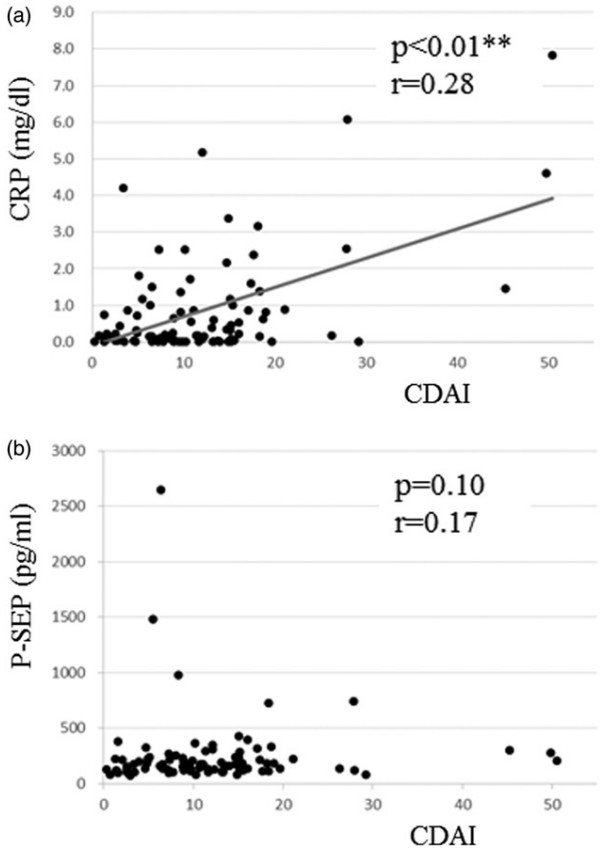

Figure 2. Correlation of CDAI in the non-inf RA with CRP (a), P-SEP (b) by using the Spearman rank correlation coefficient.

only 13 cases of viral infections in our RA patients, and when compared with the non-inf RA group, no statistical difference was seen (see Supplemental Figure 4). P-SEP levels were not significantly reduced after anti-viral treatment (data not shown).

In compromised hosts, singling out viral or fungal infections is difficult, and this will be a topic that will be further discussed in future reports. Furthermore, procalcitonin (PCT) has replaced CRP in the diagnosis of infection in some recent cases. It has been reported that P-SEP has a higher sensitivity and specificity compared to PCT in diagnosing infection [1]. PCT is also known

to increase in cases of systemic inflammatory response syndrome (SIRS) even without bacterial infection [5]. For our current study, only cases of known bacterial infections were considered, so PCT would have sufficed instead of P-SEP.

In general, P-SEP levels in RA patients are higher than healthy controls. P-SEP levels are not affected by low dose corticosteroids or methotrexate and are not dependent on the type of bacterial organism but are higher in sepsis than in local infections. As been previously reported [2,6] in non-RA patients, our study also found that P-SEP is useful in diagnosing bacterial infections, especially sepsis, and provides for an effective biomarker of response to treatment for the infection. In conclusion, P-SEP is a promising novel infection marker for diagnosis of bacterial infection in RA patients, regardless of RA disease activity.

Conflict of interest

S.Tsuji has received cartridges of P-SEP for PATHFAST. The other authors declare no conflict of interest.

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ORIGINAL ARTICLE

Only rheumatoid factor-positive subset of anti-citrullinated peptide/protein antibody-negative rheumatoid arthritis may seroconvert to anti-citrullinated peptide/protein antibody-positive

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Abstract

Aim: Anti-citrullinated peptide/protein antibody (ACPA) has been reported to occur in about 60% of patients with early rheumatoid arthritis (RA), and about 80% in patients with established RA. While ACPA seroconversion is possible, previous reports have shown that it rarely occurs. We retrospectively determined the proportion of patients who underwent ACPA seroconversion and described the clinical characteristics of these cases.

Methods: ACPA-negative RA patients who had undergone ACPA assessment more than once with an interval of 3 months or longer were investigated for ACPA seroconversion. The clinical characteristics of seroconverted patients were assessed.

Results: In 149 ACPA-negative RA patients, only eight patients (5.4%) converted to ACPA-positive during follow-up. We found that all eight of the seroconverted cases were positive for rheumatoid factor (RF) and showed bone erosions by X-ray. Of 56 ACPA-negative RF-positive RA patients, 14.3% of them seroconverted to ACPA-positive. None of the ACPA-negative RF-negative RA patients seroconverted to ACPA-positive.

Conclusion: The proportion of total RA patients who experienced seroconversion from ACPA-negative to ACPA-positive was 5.4%. When ACPA-negative RA patients were subdivided into RF-negative and RF-positive subsets, only the RF-positive subset seroconverted to ACPA-positive. These results imply that RF-negative and RF-positive patients are distinct subsets within ACPA-negative RA patients.

Key words: anti-citrullinated peptide/protein antibody, rheumatoid arthritis, rheumatoid factor, seroconversion.

INTRODUCTION

Anti-citrullinated peptide/protein antibody (ACPA) is a highly specific serological marker of rheumatoid arthritis (RA). It has been reported that average ACPA sensitivity and specificity for RA is 67% and 95%, respectively.[1,2] However, in early RA, the sensitivity of ACPA drops to 50–60%, whereas in established RA, it

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rises to 80%.[1–4] The discrepancy between these figures has been explained by the seroconversion from ACPA-negative to ACPA-positive in RA patients. However, previous reports have shown ACPA seroconversion seems to be rare, with a frequency ranging from 0.4% to 7%.[5] We hypothesized that patients misdiagnosed with non-RA arthritides may have been included in the early RA population and counted as ACPA-negative RA, and these cases were then excluded from the established RA population when the disease remitted or the physician changed the diagnosis. Another possibility is that ACPA-negative patients could reach remission and leave the cohort.

The aim of this study was to determine the proportion of patients who underwent ACPA seroconversion in our cohort and to clarify the characteristics of these cases.

PATIENTS AND METHODS

RA patients were recruited from January 2007 to November 2012 at Kyoto University Hospital; all patients were Japanese. Patients included in this study were diagnosed with RA by rheumatologists based on the 1987 American College of Rheumatology (ACR)-revised criteria or 2010 ACR/EULAR (European League Against Rheumatism) criteria. Patients who had not taken any anti-rheumatic drugs were excluded from this study. ACPA-negative RA patients who underwent ACPA assessment more than once with an interval of 3 months or longer were investigated for ACPA seroconversion. The clinical characteristics of patients who seroconverted to ACPA-positive were also assessed. All patients provided informed consent in accordance with the Declaration of Helsinki, before of collecting samples. Titer of ACPA in sera or plasma was measured with the second generation anti-cyclic citrullinated peptide (CCP) antibody enzyme-linked immunosorbent assay (ELISA) kit (MESACUP™-2 test CCP; Medical & Biological Laboratories Co. Ltd, Nagoya, Japan), according to the manufacturer's instructions with a cut-off value of 4.5 U/mL. A latex agglutination turbidimetric immunoassay was used for quantitating serum rheumatoid factor (RF) with a cut-off value of 11.7 IU/mL. RF was measured at almost all visits. The cases which were RF-negative all the time in our hospital were defined as 'RF negative'. Anti-nuclear antibody (ANA) was detected using a HEp-2 indirect immunofluorescent assay (ANA IFA [SRL]; Fujirebio Inc., Tokyo, Japan), according to the manufacturer's instructions. Clinical characteristics were compared between seroconverted and non-seroconverted RA patients. Continuous variables in two groups were assessed by Mann–Whitney *U*-test and frequencies were assessed by Fisher's exact probability test.

RESULTS

One thousand two hundred and forty-six RA patients were included in the present study; 216 (17.3%) patients were negative for ACPA and 149 of the 216 ACPA-negative RA patients underwent ACPA assessment more than once, and only eight of these patients (5.4%) seroconverted to ACPA-positive during follow-up (Fig. 1). Table 1 shows the clinical characteristics of the eight seroconverted cases. Of these cases, seven were female. Disease duration from onset to first ACPA measurement was between 0.2–26 years. Interval of seroconversion ranged from 0.4 to 5 years. Interestingly, we found these cases were all positive for RF and showed bone erosions by X-ray. Biologic disease-modifying antirheumatic drugs (DMARDs) were used in half of these cases. ACPA was measured more than twice in six cases. ACPA remained positive in four cases and turned into negative again in two cases (Fig. 2). ACPA titer fluctuated in a similar tendency as the fluctuation of RF in four patients (Fig. 3, patient nos. 2, 4, 7 and 8).

Comparison of basic clinical information between seroconverted and non-seroconverted cases are shown in Table 2. Age and sex of both groups did not differ significantly. RF positivity and the proportion of patients with bone erosions were lower in the non-seroconverted group (34.0% and 51.1%, respectively). When ACPA-negative cases were subdivided into RF-positive and RF-negative subgroups, none of the ACPA-negative RF-negative cases seroconverted to ACPA-positive. Of the ACPA-negative RF-positive RA patients, 14.3% (8/56 cases) seroconverted to ACPA-positive. This result is not due to frequent measurements of ACPA in RF-positive patients, because the number of ACPA measurements is not significantly different among the RF-positive subset and RF-negative subset (2.5 ± 0.84 *vs.* 2.8 ± 1.4, $P = 0.42$). As shown in Table 2, ANA positivity and biologic DMARDs usage tended to be higher in the seroconverted group than in the non-seroconverted group, but the differences were not significant (37.5% *vs.* 20.6% and 50.0% *vs.* 16.3%, respectively). Additionally, disease duration and interval between ACPA assays did not differ significantly between the seroconverted and non-seroconverted group (9.30 ± 10.15 years *vs.* 5.24 ± 7.73 years and 2.69 ± 1.92 years *vs.* 2.27 ± 1.94 years, respectively).

We next investigated whether ACPA-negative RA patients with relatively short disease duration tended to

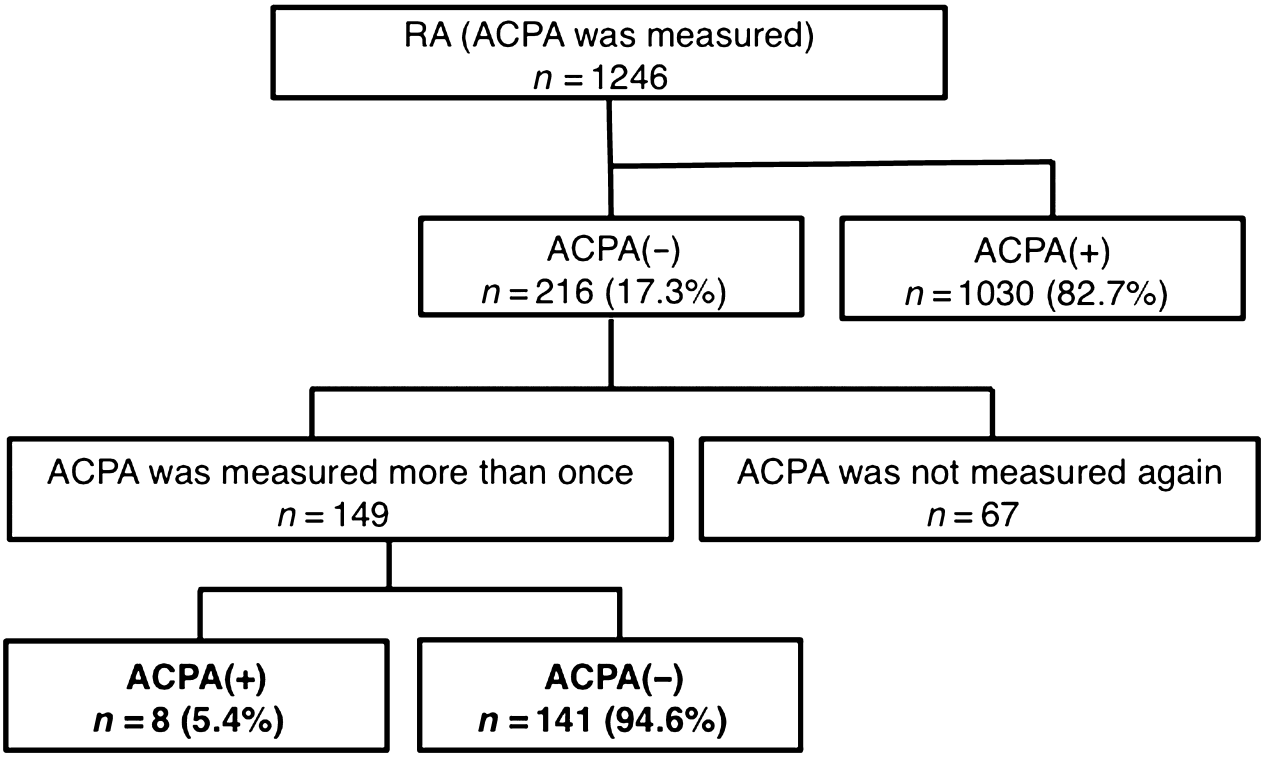

Figure 1 Seroconversion of anti-citrullinated peptide/protein antibody (ACPA). Of the 149 cases of ACPA-negative rheumatoid arthritis patients who underwent ACPA assessment more than once, only eight patients (5.4%) seroconverted to ACPA-positive during follow-up.

Table 1 Clinical characteristics of each patient who seroconverted to ACPA-positive

Pt no.	Age, sex	Disease duration† (years)	ACPA titer (U/mL)		Interval of seroconversion (years)	RF (IU/mL)	Stage	Treatment
			1st	2nd‡				
1	76, F	0.2	3.3	20.4	0.8	104.9	II	MTX, SSZ
2	60, F	0.3	3.2	180.0	4	18.9	II	SSZ, PSL
3	39, M	1	3.0	10.8	0.4	420.8	II	MTX
4	36, F	4	2.6	8.4	3.3	79.2	II	LEF
5	64, F	6	3.2	8.3	2.4	942.0	IV	TCZ
6	63, F	18	3.7	6.1	0.7	55.5	IV	IFX, MTX
7	64, F	19	4.0	11.7	5	49.1	IV	TCZ, PSL
8	64, F	26	< 0.6	14.2	5	23.0	IV	TCZ, SSZ, PSL

†From disease onset to first ACPA measurement. ‡ACPA titer when ACPA seroconversion was determined. RF titer and stage when ACPA was first measured. ACPA, anti-citrullinated peptide/protein antibody; MTX, methotrexate; SSZ; sulfasalazine; PSL, prednisolone; LEF, leflunomide; TCZ, tocilizumab; IFX, infliximab.

seroconvert more frequently. In the 16 very early RA patients (disease duration < 3 months), two patients (12.5%) seroconverted, whereas 0/18 (0%) early RA patients (disease duration 3–6 months) and 6/115 (5.2%) established RA patients (disease duration > 6 months), seroconverted to ACPA-positive (Table 3). Thus, very early RA may tend to seroconvert more frequently.

DISCUSSION

The proportion of all RA patients who seroconverted from ACPA-negative to ACPA-positive was 5.4%. This result is consistent with previous reports. Mjaavatten *et al.*[5] reported that 175 ACPA-negative early arthritis patients underwent ACPA assessment several times over a span of 12 months, and only one patient (0.6%)

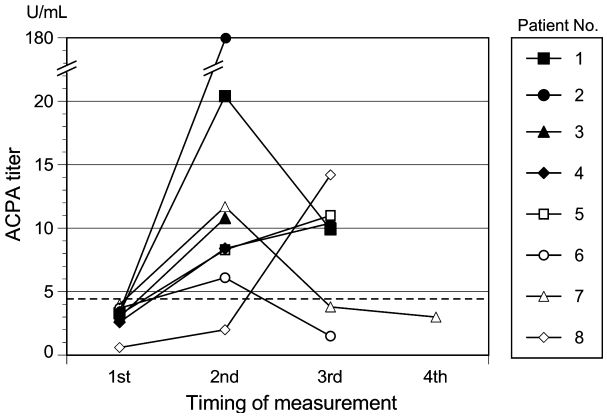

Figure 2 Fluctuation of anti-citrullinated peptide/protein antibody (ACPA) titers in each seroconverted case. Serial measurements of ACPA titers in each seroconverted case are shown. Patient numbers correspond to those in Table 1. The broken line shows the cut-off value of ACPA (4.5 U/mL).

seroconverted to ACPA-positive during follow-up. Ursum *et al.*[6] reported that 18/442 (4%) ACPA-negative RA patients seroconverted to ACPA-positive with a mean ACPA assessment interval of 11 months. Ursum *et al.*[7] further reported the seroconversion rate was 2%, in which 241 ACPA-negative early arthritis patients were assessed for ACPA at inclusion and 1 year later and only five patients became positive for ACPA after a 1-year follow-up. Meyer *et al.*[8] reported that 44 ACPA-negative RA patients with a disease duration of < 1 year and no history of DMARDs therapy were followed prospectively for 3 years, and 7/44 ACPA-negative RA patients (15.9%) seroconverted to ACPA-positive.

As previously reported, ACPA can be detected years before disease manifestation.[9,10] It is possible that ACPA might have not developed yet and might seroconvert to ACPA-positive later. However, the present study showed the proportion of total RA patients who seroconverted from ACPA-negative to ACPA-positive was 5.4%. Because the sensitivity of ACPA is about 60% in early RA patients and about 80% in patients with established RA, theoretically 50% of ACPA-negative RA patients should undergo seroconversion if the discrepancy in sensitivity were to be completely due to seroconversion. However, our observation is far from this estimated seroconversion rate. This discrepancy in sensitivity may be explained by the following reasons: (i) patients with non-RA arthritides may have been misdiagnosed as ACPA-negative RA; (ii) some patients may have remitted during the natural course of the disease; and (iii) physicians may have changed the diagnosis during the follow-up. Such cases would have then been

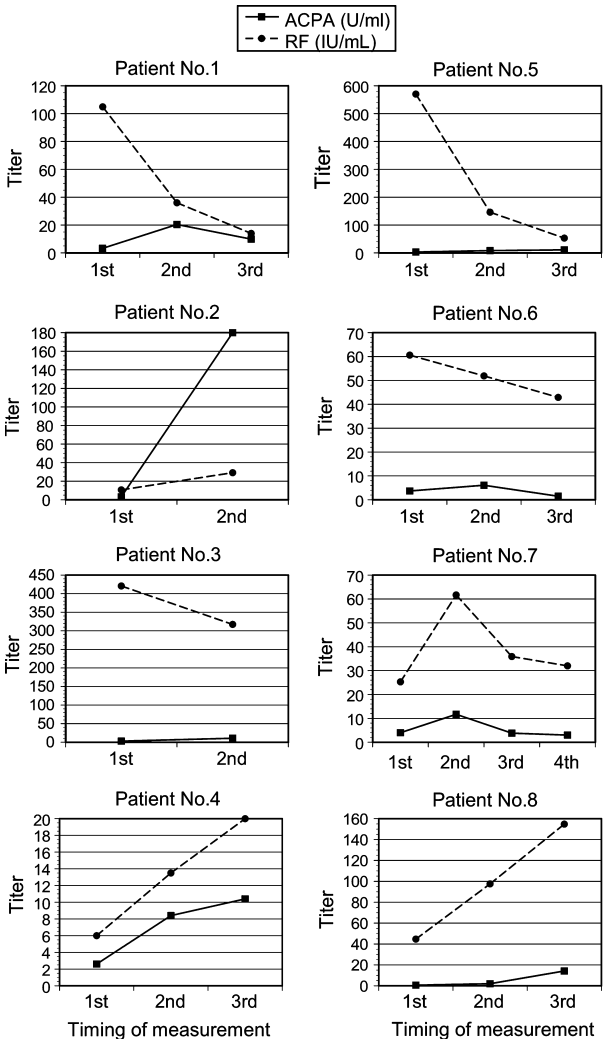

Figure 3 Co-fluctuation of anti-citrullinated peptide/protein antibody (ACPA) and rheumatoid factor (RF) titers in each seroconverted case. Serial measurements of ACPA and RF titers in each seroconverted case are shown. Continuous lines show the titers of ACPA. Broken lines show the titers of RF. Patient numbers correspond to those in Table 1 and Figure 2.

excluded from the RA population and the proportion of ACPA positivity would have increased.

On the other hand, we found that very early RA patients tended to seroconvert more frequently. The seroconversion rate was 12.5% in very early RA patients, while the overall rate was 5.4%. Based on this result, repeat assessment of ACPA may be useful for patients with suspected early arthritis if the diagnosis was unclear.

More importantly, we found that only the RF-positive subset of patients seroconverted to ACPA-positive. We

Table 2 Basic clinical information of seroconverted and non-seroconverted RA patients

	Seroconverted	Non-seroconverted	*P*-value
n	8	141	
Age, years	58.3 ± 13.7	65.6 ± 13.4	0.11
Female	7 (87.5%)	109 (77.3%)	0.44
RF positive	8 (100%)	48 (34.0%)	0.00029
RF titer	211.7 ± 322.9	97.6 ± 244.3	0.16
Presence of bone erosion	8 (100%)	72 (51.1%)	0.00058
ANA ≧1 : 160	3 (37.5%)	29 (20.6%)	0.23
MTX administration	3 (37.5%)	78 (55.3%)	0.27
Biologic DMARDs	4 (50.0%)	23 (16.3%)	0.036
Onset to 1st ACPA, years	9.3 ± 10.2	5.2 ± 7.7	0.38
1st ACPA to 2nd ACPA, years	2.7 ± 1.9	2.3 ± 1.9	0.58
No. of ACPA measurements	2.8 ± 0.64	2.7 ± 1.3	0.087

RF titer and presence of bone erosion when ACPA was first measured. ACPA, anti-citrullinated peptide/protein antibody; ANA, antinuclear antibody; DMARDs, disease-modifying antirheumatic drugs; MTX, methotrexate; RA, rheumatoid arthritis; RF, rheumatoid factor.

Table 3 Frequency of ACPA seroconversion among different disease duration

Duration (months)	< 3	3–6	6–12	12–24	> 24
No. of ACPA (−) cases	16	18	16	16	83
No. of seroconverted cases	2	0	1	0	5
Seroconversion rate (%)	12.5	0	5.2	0	6.0

ACPA, anti-citrullinated peptide/protein antibody.

previously showed that ACPA-negative RA patients consisted of two genetically distinct subsets according to RF positivity.[11] With regard to ACPA seroconversion, the results of the present study support the notion that the RF-positive and RF-negative subsets of ACPA-negative patients are clinically distinct. In some patients, ACPA titer fluctuated in a similar tendency as the fluctuation of RF. In general, ACPA titer and RF titer do not correlate during the treatment of established RA.[12] However, ACPA titer may be correlated with RF titer in the relatively early phase of seroconversion as in the seroconversion phase before the onset of RA.[10] That will be the reason why ACPA-negative RF-positive patients tend to seroconvert to ACPA-positive rather than ACPA-negative RF-negative cases. Because some of the anti-CCP-negative RA patient sera contains antibodies against citrullinated proteins other than the citrullinated peptides detected by the anti-CCP antibody ELISA kit,[13] there is a possibility that some anti-CCP-negative RF-positive patients may have already had ACPA and become anti-CCP-positive later via epitope spreading; this hypothesis should be further explored with an early RA cohort in the future.

This study has several limitations as follows. This study is retrospective and therefore may contain selection biases. Patients with established RA were included, and we also cannot deny the possibility of seroconversion before the follow-up intervals. In addition, this cohort is aged and a part of patients was diagnosed as RA before the second-generation anti-CCP antibody was developed. Physicians may have also chosen to repeat ACPA assessment in patients with higher disease activity.

CONCLUSIONS

The proportion of total RA patients who seroconverted from ACPA-negative to ACPA-positive was 5.4%. When ACPA-negative cases were subdivided into RF-positive and RF-negative subgroups, only the RF-positive subset contained patients who seroconverted to ACPA-positive. These results imply that RF-negative and RF-positive are distinct subsets of ACPA-negative RA patients.

CONFLICTS OF INTEREST

None.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: RH, KO. Analyzed the data: RH. Contributed reagents/materials/analysis tools: SN, CT, KM, RN, YI, NY, HY, MH, MF, HI, TF and TM. Wrote the paper: RH, KO.

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