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"Dall'inflammasoma alle malattie autoimmuni: criopirinopatie (CAPS) un modello fisiopatologico e terapeutico"

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1.1 INTRODUZIONE

Circa un secolo fa Paul Ehrlich propose che le reazioni immuni contro il self, che aveva definito come "orrore autotossico", attualmente definite “autoimmunità”, dovevano essere condizioni incompatibili con la vita a causa delle conseguenze potenzialmente devastanti per l'ospite. Successivamente, con l’identificazione degli autoanticorpi e la comprensione delle basi teoriche dell’autoreattività [1], la tesi di Ehrlich è stata confutata ed è nato il concetto di “autoimmunità”.

Concettualmente, l’autoimmunità è considerata come un difetto di selezione di linfociti B o T, con risposta linfocitaria aberrante verso autoantigeni [2].

Le malattie autoinfiammatorie, chiamate anche sindromi da febbre periodica, si riferiscono ad un gruppo di rari disturbi infiammatori su base genetica, che si verificano in assenza di infezione [3-5].

La forma più nota di malattia autoinfiammatoria è la febbre mediterranea familiare (FMF), ma rientrano in questo gruppo la sindrome periodica associata al recettore del TNF (TRAPS) e le Cryopyrin-associated periodic syndrome (CAPS) definite anche Criopirinopatie che comprendono 3 quadri clinici a differente gravità come l'orticaria familiare da freddo (FCAS), la Sindrome di Muckle Wells (MWS) e la sindrome cronica infantile neurologica articolare e cutanea (CINCA syndrome) anche nota come sindrome infiammatoria multisistemica ad esordio neonatale (NOMID).

Altre malattie caratterizzate da episodi di infiammazione acuta in assenza di autoanticorpi sono state recentemente classificate in questo gruppo, ed includono le malattie piogeniche: Sindrome artrite piogenica, pioderma gangrenoso e acne (PAPA), l'osteomielite cronica multifocale ricorrente (CRMO), la sindrome di Majeed, le mallattie immunomediate granulomatose (sindrome di Blau e morbo di Crohn), e sindromi febbrili idiopatiche come l'artrite idiopatica giovanile ad esordio sistemico (AIGs), la febbre periodica con stomatite aftosa, faringite e adenopatia cervicale (PFAPA) e la sindrome di Behçet.

L’identificazione delle malattie autoinfiammatorie come entità nosografiche a se stanti ha indotto a collegare i quadri clinici alle patologie autoimmuni.

Da un'iniziale osservazione questi due tipi di malattie, malattie autoinfiammatorie e malattie autoimmuni, condividono alcune caratteristiche: iniziano con il prefisso "auto" per definire un processo patologico diretto contro il self, sono malattie sistemiche che frequentemente coinvolgono la cute e il sistema muscolo-scheletrico, includono entrambe malattie monogeniche e poligeniche.

Dal punto di vista patogenetico, sono caratterizzate da una attivazione cronica del sistema immunitario, che porta all'infiammazione dei tessuti in individui geneticamente predisposti. Tuttavia, gli effettori specifici del danno sono differenti nei due gruppi di malattie: nelle malattie autoinfiammatorie il sistema immunitario innato provoca direttamente l'infiammazione dei tessuti, mentre nelle malattie autoimmuni il sistema immunitario innato attiva il sistema immunitario adattativo che, a sua volta, è responsabile del processo infiammatorio [6].

Le malattie autoimmuni mostrano una chiara suscettibilità associata all'aplotipo del complesso maggiore di istocompatibilità (MHC) [6], mentre le malattie autoinfiammatorie non hanno associazioni con aplotipi MHC di classe II.

I pazienti affetti da patologie autoinfiammatorie non hanno anticorpi autoreattivi o cellule T antigene-specifiche che guidano il processo della malattia, nelle patologie autoinfiammatorie sono i monociti -macrofagi, piuttosto che le cellule T e B ad essere responsabili di infiammazione e danno [6].

1.2 Immunoeffettori innati e adattivi coinvolti nelle malattie autoinfiammatorie e autoimmunitarie

L'immunità innata rappresenta la prima barriera di difesa immunitaria dell'organismo; identifica patogeni o altri triggers dannosi che possono indurre un processo infiammatorio con lo scopo di bloccare la loro diffusione, e attiva l'immunità adattativa.

Le cellule effettrici dell'immunità innata sono i fagociti, inclusi i macrofagi, le cellule dendritiche e le cellule presentanti gli antigeni (APC) [6]. L'immunità innata agisce attraverso i recettori dell'immunità innata «pattern recognition receptors» (PRR) che si legano a strutture altamente conservate espresse dai patogeni (Pathogen Associated Molecular Patterns, PAMPs) o dalle cellule danneggiate (Damage associated molecular patterns, DAMPs).

Sono state individuate tre classi di PRR: i recettori Toll -like (TLR), i recettori del gene -I (RIG - I), i recettori like (RLRs) e il dominio di oligomerizzazione legante il nucleotide (NOD) e i recettori nodlike (NLRs) [7].

Il riconoscimento di molecole estranee è seguito dalla trasduzione del segnale intracellulare, che induce l'espressione di geni, incluso l'interferone (IFN) α, IFN-β, TNF e sequenze geniche dell'interleuchina 1 (IL-1). Sia la disregolazione eccessiva che l'attivazione prolungata di tali recettori, possono portare allo sviluppo di malattie autoinfiammatorie o di malattie autoimmuni [8].

In modelli sperimentali [9] è stato dimostrato che il coinvolgimento dei TLRs nelle malattie autoimmuni, come il lupus eritematoso sistemico (LES), comporta la produzione di IFNs tipo I [10].

L'attivazione delle proteine NLR, NLRP3 (nota anche come NALP3 o criopirina), NLRP1, e NLRC4 risulta nella formazione di grandi complessi proteici definiti inflammasomi. Sono stati descritti due tipi di inflammasoma: inflammasoma NALP1 e NALP3, o criopirina inflammasoma [7].

L'inflammasoma funge da piattaforma molecolare che media l'attivazione della pro-caspasi-1 in caspasi 1, che scinde le forme inattive delle citochine pro-infiammatorie IL- 1β e IL- 18, a forme biologicamente attive.

L'attivazione dell'inflammasoma è un punto cruciale per la difesa dell'organismo dagli agenti patogeni.

Le malattie autoinfiammatorie sono state fortemente legate a mutazioni del complesso inflammasoma-NLR [11].

Il ruolo dell'inflammasoma nell'autoimmunità è meno chiaro. Ad oggi non ci sono legami genetici convincenti tra NLRs, malattie autoimmuni e il ruolo dell'inflammasoma.

Tuttavia è possibile immaginare un ruolo per dell'inflammasoma in alcune forme di patologie autoimmuni, considerato l' ampio spettro di stimoli di pericolo endogeni che inducono l'attivazione degli NLRs [12,13], e considerando il ruolo che i prodotti dell'inflammasoma, tra cui IL-1β, possono giocare nell'attivazione dell'immunità adattativa [14].

L'IL-1β può agire sulle cellule B e T: prolunga la sopravvivenza delle cellule T attraverso l'aumento dell'attività recettoriale dell'IL-2, incrementa la proliferazione delle cellule B, e rafforza la produzione di anticorpi da parte delle cellule B. Inoltre IL-1β guida anche la differenziazione di cellule Th17 [15].

Pertanto, l'IL- 1β amplifica la risposta delle cellule T e B e può fungere da collegamento cruciale tra l'attivazione NLR e le risposte dell'immunità adattativa.

L'immunità adattativa necessita dai 3 ai 5 giorni per maturare, coinvolge le cellule B, le cellule T, le cellule T citotossiche, la produzione di anticorpi ed è caratterizzata dal riconoscimento altamente specifico dell'antigene attraverso i recettori antigene specifici, tra i quali i più importanti sono i recettori cellulari B e T (BCR e TCR).

L'immunità innata rappresenta quindi il principale ostacolo, più rapido ma meno specifico contro DAMPs e PAMPs, mentre l'immunità adattativa è un meccanismo di difesa più efficiente ma più lento.

L'immunità adattativa svolge un ruolo importante nello sviluppo e mantenimento delle patologie autoimmuni. La risposta immunitaria innata invece contribuisce alla malattia attraverso meccanismi differenti. Infatti, il processo autoimmune evolve schematicamente attraverso due fasi: nella prima fase (fase di avvio) gli acidi nucleici auto rilasciatisi durante il processo apoptotico sono riconosciuti e internalizzati dalle cellule dendritiche (DC) attraverso i TLR, causando la produzione di IFN-α da parte di queste

cellule. L'IFN-α stimola la maturazione delle cellule dendritiche, la presentazione degli autoantigeni, il reclutamento delle cellule B e T e la produzione di autoanticorpi [10,7]. In una seconda fase (amplificazione self-sustainig), le cellule dendritiche plasmocitoidi interiorizzano gli immuno-complessi contenenti autoanticorpi e il recettore Fcy (FcγR) e producono IFN-α , che stimola e attiva le cellule T e DC, che portano alla auto perpetuazione della produzione di anticorpi e dell' infiammazione [9].

1.3 Inflammasoma e risposta immunitaria

I membri della famiglia dei NOD-like receptor (NLR), sono recettori citosolici che riconoscono componenti microbici e segnali di pericolo. Un sottoinsieme dei NLRs controlla l'assemblaggio dell'inflammasoma che risulta nell'attivazione della caspasi-1, che a sua volta, regola la produzione di IL-1β e IL-18. L'eccessiva attivazione dell'inflammasoma può causare malattie autoinfiammatorie, comprese le febbri periodiche ereditarie. Malattie autoinfiammatorie e autoimmuni formano uno spettro di malattie caratterizzate da infiammazione immuno-mediata contro il self, che si esplica attraverso l'immunità innata e adattativa. Tuttavia, il ruolo dell' inflammasoma nelle malattie autoimmuni è meno chiaro che nell'infiammazione, nonostante i numerosi effetti che IL-1β e IL-18 possono avere sulla risposta dell'immunità adattativa.

1.4 I NLR inducono l'assemblaggio dell'inflammasoma

Gli organismi multicellulari hanno sviluppato una fitta rete di segnali innati e adattativi per creare risposte efficaci ad entrambi gli insulti sia endogeni che esogeni.

La famiglia di proteine dei Nod-like receptor (NLR) è un gruppo di recettori intracellulari i "pattern recognition receptors" (PRRS) del sistema immunitario che svolgono un ruolo fondamentale nel riconoscimento di un ampio spettro di "danger e pathogen-associated molecular patterns" (DAMPs e PAMPs, rispettivamente) [16].

Negli esseri umani, la famiglia NLR è composta da 22 geni, mentre il genoma murino contiene almeno 34 geni codificanti NLR [17]. Delle proteine NLR, l'attivazione di NLRP3 (anche noto come NALP3 o criopirina), NLRP1, NLRC4 e "absence in melanoma 2" (AIM2) è coinvolta nella formazione di grandi complessi proteici chiamati inflammasoma. Il meccanismo di attivazione degli NLRs è ancora oggetto di dibattito, ma una volta attivati NLRP3, NLRP1, NLRC4 e AIM2 subiscono un cambiamento conformazionale che consente l'interazione con un

adattatore “inflammasoma-proteina “, ASC (PYCARD), che, a sua volta, interagisce con caspasi-1.

L’attivazione dell’inflammasoma è punto cruciale per la difesa dell’ospite dagli agenti patogeni, ma recentemente è stato messo in evidenza il ruolo dell'inflammasoma anche nella patogenesi di varie malattie con una componente infiammatoria, come il diabete di tipo 2 (DT2), le malattie infiammatorie intestinali (IBD) e l’aterosclerosi [18-21]. Tuttavia, si è anche evidenziato il ruolo dell’inflammasoma nella patogenesi delle malattie autoimmuni.

1.5 Attivazione dell'inflammasoma e il continuum di malattie causate da infiammazione immuno-mediata contro il self

Malattie classificate da eccessiva attivazione o attivazione cronica del sistema immunitario possono essere collocate in un continuum di malattia, con disordini autoinfiammatori a un'estremità dello spettro di malattia e malattie autoimmuni dall'altra [22].

Le malattie autoinfiammatorie sono disturbi clinici che si presentano caratterizzati da infiammazione ricorrente e febbre come parte del loro fenotipo, a causa di un anomalo aumento dell'infiammazione mediata dalle cellule del sistema immunitario innato [23]. Nelle malattie autoinfiammatorie, i danni ai tessuti sono il risultato di un processo di auto-infiammazione, a causa dell'attivazione di cellule dell'immunità innata, che includono macrofagi e neutrofili. Per esempio, alterazioni dell'omeostasi della cascata citochinica nelle febbri periodiche, predispongono ad un'infiammazione sito-specifica che è largamente

indipendente dalla risposta immunitaria adattativa. Per contro, le malattie autoimmuni possono essere classificate come infiammazioni contro il self mediate dal sistema immunitario adattativo, con sviluppo di reattività immunitaria verso antigeni nativi. L'Iper-reattività delle cellule T e B (come pure cellule dendritiche) si osserva tipicamente in combinazione con presenza di autoanticorpi e cellule T antigene-specifiche rivolte contro il self, con conseguente distruzione dei tessuti.

Le malattie autoimmuni possono causare coinvolgimento multiorgano, ma il principale organo bersaglio in genere domina la presentazione clinica e la definizione della malattia.

Ad oggi, diversi prototipi di malattie autoinfiammatorie sono stati collegati con mutazioni nel complesso inflammasoma-NLRs [11]. Mutazioni che provocano iperattivazione del complesso NLRP3-inflammasoma causano l'aumentato rilascio di IL-1β e sono la causa delle sindromi periodiche associate alla criopirina (CAPS) [24], che comprendono la Sindrome autoinfiammatoria familiare da freddo (FCAS), la Sindrome di Muckle-Wells (MWS) e la Malattia multisistemica infiammatoria ad esordio

neonatale (NOMID), queste sono provocate da mutazioni del gene NLRP3, di cui sono state identificate oltre 50 mutazioni [11,24]. Queste tre sindromi sono caratterizzate da similitudini cliniche, ma si distinguono per la loro gravità fenotipica. Segni e sintomi di queste malattie comprendono manifestazioni cutanee ricorrenti, febbre/brividi, dolori articolari, astenia, sordità, amiloidosi sistemica, alterazioni del sistema nervoso centrale, perdita della vista e deformazioni ossee e cartilaginee.

L'utilizzo di farmaci biologici quali anakinra, rilonacept o canakinumab, che agiscono tramite continua inibizione dell'IL-1β, migliorano le manifestazioni cliniche della malattia, tranne la proliferazione ossea nella sindrome NOMID [25-26].

Il complesso NLRP3-inflammasoma è stato anche collegato con malattie infiammatorie poligeniche, come la gotta e pseudogotta, che dipendono dalla interazione sinergica tra acidi grassi liberi e cristalli di urato monosodico che guida l'infiammazione attraverso il complesso NLRP3-inflammasoma [27]. L'infiammazione risultante provoca l'attivazione dei neutrofili e dei macrofagi, specialmente a livello articolare [28]. L'inibizione dell'IL-1β con Anakinra

ha un effetto sostanziale, esemplificando il ruolo critico dell'attivazione dell'inflammasoma nella progressione della malattia [29].

La comprensione del ruolo dell'inflammasoma nell'autoimmunità è meno chiaro. Il sistema immunitario innato influenza lo sviluppo della risposta autoimmune [30], una fase indipendente dalle cellule T è stata proposta per l'esordio dell'artrite reumatoide (RA), una malattia sistemica autoimmune [31].

Legami genetici tra il complesso inflammasoma-NLRs e malattie autoimmuni sono limitati, ma questo non esclude un ruolo potenziale per l'attivazione dell'inflammasoma nella progressione di queste malattie; peraltro un ruolo dell'inflammasoma in alcune malattie autoimmuni è probabile, considerando l'ampio spettro di segnali endogeni dannosi che attivano gli NLRs [32] e il ruolo che i prodotti dell'inflammasoma, quali IL-1β e IL-18, possono svolgere nella definizione dell'immunità adattativa [33].

REFERENZE

1. Burnet F (1957). A modifi cation of Jerne's theory of antibody production using the concept of clonal selection. Aust J Sci 20:67–69.
2. Davidson A, Diamond B. Autoimmune diseases. N Engl J Med 2001;345:340–350.
3. Galeazzi M, Gasbarrini G, Ghirardello A, Grandemange S, Hoffman HM, Manna R, et al. Autoinflammatory syndromes. Clin Exp Rheumatol 2006;24:S79-85.
4. Touitou I, Koné-Paut I. Autoinflammatory diseases. Best Pract Res Clin Rheumatol 2008;22:811-29.
5. Farasat S, Aksentijevich I, Toro JR. Autoinflammatory diseases. Clinical and genetic advances. Arch Dermatol 2008;144:392-402.
6. Theofilopoulos AN, Gonzalez-Quintial R, Lawson BR, Koh YT, Stern ME, Kono DH, et al. Sensors of the innate immune system: their link to rheumatic diseases. Nat Rev Rheumatol 2010;6:146-56.
7. Martinon F, Mayor A, Tschopp J. The inflammasomes: guardians of the body. Annu Rev Immunol 2009;27:229-65.

8. McGonagle D, Sinisa S, McDermott MF. The NLR network and the immunological disease continuum of adaptive and innate immune-mediated inflammation. Semin Immunopathol 2007;29:303-13.
9. Leadbetter EA, Rifkin IR, Hohlbaum AM, Beaudette BC, Shlomchik MJ, Marshak-Rothstein A. Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. Nature 2002;416:603-7.
10. Kawasaki T, Kawai T, Akira S. Recognition of nucleic acids by pattern-recognition receptors and its relevance in autoimmunity. Immunol Rev 2011;243:61-73.
11. Shinkai K, McCalmont TH, Leslie KS. Cryopyrin-associated periodic syndromes and autoinflammation. Clin Exp Dermatol 2008;33:1-9.
12. Faustin B, Reed JC. Sunburned skin activates inflammasomes. Trends Cell Biol 2008;18:4-8.
13. Lamkanfi M, Walle LV, Kanneganti TD. Deregulated inflammasome signaling in disease. Immunol Rev 2011;243:163-73.
14. Sims JE, Smith DE. The IL-1 family: regulators of immunity. Nat Rev Immunol 2010;10:89–102.

15. Chung Y, Chang SH, Martinez GJ, Yang XO, Nurieva R, Kang HS, et al. Critical regulation of early Th17 cell differentiation by interleukin-1 signaling. Immunity 2009;30:576-87.
16. Kanneganti TD, et al. Intracellular NOD-like receptors in host defense and disease. Immunity 2007;27:549–559.
17. Ting JP, et al. The NLR gene family: a standard nomenclature. Immunity 2008;28:285–287.
18. Bauer C, et al. Colitis induced in mice with dextran sulfate sodium (DSS) is mediated by the NLRP3 inflammasome. Gut 2010;59:1192–1199.
19. Duewell P, et al. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. Nature 2010;464:1357–1361.
20. Zaki MH, et al. The NLRP3 inflammasome protects against loss of epithelial integrity and mortality during experimental colitis. Immunity 2010;32:379–391.
21. Zhou R, et al. Thioredoxin-interacting protein links oxidative stress to inflammasome activation. Nat Immunol 2010;11:136–140.
22. McGonagle D, et al. An integrated classification of pediatric inflammatory diseases, based on the concepts

of autoinflammation and the immunological disease continuum. Pediatr Res 2009;65:38R– 45R.

23. Dickie LJ, et al. Periodic fever syndrome and autoinflammatory diseases. F1000 Med Rep 2010;2
24. Aksentijevich I, et al. The clinical continuum of cryopyrinopathies: novel CIAS1 mutations in North American patients and a new cryopyrin model. Arthritis Rheum 2007;56:1273–1285.
25. Hoffman HM, et al. Prevention of cold-associated acute inflammation in familial cold autoinflammatory syndrome by interleukin-1 receptor antagonist. Lancet 2004;364:1779–1785.
26. Neven B, et al. Cryopyrinopathies: update on pathogenesis and treatment. Nat Clin Pract Rheumatol 2008;4:481–489.
27. Joosten LA, et al. Fatty acids engagement with TLR2 drive IL-1beta production via ASC caspase- 1 activation by urate crystals in gouty arthritis. Arthritis Rheum. 2010.
28. Martinon F, Glimcher LH. Gout: new insights into an old disease. J Clin Invest 2006;116:2073–2075.

29. So A, et al. A pilot study of IL-1 inhibition by anakinra in acute gout. Arthritis Res Ther 2007;9:R28.
30. Olson JK, Miller SD. The innate immune response affects the development of the autoimmune response in Theiler's virus-induced demyelinating disease. J Immunol 2009;182:5712–5722.
31. Firestein GS, Zvaifler NJ. How important are T cells in chronic rheumatoid synovitis?: II. T cellindependent mechanisms from beginning to end. Arthritis Rheum 2002;46:298–308.
32. Schroder K, Tschopp J. The inflammasomes. Cell 2010;140:821–832.
33. Sims JE, Smith DE. The IL-1 family: regulators of immunity. Nat Rev Immunol 2010;10:89–102.

CAPITOLO 2

2.1 Cryopyrin-associated periodic syndrome (CAPS): un modello etiopatogenetico e terapeutico

Le sindromi auto infiammatorie sono un gruppo eterogeneo di affezioni, ad ereditarietà monogenica, caratterizzate da un'alterazione dei meccanismi di controllo della risposta infiammatoria responsabile di flogosi recidivante apparentemente primitiva a carico di vari organi o apparati, in particolare articolazioni e cute [1-2].

Il termine "autoinfiammatorio" è stato coniato nel 1999 da Daniel L. Kastner [3] per descrivere lo sviluppo apparentemente spontaneo di infiammazione senza la presenza di un titolo elevato di linfociti T self-reattivi o autoanticorpi specifici, tipici delle malattie autoimmuni.

Sebbene tutte queste affezioni abbiano caratteristiche genetiche distinte e peculiari, esse hanno espressioni cliniche comuni e spesso difficilmente distinguibili [4-7].

Tra queste si distinguono le Cryopyrin-associated periodic syndrome (CAPS) definite anche Criopirinopatie. Si tratta di un gruppo di rare affezioni monogeniche, autosomiche

dominanti, secondarie a mutazioni differenti a carico dello stesso gene (CIAS1) [8-11], che codifica per una proteina denominata criopirina che è coinvolta, nella regolazione della secrezione e attivazione di IL-1β [12-14]. Almeno 3 sindromi cliniche sono legate a diverse mutazioni del gene CIAS1 [15-18]. La forma più lieve è rappresentata dalla sindrome auto infiammatoria da freddo familiare (Familial Cold Autoinflammatory syndrome, FCAS, MIM 120100), una affezione maggiormente tipica dell'età adulta, caratterizzata da accessi febbrili e lesioni orticariodi scatenati dall'esposizione al freddo; talvolta si possono associare artralgia, addominalgia e congiuntivite. Altri sintomi osservati a seguito di esposizione a freddo includono sudorazione profusa, sonnolenza, cefalea, sete eccessiva e nausea [11, 19].

La sindrome di Muckle-Wells (MWS, MIM 191900), che è caratterizzata da lesioni simil-orticarioidi, non sempre pruriginose, ad esordio nei primi anni di vita, di natura non vasculitica. Con il tempo possono comparire una poliartrite non erosiva, sordità neurosensoriale e amiloidosi renale, che è una complicazione della fase tardiva della malattia [11, 20-22].

La forma più severa è rappresentata dalla sindrome CINCA (chronic infantile neurological cutaneous and articular, MIM 607115), affezione febbrile infiammatoria sistemica ad andamento cronico caratterizzata da lesioni cutanee tipo orticarioide ad esordio già in età neonatale, associate ad un costante quadro infiammatorio sistemico (febbre di intensità variabile, persistente elevazione degli indici di flogosi, anemia ipocromica) [10, 23-25]. Possono comparire un severo interessamento osteoarticolare (artrite e displasie diafisarie e metafisarie), sordità neurosensoriale, deficit intellettivo di gravità variabile, cefalea subcronica legata ad un quadro di meningite asettica [11, 26] e coinvolgimento infiammatorio oculare (iridociclite, vasculite retinica) [10]. L'insieme delle gravi manifestazioni cliniche è legato all'alterazione dei meccanismi di controllo dell'attivazione e secrezione dell' IL-1β.

IL-1β è una citochina pro-infiammatoria prevalentemente prodotta dai macrofagi in risposta a noxae infettive o infiammatorie. L'attività biologica di questa citochina è codificata da 2 diversi geni. La secrezione di IL-1 prevede l'interazione di un complesso di proteine noto con il nome di IL-1-inflammasoma che determina l'attivazione di

caspasi -1, responsabile della secrezione, a partire dalla pro-IL-1, della forma biologicamente attiva [27].

Una delle proteine costituente l'inflammosoma è la criopirina che è codificata dal gene NALP3/CIAS1. Singole mutazioni di questo gene, danno luogo ad aumentata secrezione di IL-1β da parte dei monociti circolanti [28-29] .

Negli anni più recenti, grazie ai notevoli progressi compiuti dalla ricerca biologica, sono stati chiariti nel dettaglio molti dei processi fisiopatologici responsabili dell'infiammazione. Questi sviluppi hanno consentito di mettere a punto nuove molecole capaci di neutralizzare in maniera selettiva e mirata i principali mediatori del processo flogistico e del danno tissutale conseguente. Numerosi studi hanno dimostrato che l'interleuchina 1 (IL-1) è un mediatore chiave della flogosi, del riassorbimento osseo e della distruzione cartilaginea, che sono i principali determinanti del danno articolare nelle artriti croniche [30]. L'azione pro-infiammatoria dell'IL-1 è contrastata da un inibitore naturale, che, quando presente in eccesso, impedisce il legame della citochina con il suo recettore e, conseguentemente, la trasduzione del segnale alle cellule effettrici. L'anakinra è una forma ricombinante

dell'antagonista recettoriale umano, che, analogamente a quest'ultimo, frena con effetto terapeutico le attività biologiche dell'IL-1 attraverso l'inibizione competitiva della sua interazione recettoriale. L'utilità del blocco dell'IL-1 in queste patologie è stata suggerita dalla dimostrazione che il gene CIAS1 è coinvolto nella produzione del'IL-1 e dal riscontro di una produzione spontanea di questa citochina da parte dei monociti dei pazienti. D'altra parte, la dimostrazione della capacità dell'anakinra di sopprimere i sintomi clinici e di diminuire la risposta flogistica ha confermato, in maniera indiretta, il ruolo prominente dell'IL-1 nella patogenesi di queste condizioni [31-34]. L'effetto collaterale principale dell'anakinra è dato dal dolore, spesso accompagnato da reazioni eritematose, nel sito di iniezione [35]. Le reazioni locali rendono spesso problematica la somministrazione continuativa di questo trattamento, che richiede l'esecuzione di iniezioni sottocutanee con frequenza quotidiana. Negli ultimi anni la ricerca farmacologica ha messo a punto nuove molecole, capaci di antagonizzare l'IL-1analogamente all'anakinra, ma dotate di emivita più lunga e quindi somministrabili ad intervalli più lunghi. Una di queste molecole è rappresentata

dal canakinumab, un anticorpo monoclonale interamente umano capace di neutralizzare efficacemente le attività biologiche dell'IL-1β, senza peraltro prevenire il legame dell'inibitore naturale, né il legame all'IL-1α [36]. Può essere infuso per via endovenosa o sottocutanea. Uno studio randomizzato contro placebo nelle criopirinopatie ha mostrato che il trattamento con canakinumab sottocute ogni 8 settimane si è associato a remissione dei sintomi nella maggior parte dei pazienti [14]. I risultati soddisfacenti ottenuti nelle criopirinopatie suggeriscono che l'inibizione dell'IL-1 possa avere un ruolo terapeutico anche per le altre patologie autoinfiammatorie legate ad un difetto genico di proteine coinvolte nella regolazione dell'attivazione di IL-1β.

È molto verosimile che le malattie autoinfiammatorie attualmente note rappresentino solo una piccola parte di quelle esistenti. Sono inoltre di estremo interesse i risultati, sull'impiego dell'anakinra nella forma sistemica di artrite idiopatica giovanile (AIG) [37-39] e nel morbo di Still dell'adulto (equivalente della forma sistemica nell'adulto) [40].

REFERENZE

1. Hull KM, Shoham N, Chae JJ, Aksentijevich I, Kastner DL. The expanding spectrum of systemic autoinflammatory disorders and their rheumatic manifestations. Current opinion in rhe umatology 2003 Jan;15(1):61-69.
2. Touitou I, Kone-Paut I. Autoinflammatory diseases. Best practice & research.Clinical rheumatology 2008 Oct;22(5):811-829.
3. McDermott MF, Aksentijevich I, Galon J, McDermott EM, Ogunkolade BW, Centola M, et al. Germline mutations in the extracellular domains of the 55 kDa TNF receptor, TNFR1, define a family of dominantly inherited autoinflammatory syndromes. Cell 1999 Apr 2;97(1):133-144.
4. Drenth JP, van der Meer JW. Hereditary periodic fever. The New England journal of medicine 2001 Dec 13;345(24):1748-1757.
5. Galeazzi M, Gasbarrini G, Ghirardello A, Grandemange S, Hoffman HM, Manna R, et al. Autoinflammatory

syndromes. Clinical and experimental rheumatology 2006 Jan-Feb;24(1 Suppl 40):S79-85.

6. McDermott MF, Aksentijevich I. The autoinflammatory syndromes. Current opinion in allergy and clinical immunology 2002 Dec;2(6):511-516.

7. Stankovic K, Grateau G. Auto inflammatory syndromes: Diagnosis and treatment. Joint Bone Spine, 2007 12;74(6):544-550.

8. Kile RL, Rusk HA. A case of cold urticaria with an unusual family history. JAMA. 1940;(114):1067–8.

9. Muckle TJ, WELLSM. Urticaria, deafness, and amyloidosis: a new heredo-familial syndrome. Q J Med. 1962;31:235–48.

10. Prieur AM, Griscelli C. Arthropathy with rash, chronic meningitis, eye lesions, and mental retardation. J Pediatr. 1981;99(1):79–83.

11. Hoffman HM. Hereditary immunologic disorders caused by pyrin and cryopyrin. Curr Allergy Asthma Rep. 2007 Sep;7(5):323-30.

12. Feldmann J, Prieur AM, Quartier P, Berquin P, Certain S, Cortis E, et al. Chronic infantile neurological cutaneous and articular syndrome is caused by mutations

in CIAS1, a gene highly expressed in polymorphonuclear cells and chondrocytes. Am J Hum Genet. 2002;71(1):198–203.

13. Dinarello CA. Mutations in cryopyrin: bypassing roadblocks in the caspase 1 inflammasome for interleukin-1beta secretion and disease activity. Arthritis Rheum. 2007;56(9):2817–22.

14. Lachmann HJ, Kone-Paut I, Kuemmerle-Deschner JB, Leslie KS, Hachulla E, Quartier P, Gitton X, Widmer A, Patel N, Hawkins PN; Canakinumab in CAPS Study Group. Use of canakinumab in the cryopyrin-associated periodic syndrome. N Engl J Med. 2009 Jun 4;360(23):2416-25.

15. Hoffman HM, Mueller JL, Broide DH, Wanderer AA, Kolodner RD. Mutation of a new gene encoding a putative pyrin-like protein causes familial cold autoinflammatory syndrome and Muckle-Wells syndrome. Nat Genet 2001;29:301-5.

16. Feldmann J, Prieur AM, Quartier P, et al. Chronic infantile neurological cutaneous and articular syndrome is caused by mutations in CIAS1, a gene highly

expressed in polymorphonuclear cells and chondrocytes. Am J Hum Genet 2002;71:198-203.

17. Neven B, Callebaut I, Prieur AM, et al. Molecular basis of the spectral expression of CIAS1 mutations associated with phagocytic cell-mediated autoinflammatory disorders CINCA/NOMID, MWS, and FCU. Blood 2004;103:2809-15.

18. Aganna E, Martinon F, Hawkins PN, Ross JB, Swan DC, Booth DR, Lachmann HJ, Bybee A, Gaudet R, Woo P, Feighery C, Cotter FE, Thome M, Hitman GA, Tschopp J, McDermott MF. Association of mutations in the NALP3/CIAS1/PYPAF1 gene with a broad phenotype including recurrent fever, cold sensitivity, sensorineural deafness, and AA amyloidosis. Arthritis Rheum. 2002 Sep;46(9):2445-52. Erratum in: Arthritis Rheum 2002 Dec;46(12):3398.

19. Hoffman HM, Wanderer AA, Broide DH. Familial cold autoinflammatory syndrome: phenotype and genotype of an autosomal dominant periodic fever. J Allergy Clin Immunol. 2001;108(4):615–20.

20. Muckle TJ, WELLSM. Urticaria, deafness, and amyloidosis: a new heredo-familial syndrome. Q J Med. 1962;31:235–48.

21. Nazzari G, Desirello G, Crovato F. Recurrent urticarial skin eruption since infancy. Muckle-Wells syndrome (MWS). Arch Dermatol. 1995;131(1):81–5.

22. Leslie KS, Lachmann HJ, Bruning E, McGrath JA, Bybee A, Gallimore JR, et al. Phenotype, genotype, and sustained response to anakinra in 22 patients with autoinflammatory disease associated with CIAS-1/NALP3 mutations. Arch Dermatol. 2006;142(12):1591–7.

23. Prieur AM, Griscelli C, Lampert F, Truckenbrodt H, Guggenheim MA, Lovell DJ, et al. A chronic, infantile, neurological, cutaneous and articular (CINCA) syndrome. A specific entity analysed in 30 patients. Scand J Rheumatol Suppl. 1987;66:57–68.

24. Aksentijevich I, Nowak M, Mallah M, Chae JJ, Watford WT, Hofmann SR, et al. De novo CIAS1 mutations, cytokine activation, and evidence for genetic heterogeneity in patients with neonatal-onset multisystem inflammatory disease (NOMID): a new

member of the expanding family of pyrin-associated autoinflammatory diseases. Arthritis Rheum. 2002;46(12):3340–8.

25. Caroli F, Pontillo A, D'Osualdo A, Travan L, Ceccherini I, Crovella S, et al. Clinical and genetic characterization of Italian patients affected by CINCA syndrome. Rheumatology. (Oxford) 2007;46(3):473–8.

26. Gattorno M, Federici S, Pelagatti MA, Caorsi R, Brisca G, Malattia C, Martini A. Diagnosis and management of autoinflammatory diseases in childhood. J Clin Immunol. 2008 May;28 Suppl 1:S73-83.

27. Church LD, McDermott MF. Canakinumab: a human anti-IL-1β monoclonal antibody for the treatment of cryopyrin-associated periodic syndromes. Expert Rev Clin Immunol. 2010 Nov;6(6):831-41.

28. Agostini L, Martinon F, Burns K, McDermott MF, Hawkins PN, Tschopp J. NALP3 forms an IL-1beta-processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder. Immunity 2004;20:319-25.

29. Janssen R, Verhard E, Lankester A, Ten CR, van Dissel JT. Enhanced interleukin-1beta and interleukin-18

release in a patient with chronic infantile neurologic, cutaneous, articular syndrome. Arthritis Rheum 2004;50:3329-33.

30. Dinarello CA. Blocking IL-1 in systemic inflammation. J Exp Med. 2005 May 2;201(9):1355-9. Review.

31. Hoffman HM, Patel DD. Genomic-based therapy: targeting interleukin-1 for autoinflammatory diseases. Arthritis Rheum. 2004 Feb;50(2):345-9.

32. Ross JB, Finlayson LA, Klotz PJ, Langley RG, Gaudet R, Thompson K, Churchman SM, McDermott MF, Hawkins PN. Use of anakinra (Kineret) in the treatment of familial cold autoinflammatory syndrome with a 16-month follow-up. J Cutan Med Surg. 2008 Jan-Feb;12(1):8-16.

33. Hawkins PN, Lachmann HJ, McDermott MF. Interleukin-1-receptor antagonist in the Muckle-Wells syndrome. N Engl J Med. 2003 Jun 19;348(25):2583-4.

34. Goldbach-Mansky R, Dailey NJ, Canna SW, Gelabert A, Jones J, Rubin BI, Kim HJ, Brewer C, Zalewski C, Wiggs E, Hill S, Turner ML, Karp BI, Aksentijevich I, Pucino F, Penzak SR, Haverkamp MH, Stein L, Adams BS, Moore TL, Fuhlbrigge RC, Shaham B, Jarvis JN,

O'Neil K, Vehe RK, Beitz LO, Gardner G, Hannan WP, Warren RW, Horn W, Cole JL, Paul SM, Hawkins PN, Pham TH, Snyder C, Wesley RA, Hoffmann SC, Holland SM, Butman JA, Kastner DL. Neonatal-onset multisystem inflammatory disease responsive to interleukin-1beta inhibition. N Engl J Med. 2006 Aug 10;355(6):581-92.

35. Hawkins PN, Lachmann HJ, Aganna E, McDermott MF. Spectrum of clinical features in Muckle-Wells syndrome and response to anakinra. Arthritis Rheum. 2004 Feb;50(2):607-12.

36. Alten R, Gram H, Joosten LA, van den Berg WB, Sieper J, Wassenberg S, Burmester G, van Riel P, Diaz-Lorente M, Bruin GJ, Woodworth TG, Rordorf C, Batard Y, Wright AM, Jung T. The human anti-IL-1 beta monoclonal antibody ACZ885 is effective in joint inflammation models in mice and in a proof-of-concept study in patients with rheumatoid arthritis. Arthritis Res Ther. 2008;10(3):R67.

37. Verbsky JW, White AJ. Effective use of the recombinant interleukin 1 receptor antagonist anakinra in therapy

resistant systemic onset juvenile rheumatoid arthritis. J Rheumatol 2004;31:2071-5.

38. Pascual V, Allantaz F, Arce E, et al. Role of interleukin-1 (IL-1) in the pathogenesis of systemic onset juvenile idiopathic arthritis and clinical response to IL-1 blockade. J Exp Med 2005;201:1479-86.

39. Gattorno M, Piccini A, Lasigliè D, Tassi S, Brisca G, Carta S, Delfino L, Ferlito F, Pelagatti MA, Caroli F, Buoncompagni A, Viola S, Loy A, Sironi M, Vecchi A, Ravelli A, Martini A, Rubartelli A. The pattern of response to anti-interleukin-1 treatment distinguishes two subsets of patients with systemic-onset juvenile idiopathic arthritis. Arthritis Rheum. 2008 May;58(5):1505-15.

40. Fitzgerald AA, Leclercq SA, Yan A, Homik JE, Dinarello CA. Rapid responses to anakinra in patients with refractory adult-onset Still's disease. Arthritis Rheum 2005;52:1794-803.

2.2 The schedule of administration of Canakinumab in cryopyrin associated periodic syndrome is driven by the phenotype severity rather than the age

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The schedule of administration of canakinumab in cryopyrin associated periodic syndrome is driven by the phenotype severity rather than the age

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Abstract

Introduction: Interleukin-1 (IL-1) blockade is the treatment of choice of cryopyrin associated periodic syndromes (CAPS). Anti-IL-1 monoclonal antibody (canakinumab) was recently registered. However no clear data are available on the optimal schedule of administration of this drug. The aim of the present study was to analyse the impact of canakinumab on CAPS patients in daily clinical practice and to identify the best schedule of administration according to age and phenotype.

Methods: 13 CAPS patients (10 children and 3 young adults) treated with canakinumab were followed for 12 months. Clinical and laboratory parameters were collected at each visit. Health-related quality of life (HRQoL) was recorded at month 12. Complete response was defined as absence of clinical manifestations and normal examinations. Clinical and laboratory variables at last follow-up were compared with those registered at the moment of anakinra discontinuation.

Results: seven patients with chronic infantile neurological cutaneous articular (CINCA) syndrome, four patients with Muckle-Wells syndrome (MWS) and two patients with an overlapping MWS/CINCA phenotype were analysed. CINCA patients experienced a higher number of modifications of the treatment (increased dosage or decreased dosing interval) in respect to MWS patients. At the end of the follow-up CINCA patients displayed a higher frequency of administration with a median dose of 3.7 mg/kg (2.1 mg/kg for MWS patients). Canakinumab was withdrawn in a patient with CINCA for incomplete response and poor compliance. The effect of canakinumab on HRQoL was similar to that observed during treatment with anakinra, with the exception of an improvement of the psychosocial concepts after the introduction of canakinumab.

Conclusions: The use of canakinumab in daily practice is associated with persistent satisfactory control of disease activity but needs progressive dose adjustments in more severe patients. The clinical phenotype, rather than the age, represents the main variable able to determine the need of more frequent administrations of the drug at higher dosage.

Introduction

Familial cold autoinflammatory syndrome (FCAS), Muckle-Wells syndrome (MWS) and chronic infantile neurological cutaneous and articular syndrome (CINCA) represent the clinical spectrum associated to mutations in *NLRP3* gene coding for the cryopyrin protein [1,2] and are collectively known as cryopyrin-associated periodic syndrome (CAPS).

FCAS is characterized by urticarial rash, arthralgia and fever spikes of short duration induced by cold exposure [3]. In MWS recurrent episodes of urticaria-like eruptions, fever, chills, malaise and limb pain occur from childhood onwards and are associated with the late development of sensorineural hearing loss and amyloidosis [4].

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CINCA (or neonatal onset multi-systemic inflammatory disease, NOMID) represents the most severe condition and is characterized by a neonatal onset urticarial-like rash, fever, central nervous system (CNS) involvement (mental retardation, chronic aseptic meningitis, increased intracranial pressure, cerebral atrophy, ventriculomegaly, sensorineural hearing loss and chronic papilledema), chronic inflammatory arthropathy, skeletal dysplasia and specific facial and dysmorphic features [5].

Cryopyrin is involved in the assembly of an intracellular multi-protein complex (called inflammasome) that plays a pivotal role in the induction and secretion of the biologically active 17 kD form of IL-1β [6,7]. Anti-IL-1 blockers are highly effective in CAPS. The short- [8-10] and long-term [11-13] effectiveness of the IL-1 receptor antagonist (anakinra) in CAPS have been already described in the last few years. Other IL-1 inhibitors, such as rilonacept, a human dimeric fusion protein that incorporates the extra-cellular domain of both IL-1 receptor type I and IL-1 receptor accessory protein [14], and a fully human anti-IL-1β monoclonal antibody, canakinumab are also available [15].

In a recent trial the use of subcutaneous doses of 150 mg (or 2 mg/kg) of canakinumab every 8 weeks for 24 weeks was generally associated with complete control of clinical manifestations and laboratory parameters in patients with a prevalent MWS phenotype [15]. These positive results were confirmed in the following 24-month phase III trial [16]. Interestingly, in this latter study a relevant percentage of patients required modification of the treatment schedule by means of increased dosage and/or frequency of administration [16]. This was mainly observed in pediatric and CINCA patients who were not included in the previous trial. However, the description of the pattern of disease activity and the strategy used for the modified treatment schedule were not reported [16].

In this retrospective multicenter study we describe one year of follow-up in a cohort of pediatric and CAPS patients treated with canakinumab. The main aims were to 1) verify the efficacy and safety of the drug in everyday clinical practice, 2) evaluate the impact of the drug on the quality of life, and 3) identify the best schedule for CAPS patients according to their age and phenotype.

Materials and methods

Thirteen unrelated CAPS patients (female:male ratio 7:6; 10 children, 3 adults; mean age 14.6 years, range 8.7 to 38 years) were enrolled in the study from five pediatric rheumatology centers. Twelve of these patients were previously enrolled in the CACZ885D2306 trial [16]. According to their phenotype, seven patients were classified with CINCA for the presence of early-onset urticarial skin rash associated with involvement of the CNS (papilledema, early-onset hearing loss, brain atrophy at MRI, severe headache) and bone dysplasia [2,17]. Four patients were classified with MWS syndrome (absence of CNS involvement and bone dysplasia). Two patients were defined as having an overlapping phenotype between MWS and CINCA for the presence of early-onset hearing loss and/or papilledema in the absence of brain atrophy, mental retardation or bone dysplasia. Ten patients carried mutations in the *NLRP3* gene (Table 1).

At the end of the CACZ885D2306 study (July 2010), all patients continued treatment with canakinumab in an open fashion with the last schedule (dose and frequency) used during the trial. Before the registration of the drug in Italy (November 2010), patients received the treatment for compassionate use, provided by Novartis, Italy. An additional patient was treated soon after the registration of the drug. The study was approved by the Ethical Board of the G. Gaslini Institute. Local ethical boards also approved the study in each center. Informed consent was obtained from patients or families. Patients were followed at their center with monthly or bi-monthly visits. According to normal daily clinical practice used in Italian centers for patients with CAPS the following parameters were recorded: 1) presence of disease-associated manifestations (skin rash, arthralgia/arthritis, myalgia, headache and conjunctivitis) and the global evaluation of disease activity with a four-point scale as absent (0), minimal (1), moderate (2), severe (3) [16]; 2) laboratory parameters, including hemogram, C-reactive protein (CRP), serum amyloid A protein (SAA); 3) eye examination and audiogram (with a frequency of 3 to 6 months according the policy at each center), and 4) a national language version of the parent-administered 50-item version of the Child Health Questionnaire (CHQ-PF 50) at month 12, as previously described [11].

Modifications of dose and/or frequency of administration of canakinumab were performed according to the judgment of the physician in charge based on the clinical picture and/or laboratory parameters. Adverse events were also registered. After 12 months of treatment, data from clinical charts were retrospectively evaluated. Response to treatment was evaluated as follows: i) complete response: absent or minimal disease activity at the global assessment with acute phase reactants within the normal range, ii) partial response: absent or minimal global disease activity associated with elevation of acute phase reactants, or iii) inadequate response: moderate or severe global disease activity associated with elevated acute phase reactants.

Intra-individual comparison between anakinra and canakinumab

Twelve out of thirteen patients were previously treated with anakinra for a median period of 42 months (range

Table 1 Demographic data, duration of treatment, schedule (dose and frequency) of canakinumab and response to treatment at the beginning of the follow-up period

Patient number	Phenotype	NLRP3 mutation	Disease onset	Duration of treatment (months)	Body weight (Kg)	Dose (mg/kg)	Dose at each Administration, mg	Canakinumab frequency, weeks	Response
1	CINCA	N477K	birth	5	65	4.6	300	8	partial
2	CINCA	F573S	birth	5	38	4.0	152	8	inadequate
3	CINCA	Negative	2 months	5	50	6.0	300	8	partial
4	CINCA	Negative	birth	6	54	2.8	150	8	partial
5	MWS/ CINCA	D303N/ V198M	1 week	12	29	2.0	72	8	complete
6	MWS/ CINCA	T348M	6 months	11	85	3.5	300	8	partial
7	CINCA	E304K	birth	12	78	3.8	300	8	partial
8	MWS	T348M	birth	12	63	2.4	150	8	partial
9	MWS	E525K	15 months	17	62	2.4	150	8	complete
10	MWS	D303N	3 weeks	20	37	2.0	74	8	complete
11	CINCA	T348M	birth	15	23	2.0	46	8	partial
12	CINCA	Negative	birth	7	61	2.5	150	8	complete
13	MWS	V198M	9 years	-	38	2.0	76	8	complete

CINCA: chronic infantile neurological cutaneous articular syndrome; MWS: Muckle-Wells syndrome; MWS/CINCA: overlap syndrome.

12 to 60 months) [11]. For these patients, clinical and laboratory variables at the time of anakinra withdrawal were retrospectively evaluated. The CHQ-PF 50 administered after 12 months of treatment with canakinumab was compared with the same evaluation performed in the same patients during anakinra treatment [11], using the non-parametric Wilcoxon pairs test.

Results

Baseline

At the end of the ACZ885D2306 trial seven patients (three with MWS, one with MWS/CINCA and three with CINCA) were treated at the dose of 2 mg/kg (or 150 mg if body weight was > 40 Kg) every 8 weeks, which was the starting dose used in the trial [16]. Due to incomplete control of disease activity, five patients (one with MWS/CINCA and four with CINCA) experienced increasing dosing during the trial and were therefore receiving 4 mg/kg (or 300 mg, if body weight was > 40 Kg) every 8 weeks (Table 1). The MWS patient (number 13) who was treated after the registration of the drug received the starting dose of 2 mg/kg every 8 weeks according to the terms of the marketing authorization.

At baseline four patients with the MWS or MWS/CINCA phenotype (patients number 5, 9, 10 and 13) were in complete remission, while two of them (patients 6 and 8) were in partial remission (Table 1). Among patients with CINCA, a complete or partial response was observed in one (patient 12) and five (patients 1, 3, 4, 7 and 11), respectively (Table 1). One patient (number 2) was considered a non-responder due to the presence of both clinical manifestations and elevation of acute phase reactants.

Follow-up period

In Figure 1 the clinical and laboratory variables during the 12 months of follow-up in patients with less severe (MWS and MWS/CINCA, panel A) and more severe (CINCA, panel B) phenotypes are shown. Of the six patients with the MWS and MWS/CINCA phenotypes, three (patients 5, 9, and 10) did not require any adjustment of the therapy due to a persistent optimal control of both clinical and laboratory parameters during the whole follow-up period (Figure 1A). Two others (patients 6 and 8) required at least one modification of the treatment schedule due to a persistent elevation of acute phase reactants (Figure 1A). After three doses, due to the persistent control of clinical and laboratory parameters, the dosing frequency for patient number 13 was decreased to every 10 weeks. At the following visit, in the light of persistent and complete wellbeing, both the physician and the parents of the patient agreed to discontinue the treatment with the aim of using the drug only on demand. At the end of follow-up the majority of MWS and MWS/CINCA patients were treated with a dose below 2.5 mg/kg. Only patient number 6, presenting a MWS/CINCA phenotype with a T348M mutation, required a higher dose (3.7 mg/kg) due to persistent elevation of acute phase reactants (Table 2).

Six out of seven patients with CINCA received at least one modification of the canakinumab schedule. Due to a

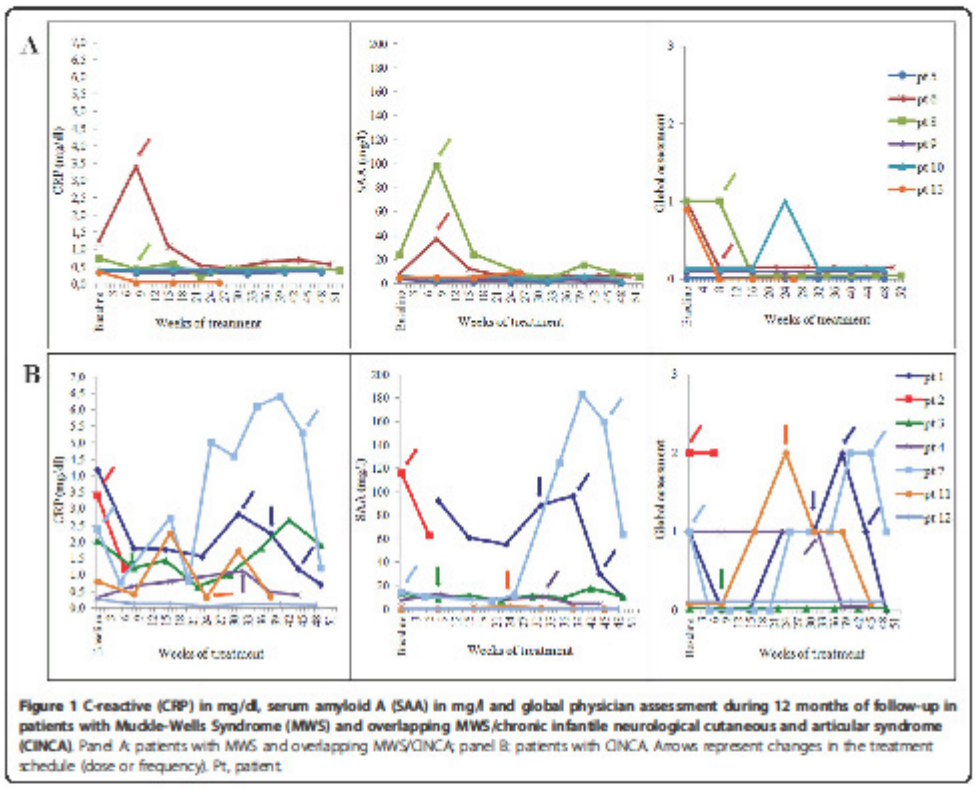

Figure 1 C-reactive (CRP) in mg/dl, serum amyloid A (SAA) in mg/l and global physician assessment during 12 months of follow-up in patients with Muckle-Wells Syndrome (MWS) and overlapping MWS/chronic infantile neurological cutaneous and articular syndrome (CINCA). Panel A: patients with MWS and overlapping MWS/CINCA; panel B: patients with CINCA. Arrows represent changes in the treatment schedule (dose or frequency). Pt, patient.

Table 2 Dose of anti-IL drug, acute phase reactants, physician assessment of disease and response to treatment at last follow-up on treatment with Canakinumab and at the moment of anakinra withdrawal

	Last follow-up on canakinumab					Last follow-up on anakinra				
Patient number	Dose, mg (mg/Kg)/ frequency	CRP, mg/dl	SAA, mg/l	Clinical assessment	Response	Dose, mg (mg/kg)/ frequency	CRP, mg/dl	SAA mg/l	Clinical assessment	Response
1[1]	300 (4.30)/5 wks	1.17	29.9	mild	partial	75 (1.30)/day	0.61	25.0	absent	partial
2[1]	150 (4.00)/6 wks	1.18	ND	moderate	inadequate	75 (2.00)/day	neg	18.0	absent	partial
3[1]	300 (5.90)/6 wks	2.67	18.1	absent	partial	100 (1.80)/day	0.47	7.50	absent	partial
4[1]	150 (2.80)/6 wks	neg	neg	absent	complete	100 (2.00)/day	neg	neg	minimal	complete
5[2]	78 (2.00)/8 wks	neg	neg	absent	complete	55 (2.00)/day	neg	neg	minimal	complete
6[2]	300 (3.75)/7 wks	0.57	6.5	absent	partial	100 (1.16)/day	0.63	neg	mild	partial
7[1]	300 (3.70)/4 wks	1.20	64.0	mild	partial	100 (1.30)/day	0.73	neg	absent	partial
8[2]	150 (2.30)/6 wks	neg	neg	absent	complete	ND	ND	ND	ND	ND
9[2]	150 (2.40)/8 wks	neg	neg	absent	complete	50 (1.00)/day	neg	neg	absent	complete
10[2]	100 (2.00)/8 wks	neg	neg	absent	complete	20 (0.66)/day	neg	neg	absent	complete
11[1]	60 (2.00)/7 wks	neg	neg	absent	complete	25 (1.00)/day	neg	13.2	mild	partial
12[1]	150 (2.00)/8 wks	neg	neg	absent	complete	55 (1.00)/day	neg	neg	absent	complete
13[2]	78 (2.00)/10 wks	neg	neg	absent	complete	38 (1.00)/day	neg	neg	absent	complete

[1]Patients with the chronic infantile neurological cutaneous and articular syndrome (CINCA) phenotype; [2]patients with the Muckle-Wells syndrome (MWS) and MWS/CINCA phenotype; CRP, C reactive protein; SAA, serum amyloid A; neg, negative; ND, not done.

persistent elevation of acute phase reactants associated with mild clinical manifestations, patients 3, 4 and 11 required a single modification of the frequency. In patients 1 and 7 the frequency was increased three and two times to a final schedule of 300 mg every 5 and 4 weeks, respectively (Table 2). Patient 2 was treated at baseline with 300 mg every 8 weeks. The frequency of administration of canakinumab was initially increased to every 6 weeks because of the persistence of disease activity (Figure 1B). At the following evaluation both the physician in charge and parents preferred to discontinue canakinumab due to the persistence of disease-associated symptoms (arthralgia and malaise), the presence of possible side effects (dizziness) and the remarkable elevation of acute phase reactants (Figure 1B). The patient was subsequently treated with anakinra, (2 mg/kg/day) with a complete control of the clinical manifestations and normalization of acute phase reactants.

The comparison between the two subgroups (MWS and MWS/CINCA vs CINCA) did not reveal any difference in their median age (Table 3). Conversely, during the course of the study CINCA patients experienced a higher number of adjustments (increased dose or decreased dosing interval) in respect to MWS patients (Table 3). At the end of the follow-up CINCA patients had required a higher frequency of administration and a trend towards the need for a higher median dose (3.7 vs 2.1 mg/kg) (Table 3).

During the 12 months of follow-up repeated audiograms and eye examinations did not show substantial changes from baseline. Flares of headache, or modifications in behavior or mental performance were not observed. Compliance with treatment was good, and no reactions at the site of the injection were recorded. Patient number 8 presented with cellulitis of the leg at month 11, which required hospitalization for intravenous antibiotic treatment. Methicillin-resistant staphylococcus aureus was isolated. The administration of canakinumab was postponed until the complete resolution of the infection, which occurred without complications.

The 15 subscales of the CHQ and the two summary scores, the physical summary score (PhS) and the psychosocial summary score (PsS), recorded at the time of the last follow-up and the reference values for healthy controls are reported in Figure 2. Overall, the health-related quality of life (HRQoL) of canakinumab-treated CAPS patients was generally comparable to that recorded in the control group. Patients with CAPS still presented a significantly impaired perception of general health ($P < 0.001$) and physical functioning ($P = 0.02$) when compared with healthy controls.

Within-patient comparison of anakinra and canakinumab

Twelve out the thirteen patients treated with canakinumab were previously treated with anakinra. We therefore evaluated for each patient whether the pattern of response to canakinumab was different from that registered at the time of anakinra withdrawal. At the time of the last administration of anakinra six patients (two with CINCA, one with MWS/CINCA and three with MWS) displayed a complete response (Table 2). Six patients (five with CINCA, one with MWS/CINCA) displayed a partial response due to a slight increase of acute phase reactants (Table 2). All five patients presenting a partial response to canakinumab already showed a partial or inadequate response to anakinra (Table 2).

We also had the possibility of comparing HRQoL at the last follow-up of treatment with canakinumab to that obtained at the time of anakinra withdrawal (Figure 2) [11]. The comparison did not reveal a significant difference of the impact of the two drugs on physical concepts. Conversely, canakinumab treatment was associated with a significant amelioration of psychosocial concepts ($P = 0.03$), especially for the parental emotional perception of the disease ($P = 0.045$) with a trend towards significance for self-esteem ($P = 0.09$) and parental time ($P = 0.1$) (not shown).

Discussion

In the present paper we describe the impact of canakinumab in the daily clinical practice in a cohort of CAPS patients mostly characterized by pediatric age and/or a severe phenotype. After one year of follow-up, twelve out of thirteen patients displayed complete control of clinical manifestations, with a slight elevation of acute

Table 3 Comparison of age, duration of disease, final dose, frequency of administration of canakinumab and number of adjustments (dose and/or frequency) performed during 12 months in patients with MWS and CINCA

	Muckle-Wells (6 patients)[1]	CINCA (7 patients)	P-value[2]
Age, years, median (range)	13.6 (10.7, 23.7)	15 (8.7, 38.0)	0.56*
Disease duration, median, years (range)	13.6 (3.0, 23.2)	15 (8.7, 38.0)	0.61*
Dose, mg/kg, median (range)	2.15 (2.0, 3.7)	3.7 (2.0, 5.9)	0.16*
Frequency, weeks, median (range)	8 (6, 10)	6 (4, 8)	0.03
Adjustments/year, number	2	9	0.05

[1]Includes two patients with a Muckle-Wells syndrome (MWS)/chronic infantile neurological cutaneous and articular syndrome (CINCA) phenotype; [2]Mann-Whitney *U*-test, *not significant.

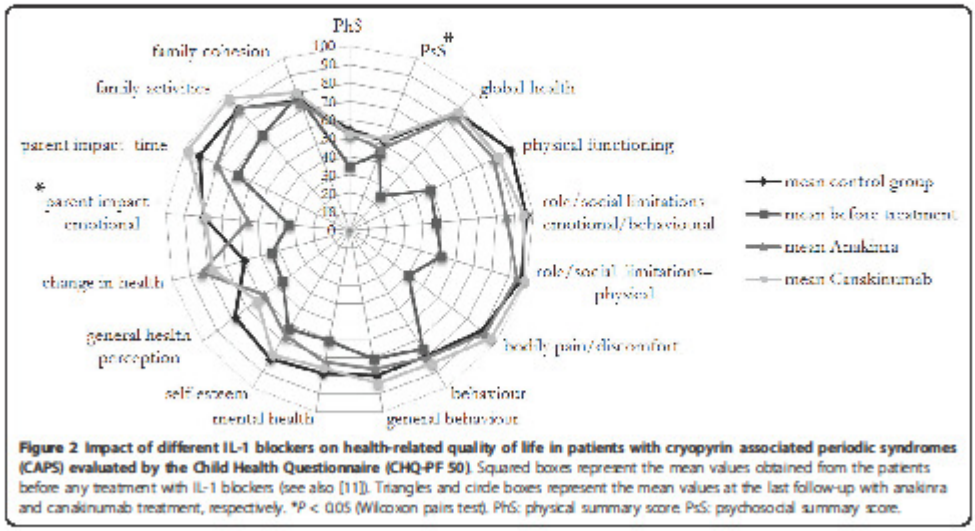

Figure 2 Impact of different IL-1 blockers on health-related quality of life in patients with cryopyrin associated periodic syndromes (CAPS) evaluated by the Child Health Questionnaire (CHQ-PF 50). Squared boxes represent the mean values obtained from the patients before any treatment with IL-1 blockers (see also [11]). Triangles and circle boxes represent the mean values at the last follow-up with anakinra and canakinumab treatment, respectively. *$P < 0.05$ (Wilcoxon pairs test). PhS: physical summary score. PsS: psychosocial summary score.

phase reactants in four of them. In relation to the recent report of Kummerle *et al.* [16] the present study analyzes in more detail the actual impact of canakinmab on disease activity according to the disease phenotype. Globally, patients with a mild-intermediate MWS phenotype display complete control of disease activity maintaining the initial dosage of 2 mg/kg (or 150 mg) every 8 weeks, independent of their age. The majority of our CINCA patients required an increase in the dose to 4 mg/kg (or 300 mg) during the ACZ885D2306 trial. For most of them this dose adjustment was not sufficient to achieve complete control of disease activity, and a progressive increase of the dosing frequency was needed in the following 12 months. In the majority of the patients the main reason for interval adjustment was the presence of a persistent elevation of acute phase reactants, despite complete control of the clinical manifestations. In one patient with severe CINCA (patient number 2), the clinical manifestations persisted along with the elevation of acute phase reactants, despite the previous increase of the dose to 300 mg every 6 weeks. In this case parents did not allow further increase in the frequency of the administration or escalation of the dose and asked for a return to the anakinra regimen.

The variability in the response to IL-1 blockers has been already observed in previous studies. The wide use of anakinra in all CAPS phenotypes has already provided evidence that patients with CINCA generally need higher doses to completely control disease activity compared to patients with a milder CAPS phenotype [11-13]. This is due to different reasons: first, the impact of different *NLRP3* mutations on the levels of secretion of IL-1β from monocytes is variable, with higher levels detected in the severe CINCA phenotype [7,18]. Second, this phenotype is characterized by the inflammatory involvement of the CNS and inner ear. In non-human primates, the diffusion of anakinra in the CNS is proportional to the systemic dose [19], supporting the need for an increasing dose to achieve complete control of CNS manifestations [13]. Third, most of the patients with CINCA are treated during childhood. Even if there are no clear data on the pharmacokinetic of IL-1 blockers in humans so far, it is conceivable that the bioavailability of these drugs in children is lower than in adults.

Interestingly in our study, all patients who did not achieve complete control of acute phase reactants at the last follow-up of treatment with canakinumab, displayed the same pattern at the moment of the anakinra withdrawal, supporting the need to identify the proper dose of IL-1 blockers on an individual basis. This issue might be due to the different impact of the *NLRP3* mutations on disease severity. This seems to be the case for the T348M mutation that in our experience determined an increased dosage regimen independently from our classification according to the disease phenotype. On the other hand the need to change the treatment schedule was also observed in CINCA patients who were negative for germ-line *NLRP3* mutations.

In any case, all partial responders displayed a CINCA phenotype, or had high body weight. In this line, the

existence of a single formulation for both anakinra (100 mg) and canakinumab (150 mg) represents a clear limitation for the proper management of the more severely affected patients.

In the present study we also compared the response to treatment in CAPS patients after 12 months of canakinumab treatment with those registered at the moment of anakinra withdrawal. This analysis has a number of limitations related to the retrospective collection of data and to the temporal gap (mean 23 months, range 18 to 36) between the last administration of anakinra and the evaluation of the patients after 12 months of treatment with canakinumab. Despite these limitations, our preliminary observations support the clinical perception of substantial equivalence of the two IL-1 blockers in the control of disease manifestations and their role in providing a sustained improvement in HRQoL [20]. With no doubt, the possibility of avoiding daily injections represented the main reason for observed better performance according to scores for the psychological items, when patients are treated with canakinumab. Due to its higher molecular weight, canakinumab has a much lower probability of reaching the CNS compared to anakinra. Despite the theoretical concern about the minor efficacy of canakinumab for CNS manifestations, none of the CINCA patients enrolled in the study had evidence of deterioration in CNS involvement during canakinumab treatment. However, in the long run further follow-up in larger series is needed to clarify this relevant issue.

So far, the safety profile reported for anakinra in the long run is rather good [11-13]. In our recent experience local erythematosus skin reaction at the injection site (28.5% of treated patients), excessive weight gain (14.2%) and severe oral aphthosis (7.1%) were the most relevant reported adverse events [11]. In this study canakinumab confirmed optimal tolerability together with a good safety profile [15,16]. Infections should be carefully and promptly recognized for proper and aggressive treatment. In the case of infectious cellulitis the previous use of canakinumab did not interfere with the time of resolution of this complication. To our knowledge this clinical manifestation has never been described in association with CAPS, or as a possible adverse event during treatment with biologic agents.

Our study shows that the CAPS phenotype, rather than patient age, represents the main variable to determine the need of more frequent administration of the drug at higher doses. Due to the age limit of 4 years in the ACZ885D2306 study, no information on very young children is available so far. Taking into account these limitations, we suggest that it is reasonable for pediatric patients above the age of 4 years who have MWS to be treated with the proposed schedule of 2 mg/kg every 8 weeks. However, patients with the severe CINCA phenotype should be more aggressively treated from the beginning. In our opinion monthly administration of 4 mg/kg should be the starting dose, with a possible progressive weekly decrease of the dosing frequency according to the clinical and laboratory response. Notably, this schedule has been recently proposed for systemic onset juvenile arthritis (SoJIA), so far with a satisfactory safety profile [21].

Further studies of both anakinra and canakinumab in larger pediatric serial studies are needed, including careful analysis of the pharmacokinetics in children younger than 4 years of age and long-term follow-up, in order to achieve a rational approach with IL-1 blockers for the broad phenotypic spectrum of CAPS in both children and adults.

Conclusions

The use of canakinumab in daily practice is associated with persistent satisfactory control of disease activity but needs progressive dose adjustments in more severely affected patients. In this respect, the clinical phenotype, rather than the patient's age, represents the main variable that determines the indication to start treatment with more frequent administration of the drug at higher doses in patients with CINCA.

Abbreviations

CAPS: cryopyrin associated periodic syndromes; CHQ-PF 50: 50-item version of the Child Health Questionnaire; CINCA: chronic infantile neurological cutaneous and articular syndrome; CNS: central nervous system; CRP: C-reactive protein; FCAS: familial cold autoinflammatory syndrome; HRQoL: health-related quality of life; IL: interleukin; JIA: juvenile arthritis; MWS: Muckle-Wells syndrome; SAA: serum amyloid A protein.

Authors' contributions

RC collected and analyzed the data and helped to draft the manuscript. FZ, LL, MA, AS, and AI contributed to the design and analysis of the study. MF, AB, GM, and CB collected the data. AM contributed to the design of the study and helped to draft the manuscript. MG conceived and coordinated the study and wrote the manuscript. All authors read and approved the final manuscript.

Competing interests

F Zulian has received consultancy fees from Novartis. A Martini has received payment from Novartis for service on speakers' bureaus. M Gattorno has received consultancy fees and honoraria Novartis and SOBI Biovitrum for meeting presentations. R Caorsi, L Lepore, M Alessio, A Stabile, A Insalaco, M Finetti, A Battagliese, G Martini and C Bibalo have no competing interests to declare.

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References

1. Hoffman HM, Mueller JL, Broide DH, Wanderer AA, Kolodner RD: **Mutation of a new gene encoding a putative pyrin-like protein causes familial cold autoinflammatory syndrome and Muckle-Wells syndrome.** *Nat Genet* 2001, 29:301-305.
2. Neven B, Callebaut I, Prieur AM, Feldmann J, Bodemer C, Lepore L, Derfalvi B, Benjaponpitak S, Vesely R, Sauvain MJ, Oertle S, Allen R, Morgan G, Borkhardt A, Hill C, Gardner-Medwin J, Fischer A, de Saint Basile G: **Molecular basis of the spectral expression of CIAS1 mutations associated with phagocytic cell-mediated autoinflammatory disorders CINCA/NOMID, MWS, and FCU.** *Blood* 2004, 103:2809-2815.
3. Kile RL, Rusk HA: **A case of cold urticaria with an unusual family history.** *JAMA* 1940, 114:1067-1068.
4. Muckle TJ, Wells M: **Urticaria, deafness, and amyloidosis: a new heredo-familial syndrome.** *Q J Med* 1962, 31:235-248.
5. Prieur AM, Griscelli C: **Arthropathy with rash, chronic meningitis, eye lesions, and mental retardation.** *J Pediatr* 1981, 99:79-83.
6. Martinon F, Tschopp J: **Inflammatory caspases: linking an intracellular innate immune system to autoinflammatory diseases.** *Cell* 2004, 117:561-574.
7. Gattorno M, Tassi S, Carta S, Delfino L, Ferlito F, Pelagatti MA, D'Osualdo A, Buoncompagni A, Alpigiani MG, Alessio M, Martini A, Rubartelli A: **Pattern of interleukin-1beta secretion in response to lipopolysaccharide and ATP before and after interleukin-1 blockade in patients with CIAS1 mutations.** *Arthritis Rheum* 2007, 56:3138-3148.
8. Hawkins PN, Lachmann HJ, McDermott MF: **Interleukin-1-receptor antagonist in the Muckle-Wells syndrome.** *N Engl J Med* 2003, 348:2583-2584.
9. Hoffman HM, Rosengren S, Boyle DL, Cho JY, Nayar J, Mueller JL, Anderson JP, Wanderer AA, Firestein GS: **Prevention of cold-associated acute inflammation in familial cold autoinflammatory syndrome by interleukin-1 receptor antagonist.** *Lancet* 2004, 364:1779-1785.
10. Goldbach-Mansky R, Dailey NJ, Canna SW, Gelabert A, Jones J, Rubin BI, Kim HJ, Brewer C, Zalewski C, Wiggs E, Hill S, Turner ML, Karp BI, Aksentijevich I, Pucino F, Penzak SR, Haverkamp MH, Stein L, Adams BS, Moore TL, Fuhlbrigge RC, Shaham B, Jarvis JN, O'Neil K, Vehe RK, Beltz LO, Gardner G, Hannan WP, Warren RW, Horn W, *et al*: **Neonatal-onset multisystem inflammatory disease responsive to interleukin-1beta inhibition.** *N Engl J Med* 2006, 355:581-592.
11. Lepore L, Paloni G, Caorsi R, Alessio M, Rigante D, Ruperto N, Cattalini M, Tommasini A, Zulian F, Ventura A, Martini A, Gattorno M: **Follow-up and quality of life of patients with cryopyrin-associated periodic syndromes treated with anakinra.** *J Pediatr* 2010, 157:310-315.
12. Neven B, Marvillet I, Terrada C, Ferster A, Boddaert N, Couloigner V, Pinto G, Pagnier A, Bodemer C, Bodaghi B, Tardieu M, Prieur AM, Quartier P: **Long-term efficacy of the interleukin-1 receptor antagonist anakinra in ten patients with neonatal-onset multisystem inflammatory disease/chronic infantile neurologic, cutaneous, articular syndrome.** *Arthritis Rheum* 2010, 62:258-267.
13. Sibley CH, Plass N, Snow J, Wiggs E, Brewer C, King K, Zalewski C, Kim H, Bishop R, Hill S, Paul SM, Kicker P, Phillips Z, Dolan JG, Widemann B, Jayaprakash N, Pucino F, Stone DL, Chapelle D, Snyder C, Butman JA, Wesley R, Goldbach-Mansky R: **Sustained response and prevention of damage progression in patients with neonatal-onset multisystem inflammatory disease (NOMID) treated with anakinra.** *Arthritis Rheum* 2012, 64:2375-86.
14. Hoffman HM, Throne ML, Amar NJ, Sebai M, Kivitz AJ, Kavanaugh A, Weinstein SP, Belomestnov P, Yancopoulos GD, Stahl N, Mellis SJ: **Efficacy and safety of rilonacept (interleukin-1 Trap) in patients with cryopyrin-associated periodic syndromes: results from two sequential placebo-controlled studies.** *Arthritis Rheum* 2008, 58:2443-2452.
15. Lachmann HJ, Kone-Paut I, Kuemmerle-Deschner JB, Leslie KS, Hachulla E, Quartier P, Gitton X, Widmer A, Patel N, Hawkins PN: **Use of canakinumab in the cryopyrin-associated periodic syndrome.** *N Engl J Med* 2009, 360:2416-2425.
16. Kuemmerle-Deschner JB, Hachulla E, Cartwright R, Hawkins PN, Tran TA, Bader-Meunier B, Hoyer J, Gattorno M, Gul A, Smith J, Leslie KS, Jiménez S, Morell-Dubois S, Davis N, Patel N, Widmer A, Preiss R, Lachmann HJ: **Two-year results from an open-label, multicentre, phase III study evaluating the safety and efficacy of canakinumab in patients with cryopyrin-associated periodic syndrome across different severity phenotypes.** *Ann Rheum Dis* 2011, 70:2095-2102.
17. Caroli F, Pontillo A, D'Osualdo A, Travan L, Ceccherini I, Crovella S, Alessio M, Stabile A, Gattorno M, Tommasini A, Martini A, Lepore L: **Clinical and genetic characterization of Italian patients affected by CINCA syndrome.** *Rheumatology (Oxford)* 2007, 46:473-478.
18. Tassi S, Carta S, Delfino L, Caorsi R, Martini A, Gattorno M, Rubartelli A: **Altered redox state of monocytes from cryopyrin-associated periodic syndromes causes accelerated IL-1beta secretion.** *Proc Natl Acad Sci USA* 2010, 107:9789-9794.
19. Fox E, Jayaprakash N, Pham TH, Rowley A, McCully CL, Pucino F, Goldbach-Mansky R: **The serum and cerebrospinal fluid pharmacokinetics of anakinra after intravenous administration to non-human primates.** *J Neuroimmunol* 2010, 223:138-140.
20. Kone-Paut I, Lachmann HJ, Kuemmerle-Deschner JB, Hachulla E, Leslie KS, Mouy R, Ferreira A, Lhertier K, Patel N, Preiss R, Hawkins PN: **Sustained remission of symptoms and improved health-related quality of life in patients with cryopyrin-associated periodic syndrome treated with canakinumab: results of a double-blind placebo-controlled randomized withdrawal study.** *Arthritis Res Ther* 2011, 13:R202.
21. Ruperto N, Quartier P, Wulffraat N, Woo P, Ravelli A, Mouy R, Bader-Meunier B, Vastert SJ, Noseda E, D'Ambrosio D, Lecot J, Chakraborty A, Martini A, Chioato A: **A phase II, multicenter, open-label study evaluating dosing and preliminary safety and efficacy of canakinumab in systemic juvenile idiopathic arthritis with active systemic features.** *Arthritis Rheum* 2012, 64:557-67.

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CAPITOLO 3

3.1 Efficacia dell'anticorpo monoclonale anti IL-1β nel trattamento dell'Artrite Idiopatica Giovanile ad esordio sistemico

L'Artrite Idiopatica Giovanile (AIG) sistemica è definita dalla presenza, accanto all'artrite, sia di una febbre quotidiana e persistente che di uno o più dei sintomi seguenti: rash, epatomegalia o splenomegalia, linfoadenomegalia generalizzata, sierositi, una sintomatologia molto simile a quella che si riscontra nelle criopirinopatie. L'AIG sistemica è una malattia caratteristica del bambino ed è di osservazione occasionale nell'adulto dove è conosciuta come malattia di Still dell'adulto.

Si tratta con molta verosimiglianza di una condizione eterogenea come suggerito dalla stessa variabile evoluzione della malattia. Mentre infatti in alcuni pazienti l'artrite è modesta e recede generalmente con il recedere della sintomatologia sistemica, in altri l'interessamento articolare domina il quadro clinico mentre la sintomatologia sistemica tende spesso ad attenuarsi fino a scomparire . La potenziale eterogeneità della artrite sistemica è stata di recente

ulteriormente avvalorata da sostanziali differenze tra i pazienti nella risposta terapeutica ad anakinra, un inibitore dell'interleuchina-1 (IL-1) [1].

Recentemente è stato descritto un importante effetto terapeutico dell'anakinra in bambini con AIG sistemica refrattaria ai preparati convenzionali, inclusi gli antagonisti del TNF [2]. Sebbene un successivo studio collaborativo francese abbia riportato risultati meno brillanti [3], l'anakinra ha conquistato negli ultimi anni un ruolo importante nel trattamento dell'AIG sistemica.

Lo studio di Gattorno M. et al del 2008 ha mostrato come il trattamento con anakinra porti alla caratterizzazione di 2 popolazioni di pz con AIG sistemica apparentemente distinta. Un gruppo di pz va incontro a una risposta brillante al trattamento, con rapida e completa normalizzazione dei sintomi clinici e dei parametri di laboratorio nell'arco di una settimana. Un secondo gruppo mostra, viceversa, una risposta modesta o nessuna risposta. L'effetto del trattamento nel primo gruppo di pz è del tutto simile a quello che si osserva in alcune malattie auto infiammatorie, quali le criopirinopatie [4]. Questo fenomeno ha indotto a ipotizzare che nell'ambito dell'AIG sistemica esistano

forme ad eziologia diversa, alcune delle quali assimilabili alle malattie auto infiammatorie. In effetti, la comparazione della severità dell'interessamento articolare nei due gruppi di pazienti inclusi nello studio evidenzia come il gruppo che ha mostrato scarsa o nulla risposta all'anakinra avesse un numero nettamente maggiore di articolazioni colpite rispetto al secondo. Ciò suggerisce che l'anakinra eserciti un'efficacia maggiore nei casi di AIG sistemica connotati da predominanza delle manifestazioni sistemiche (soprattutto febbre e rash) e da una minore estensione dell'artrite. Questi pazienti hanno, in effetti caratteristiche cliniche più simile alle sindromi auto infiammatorie rispetto a quelli con poliartrite più grave.

Tali dati suggeriscono che almeno alcuni di questi pazienti possano essere affetti da una sindrome autoinfiammatoria ancora ignota in cui sarebbero coinvolti geni che regolano, come nelle criopirinopatie, l'attivazione di IL-1 [5]. È anche interessante osservare a questo proposito che le criopirinopatie, esattamente come l'AIG sistemica [6-7], sono caratterizzate da elevati livelli circolanti di IL-6 che si normalizzano rapidamente a seguito del trattamento con

anakinra [8] il che suggerisce che la iperproduzione di IL-6 sia in questi casi secondaria a quella di IL-1.

Lo studio delle alterazioni dei meccanismi intracellulari di attivazione dell'IL-1 β nelle malattie infiammatorie potrebbe quindi in futuro non solo rappresentare un importate progresso terapeutico, ma rivelare l'esistenza di nuove entità cliniche e ridisegnare l'attuale classificazione della AIG e di altre malattie infiammatorie che, oggi ritenute multigeniche e polifattoriali, potrebbero in realtà rivelarsi sindromi autoinfiammatorie monogeniche.

3.2 Scopi

- Verificare se sia possibile identificare un gruppo di pazienti affetti da AIG sistemica responsivi alla terapia con anti IL-1β (Canakinumab)
- Verificare se in questi pz sono presenti mutazioni in CIAS 1 mediante indagine molecolare
- Valutare se esistono caratteristiche cliniche distintive fra i 2 gruppi (responder e non responder).
- Migliorare l'approccio terapeutico e la prognosi funzionale in questi pazienti

3.3 Disegno del progetto

Primo studio

Studio randomizzato, in doppio cieco, controllato verso placebo con singola somministrazione di Canakinumab, della durata di 4 settimane, per valutare l'efficacia iniziale e l'induzione da parte di Canakinumab di una risposta clinicamente significativa secondo i criteri adattati ACR 30 Pediatrici in pazienti con AIG sistemica con manifestazioni sistemiche attive

Secondo studio

Il secondo Studio è suddiviso in 2 parti:

Parte I (in aperto): a singolo braccio con trattamento attivo della durata di 32 settimane, il cui scopo è di valutare la capacità di Canakinumab di ridurre il dosaggio della terapia steroidea

Parte II (doppio cieco): si tratta di uno Studio randomizzato, in doppio cieco, controllato verso placebo, sulla prevenzione delle riacutizzazioni con Canakinumab in pazienti con AIG ad esordio sistemico con manifestazioni sistemiche attive, il cui scopo principale è quello di dimostrare l'efficacia sostenuta nel tempo sulla prevenzione delle riacutizzazioni.

Terzo studio

Studio di estensione in aperto di Canakinumab in pazienti con AIG ad esordio sistemico con manifestazioni sistemiche attive, il cui scopo è quello di raccogliere ulteriori dati sulla sicurezza ed efficacia a lungo termine di Canakinumab nell'artrite idiopatica sistemica giovanile.

3.4 Pazienti e metodi

Nell'ambito dello studio multicentrico presso il Settore di Reumatologia del Dipartimento di Pediatria dell'Università Federico II di Napoli sono stati identificati 4 pazienti affetti da AIG ad esordio sistemico, 2 maschi e 2 femmine, ricoverati in regime di Day Hospital.

Nei 4 pazienti era stata posta diagnosi di artrite idiopatica giovanile ad esordio sistemico secondo i criteri ILAR (9):

- artrite in una o piu` articolazioni con o preceduta da febbre della durata di almeno due settimane e la cui presenza sia documentata almeno per 3 giorni consecutivi con i seguenti segni:
  - rash eritematoso non fisso ed evanescente
  - ingrossamento generalizzato dei linfonodi
  - epatomegalia e/o splenomegalia
  - sierosite.

I 4 paziente con diagnosi di AIG sistemica sono stati inseriti nello studio fase III per la valutazione di efficacia e sicurezza del Canakinumab, un anticorpo monoclonale completamente umano anti- IL-1β.

Il farmaco è stato somministrato per via sottocutanea alla dose di 4 mg/kg ogni 4 settimane con dose massima singola di 300 mg.

La valutazione di sicurezza e tollerabilità comprende il monitoraggio regolare di tutti gli eventi avversi seri e non seri, il monitoraggio dei segni vitali (peso corporeo, altezza, temperatura corporea, pressione arteriosa, polso), nonché l'esame obiettivo completo con particolare attenzione a valutazione clinica di linfoadenomegalia, rash cutaneo, epatosplenomegalia, segni di sierositi, esame articolare con valutazione del numero delle articolazioni con artrite attiva e numero delle articolazioni con limitazione funzionale, l'esecuzione di ECG, esami di laboratorio ematologici, biochimici ed esame delle urine, attività della malattia secondo la valutazione globale del medico su scala VAS 0-100 mm;stato di benessere generale del paziente secondo valutazione globale del genitore o del paziente su scala VAS 0-100 mm e abilità funzionale valutata con il CHAQ.

Sono stati inoltre valutati la tollerabilità locale nella sede d'iniezione, l'insorgenza di infezioni e l'immunogenicità.

In tutti i pazienti è stata praticata analisi molecolare per ricerca mutazioni in CIAS 1. Il test genetico, veniva

praticato presso laboratori di genetica molecolare esterni alla struttura.

L'analisi molecolare si basava su DNA estratto da leucociti di sangue periferico. Il metodo di indagine utilizzato era il sequenziamento nucleotidico diretto di prodotti di amplificazione del DNA tramite PCR: per le criopirinopatie l'analisi riguardava i cinque amplimeri del gene CIAS 1 che, sovrapponendosi l'uno all'altro, coprono l'intero esone 3 e le sue sequenze introniche fiancheggianti.

3.5 Risultati preliminari

Paziente 1

Sesso maschile, età 10 aa e 4 mesi, con diagnosi di AIGs, che è stata posta all'età di 2 aa, il quadro clinico era caratterizzato da febbre persistente, rash maculo-papulare, orticarioide rosa salmone a tronco, addome ed arti, artromialgie e successivamente artrite, indici di flogosi elevati. Successivamente l'interessamento articolare si è esteso alle articolazioni di ginocchia, caviglie, gomito sin, spalla dx, polsi e MCF.

Per quanto riguarda il trattamento terapeutico, il paziente nel corso della sua storia ha assunto FANS, steroidi (metilprednisolone in bolo, poi metilprednisone per os), ciclosporina, talidomide e methotrexate, senza sostanziale beneficio.

A fine agosto 2009, nonostante terapia in atto con Indoxen (50 mg/die) e Methotrexate (10 mg/sett.), veniva riferita persistenza della febbre (TC>38°C, fino a 39°C) 2,3 gg a settimana e del rash cutaneo quotidiano, comparsa di artrite bilaterale dei polsi, delle metacarpofalangee, della caviglia destra, presenza di epatosplenomegalia e linfoadenomegalia,

inoltre gli indici di flogosi erano persistentemente aumentati, per cui ad ottobre 2009 è stato inserito nel trial clinico di fase III con Canakinumab.

Dopo la prima somministrazione di Canakinumab al dosaggio di 4 mg/kg si è assistito a netto miglioramento della sintomatologia, in particolare a rapida risoluzione della febbre e del rash cutaneo, a progressiva risoluzione della linfoadenomegalia e della epatosplenomegalia, progressivo miglioramento dell'artrite a livello di polsi, gomito sin, caviglia dx e spalla dx. Inoltre, si è asssistito a progressiva riduzione fino a negativizzazione degli indici di flogosi (PCR).

Al termine del primo studio, a gennaio 2010 il paziente è stato inserito nel secondo studio, uno studio randomizzato, in doppio cieco, controllato verso placebo, sulla prevenzione delle riacutizzazioni con Canakinumab in pazienti con AIG ad esordio sistemico con manifestazioni sistemiche attive, il cui scopo principale è quello di dimostrare l'efficacia sostenuta nel tempo sulla prevenzione delle riacutizzazioni. Durante questa fase del trial clinico al paziente è stato somministrato placebo e si è assistito a progressivo anche se lento peggioramento con ricomparsa febbre, del rash

cutaneo e dell'artrite a livello di polsi, gomito sin, caviglia dx e spalla dx e a modico aumento della PCR.

A settembre 2010 iniziava il terzo studio di estensione in aperto di Canakinumab in pazienti con AIG ad esordio sistemico con manifestazioni sistemiche attive, il cui scopo è quello di raccogliere ulteriori dati sulla sicurezza ed efficacia a lungo termine di Canakinumab nell'artrite idiopatica sistemica giovanile. Durante questa fase dello studio veniva nuovamente somministrato Cnakinumab con completa remissione della sintomatologia clinica e pronta negativizzazione della PCR.

Paziente 2

Sesso maschile, Età 11 aa e 2 mesi , con diagnosi di AIGs, che è stata posta all'età di 9 aa e 11 mesi, il quadro clinico era caratterizzato da febbre persistente, rash maculo-papulare, morbilliforme a tronco, addome, arti superiori, arti inferiori e volto, linfadenopatia laterocervicale, epatomegalia, artromialgie (algie al calcagno dx e ginocchio sin) e successivamente artrite ginocchio sin, indici di flogosi elevati con sviluppo di Sindrome da attivazione macrofagica

(MAS) confermata da aumento della ferritina, dell'LDH e dei trigliceridi plasmatici trattata con steroidi e ciclosporina.

Per quanto riguarda il trattamento terapeutico, il paziente nel corso della sua storia ha assunto indometacina, formistin, steroidi (metilprednisolone in bolo, desametasone in bolo, prednisone per os), ciclosporina, senza sostanziale beneficio.

A dicembre 2012, nonostante terapia in atto con Indoxen (150 mg/die) e ciclosporina (150 mg/die), veniva riferita persistenza della febbre, del rash cutaneo quotidiano, di artrite ginocchio sin presenza di epatomegalia e linfoadenomegalia, inoltre gli indici di flogosi erano persistentemente aumentati, per cui a dicembre 2013 veniva arruolato nel trial clinico di fase III con Canakinumab.

Dopo la prima somministrazione di Canakinumab al dosaggio di 4 mg/kg si è assistito a netto miglioramento della sintomatologia, in particolare a rapida risoluzione della febbre e del rash cutaneo, a progressiva risoluzione della linfoadenomegalia e della epatomegalia, progressivo miglioramento dell'artrite a livello del ginocchio sin, ed inoltre, si è assistito a progressiva riduzione fino a negativizzazione degli indici di flogosi (PCR).

Successivamente è stato arruolato nel secondo studio del trial clinico di fase III del Canakinumab.

Durante questa fase del trial clinico al paziente è stato somministrato il farmaco e si è assistito durante tutta la durata dello studio a mantenimento della completa remissione della sintomatologia clinica e indici di flogosi costantemente negativi.

Attualmente è stato da poco arruolato nel terzo studio del trial clinico di fase III del Canakinumab.

Paziente 3

Sesso femminile, età 10 aa e 8 mesi, con diagnosi di AIGs, che è stata posta all'età di 3 aa e 5 mesi, il quadro clinico era caratterizzato da febbre persistente, rash maculo-papulare orticarioide diffuso, artromialgie e successivamente artrite delle articolazioni metacarpofalangee della mano sinistra, epatosplenomegalia, linfoadenomegalia ed indici di flogosi elevati. Successivamente l'interessamento articolare si è esteso alle articolazioni dei polsi, alle metacarpofalangee della mano destra e del gomito dx.

Per quanto riguarda il trattamento terapeutico, la paziente nel corso della sua storia ha assunto FANS, steroidi,

indometacina, methotrexate con scarsa risposta clinico-laboratoristica.

A ottobre 2012, nonostante terapia in atto con steroidi e metotrexate veniva riferita persistenza della febbre e del rash cutaneo, comparsa di artrite alle mani e al gomito dx, inoltre gli indici di flogosi erano persistentemente aumentati, per cui veniva arruolata nel trial clinico di fase III con Canakinumab.

Dopo pochi giorni dalla prima somministrazione del Canakinumab al dosaggio di 4 mg/kg si è assistito a rapido ed assai significativo miglioramento del quadro clinico e bioumorale con netta riduzione degli indici di flogosi fino a negativizzazione della PCR e quadro clinico nettamente migliorato (scomparsa di febbre, rash cutaneo, epatosplenomegalia e sintomi articolari).

Successivamente è stata arruolata nel secondo studio del trial clinico di fase III del Canakinumab.

Durante questa fase del trial clinico alla paziente è stato somministrato il farmaco e si è assistito durante tutta la durata a mantenimento della completa remissione della sintomatologia clinica e indici di flogosi costantemente negativi.

Da luglio 2013 la paziente è entrato nel terzo studio di estensione in aperto di Canakinumab in pazienti con AIG ad esordio sistemico con manifestazioni sistemiche attive e continua a presentare remissione della sintomatologia clinica ed indici di flogosi negativi.

Paziente 4

Sesso femminile, età 4 aa e 5 mesi, con diagnosi di AIGs, che è stata posta all'età di 3 aa e 4 mesi, il quadro clinico era caratterizzato da febbre persistente, rash maculo-papulare agli arti inferiori, epatosplenomegalia, artromialgie (arti inferiori) e successivamente artrite ginocchio sin e caviglie, indici di flogosi elevati.

Per quanto riguarda il trattamento terapeutico, la paziente nel corso della sua storia ha assunto indometacina, FANS, steroidi (metilprednisolone in bolo, prednisone e desametasone per os), senza sostanziale beneficio.

A giugno 2013, nonostante terapia in atto con steroidi veniva riferita persistenza della febbre e indici di flogosi persistentemente aumentati, per cui veniva arruolata nel trial clinico di fase III con Canakinumab.

Dopo pochi giorni dalla prima somministrazione di Canakinumab al dosaggio di 4 mg/kg si è assistito a rapido ed assai significativo miglioramento del quadro clinico e bioumorale, in particolare a rapida risoluzione della febbre e del rash cutaneo, a progressiva risoluzione della epatosplenomegalia, progressivo miglioramento dell'artrite. Inoltre, si è asssistito a progressiva riduzione fino a negativizzazione degli indici di flogosi (PCR). Al termine del primo studio, della durata di 4 settimane, a luglio 2013 veniva inserita nel secondo studio, il cui scopo principale è quello di dimostrare l'efficacia sostenuta nel tempo sulla prevenzione delle riacutizzazioni. Durante questa fase del trial clinico alla paziente è stato somministrato Canakinumab e si è assistito a mantenimento di una condizione di remissione della patologia sia da un punto di vista clinico che bioumorale.
Attualmente è stata da poco arruolata nel terzo studio del trial clinico di fase III del Canakinumab.

Nei 4 pazienti è stata praticata analisi molecolare per ricerca mutazioni in CIAS 1 risultata negativa.

3.6 Dati preliminari sulla sicurezza

Nei pazienti arruolati presso il nostro Centro non si sono verificati casi di sindrome da attivazione macrofagica (MAS) durante il trattamento (1 paziente aveva sviluppato la MAS precedentemente).

In 2/4 pazienti si sono riscontrate infezioni non gravi durante il trattamento con Cnakinumab (Infiammazione delle prime vie aeree).

3.7 Risultati

Lo studio ha permesso di identificare un gruppo di pazienti affetti da AIGs responsivi alla terapia con anti IL1-β (Canakinumab), questo gruppo di pazienti presenta caratteristiche cliniche distintive rispetto agli altri gruppi: ovvero si tratta di pazienti che presentano prevalentemente manifestazioni sistemiche rispetto all'interessamento articolare.

L'utilizzo della terapia con anti IL1-β ha permesso di ridurre il dosaggio della terapia steroidea fino alla completa sospensione in tutti i pazienti fino ad allora cortico-dipendenti con conseguente riduizione degli effetti collaterali della stessa, inoltre, ha permesso di migliorare la compliance alla terapia considerando che la somministrazione del Canakinumab si effettua ogni 4 settimane.

3.8 Conclusioni

Dalla dimostrazione dell'efficacia della terapia anti IL 1-β nei pazienti affetti da AIGs si sta cercando di risalire ai meccanismi patogenetici che sono alla base di queste patologie auoimmuni. Sono necessari ulteriori studi per migliorare la comprensione del ruolo dell'inflammasoma nelle patologie autoimmuni per poter offrire suggerimenti per i futuri indirizzi di ricerca.

Ulteriori progressi in questo campo di ricerca potrebbero aprire nuove strategie nel trattamento delle malattie autoimmuni e delle loro complicanze.

REFERENZE

1. Ravelli A, Martini A. Juvenile idiopathic arthritis. Lancet. 2007 Mar 3;369(9563):767-78.
2. Pascual V, Allantaz F, Arce E, et al. Role of interleukin-1 (IL-1) in the pathogenesis of systemic onset juvenile idiopathic arthritis and clinical response to IL-1 blockade. J Exp Med 2005;201:1479-86.
3. Lequerré T, Quartier P, Rosellini D, et al. Interleukin-1 receptor antagonist (anakinra) treatment in patients with systemic-onset juvenile idiopathic arthritis or adult onset Still disease: preliminary experience in France. Ann Rheum Dis 2008;67:302-8.
4. Gattorno M, Piccini A, Lasigliè D, et al. The pattern of response to anti-interleukin-1 treatment distinguishes two subsets of patients with systemic-onset juvenile idiopathic arthritis. Arthritis Rheum 2008;58:1505-15.
5. Gattorno M, Martini A. Inherited autoinflammatory syndromes: an expanding new group of chronic inflammatory diseases. Clin Exp Rheumatol 2005;23:133-6.

6. de Benedetti F, Martini A. Is systemic juvenile rheumatoid arthritis an interleukin 6 mediated disease? J Rheumatol 1998;25:203-7.
7. de Benedetti F, Martini A. Targeting the interleukin-6 receptor: a new treatment for systemic juvenile idiopathic arthritis? Arthritis Rheum 2005;52:687-93.
8. Hoffman HM, Patel DD. Genomic-based therapy:targeting interleukin-1 for autoinflammatory diseases. Arthritis Rheum 2004;50:345-9.
9. Petty RE, Southwood TR, Manners P, et al. International League of Associations For Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001 J Rheumatol 2004;3:390-92.

CAPITOLO 4

4.1 Proposta di modifica dell'attuale classificazione dell'Artrite Idiopatica Giovanile

L'artrite Idiopatica Giovanile (AIG) è una condizione clinica eterogenea, che raggruppa tutte le forme di artrite che esordiscono prima dei 16 anni, persistono per almeno 6 settimane e hanno eziologia sconosciuta [1]. Sono stati proposti diversi sistemi di classificazione dell'AIG, nessuno dei quali è, tuttavia, universalmente accettato [2-4]. Il più recente è stato messo a punto dalla International League of Associations for Rheumatology (ILAR) nel 2001 e ha lo scopo principale di identificare gruppi omogenei di pazienti, al fine di facilitare l'esecuzione di studi genetici, ezipatogenetici, prognostici e terapeutici [4]. La classificazione ILAR riconosce le seguenti 7 categorie di malattia sulla base delle caratteristiche presenti nei primi 6 mesi di malattia: artrite sistemica, oligoartrite (suddivisa in persistente ed estesa), poliartrite fattore reumatoide (FR)-negativa, poliartrite FR-positiva, artrite psoriasica, artrite

associata ad entesite (ERA) e artrite indifferenziata. La categoria Oligoartrite è siddivisa in 2 sottogruppi: Oligoartrite persistente che interessa non più di 4 articolazioni durante tutto il decorso della malattia ed Oligoartrite estesa che interessa più di 4 articolazioni dopo i primi sei mesi di malattia.

Questa classificazione è stata recentemente sottoposta a varie critiche.

Un'osservazione frequente riguarda il numero di pazienti che finiscono con l'essere classificati nella categoria "altre artriti".

Numerosi lavori sono stati indirizzati a valutare la validità dei criteri di classificazione.

Sulla base dei risultati ottenuti alcuni autori hanno proposto modifiche all'attuale classificazione, allo scopo di diminuire il numero di pazienti che rimangono non classificati, pur garantendo omogeneità all'interno delle singole categorie AIG. In particolare, è stato suggerito che l'utilizzo di parametri come il numero di articolazioni colpite o la presenza di psoriasi non identifichi gruppi di pazienti effettivamente omogenei. E' stato inoltre ipotizzato che la presenza di anticorpi antinucleo (ANA) consenta di definire

un gruppo di pazienti dotati di caratteristiche omogenee, ma inseriti, nella classificazione vigente, in forme cliniche differenti [5-13].

In un lavoro del 2003, veniva ipotizzato, sulla base dei dati in letteratura, che un gruppo di pazienti apparentemente omogeneo, caratterizzato dalla presenza di anticorpi antinucleo (ANA), esordio precoce della malattia, forte predominanza del sesso femminile, prevalenza di artrite asimmetrica, e rischio di iridociclite, veniva classificata in 3 categorie differenti di AIG (Oligoartrite, Poliartrite FR-negativa e artrite psoriasica) e che il numero di articolazioni colpite nei primi 6 mesi di malattia come pure la presenza di psoriasi non rappresentano criteri utili per identificare entità patologiche omogenee nell'AIG [14]. Questa ipotesi è stata sostenuta da uno studio successivo, in cui è stato mostrato che i pazienti ANA-positivi raggruppati nelle categorie di Oligoartrite persistente, oligoartrite estesa e poliartrite FR-negativo erano molto simili in termini di età di esordio della malattia, rapporto maschio/femmina, frequenza di artrite asimmetrica e di iridociclite [15].

Lo scopo del nostro studio è stato quello di indagare ulteriormente il ruolo della positività degli ANA per

identificare un potenziale sottoinsieme omogeneo di malattia, esaminando una popolazione di pazienti molto più grande. Inoltre, abbiamo esteso l'analisi alle categorie ILAR artrite psoriasica e artrite indifferenziata per confermare l'ipotesi che i pazienti ANA-positivi, inclusi in queste categorie presentano le stesse caratteristiche omogenee documentate nei pazienti ANA-positivi con oligoartrite e poliartrite FR-negativo.

4.2 Antinuclear Antibody–Positive Patients Should Be Grouped as a Separate Category in the Classification of Juvenile Idiopathic Arthritis

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Antinuclear Antibody–Positive Patients Should Be Grouped as a Separate Category in the Classification of Juvenile Idiopathic Arthritis

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Objective. **We undertook this study to test the hypothesis that in the International League of Associations for Rheumatology (ILAR) classification of juvenile idiopathic arthritis (JIA), patients with similar characteristics can be classified into different categories. We sought to investigate whether antinuclear antibody (ANA)–positive patients having disease in the ILAR categories of oligoarthritis, rheumatoid factor–negative polyarthritis, psoriatic arthritis, and undifferentiated arthritis share homogeneous features and to compare these features with those of ANA-negative patients having the same categories of disease.**

Methods. **We identified JIA patients who had been followed up during a 22-year period. ANA positivity was defined as ≥2 positive results at a titer of ≥1:160. Demographic and clinical features were recorded retrospectively and compared between ANA-positive and ANA-negative patients.**

Results. **Of a total of 971 patients, 711 were ANA positive, 149 were ANA negative, and 111 had an indeterminate ANA status. Patients with indeterminate ANA status were excluded. ANA-positive patients in the different ILAR categories were similar in terms of age at disease presentation, female-to-male ratio, and frequency of asymmetric arthritis and iridocyclitis. Compared with ANA-positive patients, the ANA-negative group was older at disease presentation and had a lower prevalence of females, a lower frequency of iridocyclitis and asymmetric arthritis, a greater number of affected joints over time, and a different pattern of arthritis. The close relationship between the presence of ANAs and younger age at disease presentation, female predominance, asymmetric arthritis, development of iridocyclitis, lower number of affected joints over time, and lack of hip involvement was also confirmed by multivariate and multiple correspondence analysis.**

Conclusion. **Our findings substantiate the hypothesis that ANA-positive patients classified into different JIA categories by current ILAR criteria constitute a homogeneous patient population.**

Juvenile idiopathic arthritis (JIA) is an umbrella term that encompasses all forms of arthritis that begin before the age of 16 years, persist for more than 6 weeks, and are of unknown cause (1). Over the years, several classification systems have been developed, none of which has been universally embraced (2–4). The most recent one has been proposed by the International League of Associations for Rheumatology (ILAR) (4) and is primarily aimed at identifying homogeneous disease groups to facilitate research on etiopathogenesis and epidemiology, outcome studies, and treatment trials. The ILAR classification recognizes the following 7 disease categories on the basis of features present in the

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first 6 months of illness: systemic arthritis, rheumatoid factor (RF)–positive polyarthritis, RF-negative polyarthritis, oligoarthritis, psoriatic arthritis, enthesitis-related arthritis, and undifferentiated arthritis. The oligoarthritis category is divided into 2 subsets: persistent oligoarthritis, in which arthritis remains confined to ≤4 joints throughout the whole disease course, and extended oligoarthritis, in which arthritis extends to >4 joints after the first 6 months of illness.

The ILAR classification has the merit of having resolved the previous disparity in terminology between Europe and North America. However, it has the limitation of not being data-driven, but rather based on expert consensus. Nonetheless, it has been recommended that the current classification system be viewed as "a work in progress," and pediatric rheumatologists have been urged to participate in the process by making their opinions known and by testing the proposed criteria in their patient series (5).

In recent years, several investigators have evaluated the ILAR criteria and offered numerous suggestions for revision (6–14). In 2003, one of us hypothesized, based on the data in the literature, that a seemingly homogeneous patient group characterized by the presence of antinuclear antibodies (ANAs), early onset of disease, strong predominance of females, prevalence of asymmetric arthritis, and risk for iridocyclitis was classified into 3 different JIA categories (oligoarthritis, RF-negative polyarthritis, and psoriatic arthritis) and that the number of affected joints in the first 6 months of disease as well as the presence of psoriasis did not represent useful criteria to identify homogeneous disease entities in JIA (15). This hypothesis was supported by a subsequent study, in which we showed that ANA-positive patients grouped in the categories of persistent oligoarthritis, extended oligoarthritis, and RF-negative polyarthritis were very similar in terms of age at disease presentation, female-to-male ratio, and frequency of asymmetric arthritis and iridocyclitis (16).

In the present study, we investigated further the role of ANA in identifying a potentially homogeneous disease subset by examining a much larger patient population. Furthermore, we extended the analysis to the ILAR categories of psoriatic arthritis and undifferentiated arthritis to confirm the hypothesis that the ANA-positive patients included in these categories share the same homogeneous features documented in ANA-positive patients with oligoarthritis and RF-negative polyarthritis.

PATIENTS AND METHODS

Patient selection. This was a retrospective cohort study involving patients who were followed up at the Fondazione Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico San Matteo of Pavia or at the IRCCS G. Gaslini of Genoa between January 1987 and December 2008. Inclusion criteria were a diagnosis of JIA based on the 2001 revised ILAR criteria (4) and a disease category of persistent oligoarthritis, extended oligoarthritis, RF-negative polyarthritis, psoriatic arthritis, or undifferentiated arthritis. Patients were excluded if they met the ILAR criteria for systemic arthritis, enthesitis-related arthritis, or RF-positive polyarthritis because these ILAR categories represent well-defined and separate disease entities.

Included and excluded patients had their ILAR category classified independently by 2 investigators (SO and AMag) based on the review of clinical charts. Discordances were resolved, after discussion, by consensus on the final diagnosis among the 2 investigators and the principal investigator of the study (AR). All patients seen before the publication of ILAR criteria for JIA were reclassified using such criteria. For the sake of uniformity, patients with oligoarthritis were subclassified in the persistent or extended subtype on the basis of the course of joint disease in the first 24 months after disease presentation. The study was approved by the Ethics Committee of the IRCCS G. Gaslini of Genoa.

Classification of ANA status. Patients had their ANA status defined if they had at least 2 ANA determinations made at least 3 months apart during followup. Our gathering of this information was facilitated by our policy of repeating ANA determinations several times, generally every 6–12 months, in patients with the JIA categories under study. Patients were defined as being ANA positive if they had at least 2 positive results on indirect immunofluorescence at a titer of ≥1:160 and as ANA-negative if they had negative results in all determinations made during the entire followup period. The substrate used for the ANA determinations was rat liver during the first 2 years of the study and HEp-2 cells thereafter. ANA positivity or negativity was confirmed in at least 2 HEp-2 cell assays in all patients for whom the initial assays were done using rat liver as substrate. Patients who had only 1 ANA determination available or who had ≥2 ANA determinations but did not meet the criteria for ANA positivity or negativity were classified as having an "indeterminate" ANA status.

Clinical assessments. Patients were identified through existing databases and/or clinic files. The medical charts were reviewed for the following information: sex, age at disease presentation, duration from disease presentation until last followup visit, occurrence of iridocyclitis, presence of HLA–B27, asymmetry of arthritis at disease presentation and 6 months after presentation, number and type of joints involved in the first 6, 12, and 24 months after disease presentation, and development of radiographic joint lesions (defined as the presence of joint space narrowing and/or bone erosions in at least 1 joint).

The date of disease presentation was defined as the date when the first symptoms of arthritis were noted, obtained by history as recorded in medical charts. We routinely perform and record on a standardized form a detailed joint assessment in each patient at each clinic visit, which facilitated the

gathering of information about joint involvement at specific time intervals. All joint assessments were performed by 3 investigators (AR, SM-M, and Stefania Viola, Genoa, Italy), who used the same methodology. Arthritis was defined as symmetric if >50% of the joints involved during the first 6 months of disease were symmetric pairs. This definition of symmetry was adapted from that used in adults with rheumatoid and psoriatic arthritis, which requires that at least 50% of involved joints be symmetric pairs (17). Eye examinations were performed every 3–4 months in ANA-positive patients and every 6–12 months in ANA-negative patients. Diagnosis of uveitis was confirmed by an ophthalmologist in all patients who developed this complication. All eye examinations performed in the study centers were performed by the same ophthalmologist (Dr. A. M. Broglia in Pavia and Dr. R. De Marco in Genoa) throughout the entire study period.

Clinical data for each patient were collected by investigators using a standardized form developed specifically for this study. Data were then entered into an electronic database.

Statistical analysis. Descriptive statistics were reported as medians and interquartile ranges for continuous variables and as absolute frequencies and percentages for categorical variables. Comparisons of quantitative variables between 2 groups were made by means Mann-Whitney U test, whereas comparisons of quantitative variables between more than 2 groups were made by nonparametric analysis of variance (Kruskal-Wallis test). Dunn's test was chosen as an a posteriori test to assess the statistical significance of differences between pairs of groups. Categorical data were compared by chi-square test, or by Fisher's exact test in case of expected frequencies <5. Bonferroni adjustment was applied as a correction for multiple comparisons to explore post hoc differences between pairs of groups.

Multiple logistic regression analysis was performed, entering explanatory variables that showed significant results in univariate tests ($P < 0.05$) or were considered a priori to be of foremost importance for the study outcome, with ANA positivity as the outcome variable. Cases with missing variables were excluded from the analysis. Before the application of logistic regression procedures, some continuous variables were dichotomized to binary variables. For age at disease presentation, the cut points chosen were ≤6 years and >6 years. Cut points for the cumulative number of joints affected at 6, 12, and 24 months were obtained through receiver operating characteristic (ROC) curve analysis. The step-down strategy of analysis was chosen; this consists of examining the effect of removing variables from the saturated model. Possible explanatory variables assessed were sex, age at disease presentation, symmetric arthritis at disease presentation and at 6 months, iridocyclitis, cumulative number of affected joints at 6, 12, and 24 months, radiographic changes, and type of joints involved in the first 24 months after disease presentation (shoulder, elbow, wrist, metacarpophalangeal, proximal interphalangeal, hip, knee, ankle, foot, and finger joints). The effect was expressed in terms of odds ratios, and 95% confidence intervals were calculated; statistical significance was tested by likelihood ratio test. The area under the ROC of the best-fitting model was used as an indicator of the predictive ability of the model.

The association between categorical variables was further explored by multiple correspondence analysis (18). Multiple correspondence analysis is an exploratory analysis and descriptive technique that enables analysis of contingency tables with a large number of variables, considering measures of correspondence between rows and columns. It can be considered a variant of factor analysis, but it differs because it evaluates the relationship between categorical data organized in contingency tables, rather than continuous data. Essentially, multiple correspondence analysis converts a nonnegative data matrix into a kind of graphic representation that allows studying the relationship between the categories in a multiway contingency table. The importance of the categories of variables for constructing each axis is measured by their absolute contribution and aids in the interpretation of the axis. Categories with high absolute contribution show greater importance in the factor's formation. The relative contribution provides information as to how much of the category's variability is explained by the axis. Graphic analysis of association of variables is performed by considering their geometric proximity and the separation of categories by quadrants, because the closer the variables, the more interrelated they are, and those separated by quadrants display groups with opposite profiles. The variables that proved statistically significant in multivariate analysis were used in the multiple correspondence analysis. Only patients for whom complete information was available were included.

All statistical tests were 2-sided; P values less than 0.05 were considered significant. The statistical packages used were Statistica (version 8.0; StatSoft) for univariate analyses and Stata release 11 (StataCorp) for multivariate and cluster analysis. For multiple correspondence analysis, the software XLSTAT, 6.1.9 (Addinsoft) was used.

RESULTS

A total of 1,219 patients fulfilled the ILAR criteria for JIA. The ILAR category was systemic arthritis in 149 patients (12.2%), oligoarthritis in 649 patients (53.2%), RF-positive polyarthritis in 26 patients (2.1%), RF-negative polyarthritis in 223 patients (18.3%), enthesitis-related arthritis in 73 patients (6.0%), psoriatic arthritis in 37 patients (3.0%), and undifferentiated arthritis in 62 patients (5.1%). Of the 649 patients with oligoarthritis, 433 had the persistent subtype and 195 had the extended subtype; 21 patients could not be subclassified as having the persistent or extended subtype due to a disease duration of <6 months. For the above-mentioned reasons, patients with systemic arthritis, RF-positive polyarthritis, and enthesitis-related arthritis were excluded from the study. The remaining 971 patients in the ILAR categories of oligoarthritis, RF-negative polyarthritis, psoriatic arthritis, and undifferentiated arthritis were combined and classified according to their ANA status, as follows: 711 (73.2%) were ANA positive, 149 (15.3%) were ANA negative, and 111 (11.4%) had an indeterminate ANA status. Patients with an indeterminate ANA status were excluded from the

Table 1. Main demographic and clinical features, cumulative joint involvement over time, and frequency of involvement of specific joints in the first 24 months after disease presentation in the 860 patients with juvenile idiopathic arthritis, according to ANA status*

	ANA positive (n = 711)	ANA negative (n = 149)	P†
Female sex	579/711 (81.43)	93/149 (62.42)	<0.0001
Age at disease presentation, median (IQR) years	2.7 (1.7–5.2)	6.3 (3.6–9.7)	<0.0001‡
Age ≤6 years at disease presentation	564/711 (79.32)	72/149 (48.32)	<0.0001
Iridocyclitis ever	186/709 (26.23)	4/149 (2.68)	<0.0001
Presence of HLA–B27	18/477 (3.8)	11/121 (9.1)	0.015§
Asymmetric arthritis at disease presentation	605/696 (86.93)	94/136 (69.12)	<0.0001
Asymmetric arthritis at 6 months	538/682 (78.65)	80/136 (58.82)	<0.0001
Cumulative no. of joints affected at 6 months, median (IQR)	2.0 (1.0–4.0)	3.0 (1.0–9.0)	0.0026‡
Cumulative no. of joints affected at 12 months, median (IQR)	3.0 (2.0–5.0)	4.0 (2.0–13.5)	0.0005‡
Cumulative no. of joints affected at 24 months, median (IQR)	3.0 (2.0–7.0)	5.0 (2.0–16.0)	0.0004‡
Joints affected in the first 24 months			
Shoulder	25/706 (3.54)	20/148 (13.51)	<0.0001
Elbow	124/706 (17.56)	39/148 (26.35)	0.013
Wrist	175/707 (24.75)	70/148 (47.30)	<0.0001
Metacarpophalangeal	137/706 (19.41)	46/148 (31.08)	0.002
Proximal interphalangeal	210/704 (29.83)	61/148 (41.22)	0.007
Hip	45/705 (6.38)	44/148 (29.73)	<0.0001
Knee	634/707 (89.67)	120/148 (81.08)	0.003
Ankle	420/707 (59.41)	85/148 (57.43)	0.65
Metatarsophalangeal	65/704 (9.23)	15/148 (10.14)	0.73
Foot interphalangeal	81/706 (11.47)	18/148 (12.16)	0.81
Presence of radiographic joint changes	98/496 (19.8)	53/133 (39.8)	<0.0001§
Disease duration at last followup visit, median (IQR) years	5.0 (2.6–8.4)	5.1 (1.8–9.4)	0.45‡

* Except where indicated otherwise, values are the number/number tested (%). ANA = antinuclear antibody; IQR = interquartile range.
† By chi-square test unless otherwise specified.
‡ By Mann-Whitney U test.
§ By Fisher's exact test.

analysis. The number of ANA determinations per patient in the 860 patients who had the ANA status specified ranged from 2 to 20 (mean 5.4); the total number of determinations was 4,610. More than 90% of patients were of Italian ancestry.

Table 1 shows the comparison of demographic and clinical features, cumulative joint involvement over time, and frequency of involvement of specific joints in the first 24 months after disease presentation between ANA-positive and ANA-negative patients. The 2 patient groups were significantly different for all features, except for the frequency of involvement of ankle and small foot joints. Compared with the ANA-negative group, the ANA-positive patients had a higher proportion of females, a younger age at disease presentation, a greater prevalence of iridocyclitis and of asymmetric arthritis at disease presentation and in the first 6 months, a lower frequency of HLA–B27, a lower number of affected joints over time, a lower frequency of involvement of the shoulder, elbow, wrist, small hand joints, and hip, a greater frequency of involvement of the knee, and a lower frequency of radiographic joint changes. The duration between disease presentation and last followup visit was comparable in the 2 patient populations.

The comparison of the main demographic and clinical features of ANA-positive and ANA-negative patients by ILAR category is presented in Table 2. Overall, the differences noted when the 2 patient groups were considered as a whole were paralleled by their comparison within each category. The sole exception was the similar frequency of asymmetric arthritis in ANA-positive and ANA-negative patients with persistent oligoarthritis and psoriatic arthritis. ANA-positive patients with psoriatic arthritis and undifferentiated arthritis tended to be less often female and to be older at disease presentation than ANA-positive patients with oligoarthritis (either persistent or extended) and RF-negative polyarthritis. In the categories extended oligoarthritis, RF-negative polyarthritis, and undifferentiated arthritis, the cumulative number of joints affected in the first 24 months after disease presentation was lower in ANA-positive patients than in ANA-negative patients. The cumulative number of joints involved over time was comparable between ANA-positive and ANA-negative patients in the categories of persistent oligoarthritis and psoriatic arthritis.

Compared with their ANA-negative counterparts, ANA-positive patients in all categories had a lower frequency of involvement of the wrist, small hand joints, and hip, and a greater frequency of involvement of the

Table 2. Main demographic and clinical features by ILAR category and ANA status*

	Persistent oligoarthritis		Extended oligoarthritis		RF-negative polyarthritis		Psoriatic arthritis		Undifferentiated arthritis	
	ANA+ (n = 326)	ANA− (n = 56)	ANA+ (n = 160)	ANA− (n = 12)	ANA+ (n = 148)	ANA− (n = 49)	ANA+ (n = 25)	ANA− (n = 8)	ANA+ (n = 36)	ANA− (n = 19)
Female sex	263/326 (82.2)†	34/56 (60.7)	138/160 (86.2)	8/12 (66.67)	119/148 (80.4)	33/49 (67.3)	17/25 (68.0)‡	2/8 (25.0)	24/36 (66.7)	11/19 (57.9)
Age at disease presentation, median (IQR) years	2.8 (1.9–5.0)§	5.4 (2.9–9.0)	2.5 (1.6–4.6)†	6.8 (4.1–9.0)	2.4 (1.6–6.1)§	6.4 (4.4–9.1)	3.5 (2.0–7.1)‡	8.3 (5.2–12.7)	3.4 (1.7–8.1)	7.4 (2.8–10.2)
Agerome tric arthritis at 6 months	274/319 (85.9)	46/52 (86.5)	120/157 (76.4)‡	5/11 (45.4)	94/148 (65.7)§	13/42 (30.9)	15/21 (71.4)	7/8 (87.5)	26/32 (78.1)¶	6/18 (33.3)
Iridocyclitis ever	74/326 (23.9)†	1/56 (1.8)	43/160 (26.9)‡	0/12 (0.0)	43/148 (29.0)†	2/49 (4.1)	7/25 (28.0)	0/8 (0.0)	9/35 (25.7)	1/19 (5.3)
Cumulative no. of joints affected at 24 months, median (IQR)	2 (1–3)	2 (1–5)	5 (3–7)¶	10 (6–20.5)	10 (7–14)§	17 (9–26)	5 (2–10)	4 (2.0–6.5)	5 (2–5)	9 (2–23)
Joints affected in the first 24 months										
Wrist	19/326 (5.8)	6/56 (10.7)	52/160 (32.5)‡	8/12 (66.7)	85/148 (57.4)†	42/49 (85.7)	11/25 (44.0)	5/8 (62.5)	7/35 (19.4)‡	9/19 (47.4)
Metacarpophalangeal	8/326 (2.4)	1/56 (1.8)	45/160 (28.1)‡	7/12 (58.3)	68/148 (45.9)	27/49 (55.1)	8/25 (66.0)	3/8 (37.5)	8/36 (22.2)	8/19 (42.1)
Proximal interphalangeal	24/326 (7.4)	5/56 (8.9)	64/158 (40.5)	7/12 (58.3)	97/148 (65.5)	38/49 (77.5)	13/25 (52.0)	2/8 (25.0)	12/36 (33.3)	9/19 (47.4)
Hip	8/326 (2.4)¶	6/56 (10.7)	14/159 (8.8)¶	5/12 (41.7)	20/148 (13.5)§	23/49 (46.9)	2/25 (8.0)	1/8 (12.5)	1/36 (2.8)§	9/19 (47.4)
Knee	296/326 (90.8)	46/56 (82.1)	146/160 (91.2)	11/12 (91.7)	137/148 (92.6)¶	33/49 (77.5)	19/25 (76.0)	4/8 (50.0)	26/36 (72.2)	17/19 (89.5)
Ankle	130/326 (39.9)	19/56 (33.9)	114/160 (71.2)	10/12 (83.3)	131/148 (88.5)	42/49 (85.7)	15/25 (52.0)	4/8 (50.0)	25/36 (69.4)	10/19 (52.6)

* Except where indicated otherwise, values are the number/number tested (%). Data on 21 patients with nonspecified oligoarticular arthritis were excluded from this table. Comparisons of quantitative data were made by Mann-Whitney U test; comparisons of frequencies were made by chi-square test (or by Fisher's exact test if expected frequencies were <5). ILAR = International League of Associations for Rheumatology; ANA = antinuclear antibody; RF = rheumatoid factor; IQR = interquartile range.
† $P < 0.001$ versus ANA-negative patients.
‡ $P < 0.05$ versus ANA-negative patients.
§ $P < 0.0001$ versus ANA-negative patients.
¶ $P < 0.01$ versus ANA-negative patients.

Table 3. Main demographic and clinical features by ILAR category*

	Persistent oligoarthritis (n = 382)	Extended oligoarthritis (n = 172)	RF-negative polyarthritis (n = 197)	Psoriatic arthritis (n = 33)	Undifferentiated arthritis (n = 55)	P†	Comparisons significant on post hoc tests‡
Female sex	302/382 (79.06)	146/172 (84.88)	152/197 (77.16)	19/33 (57.58)	35/55 (63.64)	0.0006	PsA vs. OP and OE, UA vs. OE
Age at disease presentation, median (IQR) years	4.30 (1.91–5.61)	2.72 (1.66–4.85)	4.75 (1.77–6.98)	4.52 (2.33–8.33)	4.65 (2.29–9.55)	0.0008	OE vs. PsA and UA
Asymmetric arthritis at 6 months	52/371 (14.02)	43/168 (25.60)	78/185 (42.16)	7/29 (24.14)	19/50 (38.00)	<0.0001	PO vs. OP and OE, UA vs. OE, OP vs. OE
Iridocyclitis ever	79/382 (20.68)	43/172 (25.00)	45/197 (22.84)	7/33 (21.21)	10/54 (18.52)	0.78	–
Cumulative no. of joints affected at 24 months, median (IQR)	2.0 (1.0–3.0)	5.0 (4.00–7.0)	10.0 (7.0–16.0)	5.00 (2.0–10.0)	4.00 (2.0–11.0)	<0.0001	PO vs. OP, OE, PsA, and UA; OP vs. PsA, UA, and OE
Joints affected in the first 24 months							
Wrist	25/382 (6.54)	60/172 (34.88)	127/197 (64.47)	16/33 (48.48)	16/55 (29.09)	<0.0001	PO vs. OE, OP, and UA; OP vs. UA
Metacarpophalangeal	9/382 (2.36)	52/172 (30.23)	95/197 (48.22)	11/33 (33.33)	16/55 (29.09)	<0.0001	PO vs. OP and OE, OP vs. OE
Proximal interphalangeal	29/382 (7.59)	71/170 (41.76)	135/197 (68.53)	15/33 (45.45)	21/55 (38.18)	<0.0001	PO vs. UA, OP, and OE; UA vs. OP
Hip	14/382 (3.66)	19/171 (11.11)	43/197 (21.38)	3/33 (9.09)	10/55 (18.18)	<0.0001	PO vs. OP, OP vs. OE
Knee	342/382 (89.53)	157/172 (91.28)	175/197 (88.83)	23/33 (69.70)	43/55 (78.18)	0.001	PsA vs. OP and OE
Ankle	149/382 (39.01)	124/172 (72.09)	173/197 (87.82)	17/33 (51.52)	35/55 (63.64)	<0.0001	PO vs. PsA, UA, OP, and OE; UA vs. OP; OP vs. OE

* Except where indicated otherwise, values are the number/number tested (%). Data on 21 patients with nonspecified oligoarticular arthritis were excluded from this table. Comparisons of quantitative data were made by Mann-Whitney U test; comparisons of frequencies were made by chi-square test (or by Fisher's exact test if expected frequencies were <5). ILAR = International League of Associations for Rheumatology; IQR = interquartile range.
† For overall comparisons.
‡ Pairs of comparisons that were statistically significant on post hoc tests (Bonferroni adjustment or Dunn's test). PsA = psoriatic arthritis; OP = persistent oligoarthritis; OE = extended oligoarthritis; UA = undifferentiated arthritis; PO = rheumatoid factor (RF)–negative polyarthritis.

knee. Exceptions were the comparable frequency of involvement of the small hand joints in ANA-positive and ANA-negative patients with persistent oligoarthritis, the greater frequency of involvement of the same joints in ANA-positive than in ANA-negative patients with psoriatic arthritis, the comparable frequency of knee involvement in ANA-positive and ANA-negative patients with extended oligoarthritis, and the greater frequency of knee involvement in ANA-negative than in ANA-positive patients with undifferentiated arthritis. Data regarding involvement of the distal interphalangeal joints were not available. The disease duration was comparable between ANA-positive and ANA-negative patients within each category (results not shown). The values of the same demographic and clinical features for the ANA-positive and ANA-negative patients combined for each JIA category are provided in Table 3. The comparison of these data with those in Table 2 highlights the relative homogeneity of ANA-based grouping compared with grouping according to the ILAR classification.

For the multivariate analysis, complete data were available on 785 patients. The best-fitting model obtained through logistic regression procedures, in which the presence of ANAs was the dependent variable, is presented in Table 4. Independent correlations with the presence of ANAs were identified for presence or history of iridocyclitis, female sex, age ≤6 years at disease presentation, cumulative affected joints at 12 months ≤12, presence of asymmetric arthritis at 6 months, and absence of hip joint involvement. To investigate whether use of a different cutoff for ANA positivity would lead to different results, we repeated the regression analysis by adding 74 patients who were ANA positive at low titer (i.e., who had at least 2 positive ANA determinations at a titer ≥1:40) and for whom complete information was available. Compared with the original analysis, the variables presence of wrist joint involvement and presence of metacarpophalangeal joint involvement entered the best-fitting model, whereas

Table 4. Best-fitting model obtained through logistic regression procedures*

Explanatory variable	OR (95% CI)	P†
Iridocyclitis ever	22.03 (5.09–95.29)	<0.0001
Female sex	2.67 (1.69–4.21)	<0.0001
Age ≤6 years at disease presentation	2.46 (1.61–3.77)	<0.0001
Cumulative affected joints at 12 months ≤12	3.16 (1.72–5.79)	0.0003
Asymmetric arthritis at 6 months	1.69 (1.06–2.70)	0.03
Presence of hip joint involvement	0.33 (0.19–0.57)	0.0001

* The presence of antinuclear antibodies was the dependent variable. Complete data were available on 785 patients. The area under the receiver operating characteristic curve of the model was 0.82. OR = odds ratio; 95% CI = 95% confidence interval.
† By likelihood ratio test.

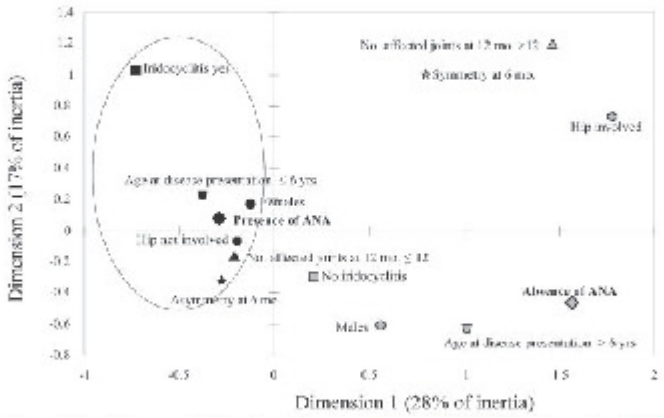

Figure 1. Graphic representation of variable categories in the first 2 dimensions of multiple correspondence analysis. ANA = antinuclear antibody.

the variable presence of asymmetric arthritis at 6 months did not (results not shown).

The association between the presence of ANA and the variables that proved statistically significant in multivariate analysis was further explored by means of multiple correspondence analysis. For the purposes of this analysis, all variables were categorized as was done in logistic regression procedures. Figure 1 depicts the 2-dimensional representation of the variables examined. This figure highlights the existence of 2 groups. One group, located in the upper and lower left quadrants, whose proximity between categories includes ANA positivity, is characterized by age at disease presentation ≤6 years, greater prevalence of females, asymmetric arthritis, lower number of affected joints over time, lack of hip involvement, and presence of iridocyclitis. A second group, located in the upper and lower right quadrants, whose proximity between categories includes ANA negativity, is characterized by greater prevalence of males, age at disease presentation >6 years, symmetric arthritis, greater number of affected joints over time, presence of hip involvement, and lack of iridocyclitis.

DISCUSSION

The results of this analysis show that ANA-positive patients classified into different disease categories (persistent oligoarthritis, extended oligoarthritis, RF-negative polyarthritis, psoriatic arthritis, and undifferentiated arthritis) by the current JIA criteria share similar characteristics (e.g., strong predominance of females, early onset of disease, asymmetric arthritis, and high risk of chronic iridocyclitis). This confirms and expands our previous findings (16) and substantiates our original hypothesis that ANA-positive patients with JIA constitute a homogeneous subgroup, irrespective of the course of joint disease and the presence of psoriatic features (15).

As compared to our former study (16), the present investigation included a much larger patient sample and extended the analysis to the psoriatic and undifferentiated arthritis categories. We found that ANA-positive patients had a lower number of affected joints over time and a lower frequency of involvement of some joints and of radiographic joint lesions than did ANA-negative patients. These differences were seen when ANA-positive and ANA-negative patients were compared either as whole groups or within the single categories. The diversities between ANA-positive and ANA-negative patients were confirmed by multivariate regression analysis and were further highlighted by the 2-dimensional scatterplot of multiple correspondence analysis.

Our findings further underscore the need to reconsider some of the JIA categories. Should the ILAR classification be used, the study patients would be placed in their respective category based on the number of joints affected over time or the presence of psoriasis or psoriatic features. As one of us previously discussed

in detail elsewhere (15), the total number of joints involved may not be an appropriate classification tool, as it may simply reflect a more rapid spread of arthritis within the same disease. Previous studies have demonstrated that the reliability of clinical examination of joints in children with JIA is poor (19). A recent analysis has shown a high prevalence of subclinical synovitis as detected by ultrasound in children with JIA. Some patients who were labeled as having oligoarthritis or who were found to have no synovitis on clinical evaluation were subsequently determined with the use of ultrasound to have polyarthritis (20).

The heterogeneity of RF-negative polyarthritis has been highlighted recently by gene expression studies. Griffin et al (21) found 3 distinct gene expression signatures with variable expression among patients with polyarticular JIA, with signature I identifying patients with a disease similar to adult rheumatoid arthritis, signature II not being associated with any disease subset, signature III identifying patients with less inflammatory disease, and patients with ANA-positive early-onset disease expressing none of these signatures.

There is evidence, as well, that juvenile psoriatic arthritis is not a unique entity. Two distinct subtypes of this condition have been recognized (15). One is very similar to early-onset ANA-positive oligoarthritis, although often with a more rapid spread of arthritis; the other shares the features of enthesitis-related arthritis and therefore belongs to the group of spondylarthritides. In accordance with this hypothesis, Stoll et al (22) recently identified 2 distinct populations of patients with juvenile psoriatic arthritis diagnosed according to the Vancouver criteria (23): one group with younger age at onset, a greater prevalence of females, and more common expression of ANAs, and another group with older age at onset, an even number of boys and girls, and an increased incidence of axial disease and enthesitis. In the ILAR classification (as opposed to the Vancouver criteria), patients with enthesitis are excluded from the category of psoriatic arthritis. Indeed, most of our patients with psoriatic arthritis had the features of ANA-positive oligoarthritis.

The choice of a family history of psoriasis as an exclusion criterion (leading to placement of a patient in the undifferentiated arthritis category) in the ILAR classification has been a matter of controversy (14,24). As many as 82% of our patients who fell into the undifferentiated arthritis category were classified as such due to the presence of a family history of psoriasis in a first-degree relative. We previously found that a positive family history of psoriasis does not affect the clinical picture and course in JIA patients with oligoarthritis, which contradicts the use of such a history as an exclusion criterion (25). In accordance with this criticism, we found that ANA-positive patients in the undifferentiated arthritis category were comparable to ANA-positive patients in the other ILAR subsets.

A number of potential limitations to the study must be acknowledged, the most important of which is its retrospective nature. Retrospective data collection is subject to missing and possibly erroneous data. In the absence of agreed-upon criteria for positivity of ANA, we relied on tests performed on rodent or HEp-2 substrates (13) and chose a cutoff of 1:160 to define positivity. However, in ~11% of our patients, ANA status could not be defined by these stringent criteria. Furthermore, although in the majority of ANA determinations in patients classified as ANA positive the titer was ≥1:160, some repeat determinations in these patients were positive at a lower titer or even negative. We recognize that our choice of the 1:160 cutoff is arbitrary and that other cutoffs may prove equally suitable in other centers or laboratories. A multinational collaborative effort is necessary to establish the optimal threshold for ANA positivity in children with JIA. The frequency of ANA positivity in our series (73.2%) may appear high. However, this frequency was assessed only in select categories of JIA. Considering all categories, the proportion of ANA-positive patients in our series drops to 59.7%, which is in accordance with that recently reported in a large sample of JIA patients followed up in a Western tertiary care pediatric rheumatology center (55.5%) (26). However, because our study population was almost exclusively of Italian ancestry, it is not certain if this ANA-positive phenotype is similarly seen in all genetic backgrounds.

The absence of RF was confirmed in most RF-negative patients with at least 2 determinations. However, we cannot ensure that the RF test was repeated many times in patients who were initially RF negative. Therefore, we cannot exclude the possibility that some patients who were initially RF negative could have become RF positive over time. Finally, we should acknowledge that our definition of joint symmetry is arbitrary. An agreed-upon definition of symmetry requires consensus among international expert pediatric rheumatologists. The main strength of our study lies in the large number of patients enrolled and in the thorough clinical and statistical analysis.

In conclusion, our findings confirm our previous hypothesis that patients with a distinct set of features, including ANA positivity, young age at disease onset,

female sex, asymmetric arthritis, and high risk of developing chronic iridocyclitis, represent a homogeneous group, irrespective of the course of joint disease or the presence of psoriatic features. Further support for our hypothesis comes from the recent observation that gene expression differences, in part representing a B cell signature, are able to distinguish early- and late-onset JIA, independently of the number of joints involved (27).

We therefore suggest the need to revise some of the present JIA classification categories in order to better identify homogeneous patient populations for future genetic and immunopathogenetic investigations, outcome studies, and clinical trials. This effort should be paralleled by an investigation of whether the ANA-defined subgroups are distinct with regard to objective biomarkers, preferably ones that are relevant to disease etiology.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Ravelli had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Analysis and interpretation of data. Ravelli, Pistorio, Ruperto, Galasso, Martini.

REFERENCES

1. Ravelli A, Martini A. Juvenile idiopathic arthritis. Lancet 2007; 369:767–78.
2. JRA Criteria Subcommittee of the Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. Current proposed revision of the JRA criteria. Arthritis Rheum 1977;20 Suppl 2:195–9.
3. Wood P. Special meeting on: nomenclature and classification of arthritis in children. In: Munthe E, editor. The care of rheumatic children. Basel: EULAR; 1987. p. 47–50.
4. Petty RE, Southwood TR, Manners P, Baum J, Glass DN, Goldenberg J, et al. International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. J Rheumatol 2004;31:390–2.
5. Petty RE. Classification of childhood arthritis: a work in progress. Baillieres Clin Rheumatol 1998;12:181–90.
6. Ramsey SE, Bolaria RK, Cabral DA, Malleson PN, Petty RE. Comparison of criteria for the classification of childhood arthritis. J Rheumatol 2000;27:1283–6.
7. Foeldvari I, Bidde M. Validation of the proposed ILAR classification criteria for juvenile idiopathic arthritis. J Rheumatol 2000; 27:1069–72.
8. Fantini F. Classification of chronic arthritides of childhood (juvenile idiopathic arthritis): criticisms and suggestions to improve the efficacy of the Santiago-Durban criteria. J Rheumatol 2001;28: 456–9.
9. Hofer MF, Mouy R, Prieur AM. Juvenile idiopathic arthritides evaluated prospectively in a single center according to the Durban criteria. J Rheumatol 2001;28:1083–90.
10. Krumrey-Langkammerer M, Hafner R. Evaluation of the ILAR criteria for juvenile idiopathic arthritis. J Rheumatol 2001;28: 2544–7.
11. Merino R, De Inocencio J, Garcia-Consuegra J. Evaluation of ILAR classification criteria for juvenile idiopathic arthritis in Spanish children. J Rheumatol 2002;28:2731–6.
12. Berntson L, Fasth A, Andersson-Gare B, Kristinsson J, Lahdenne P, Marhaug G, et al. Construct validity of ILAR and EULAR criteria in juvenile idiopathic arthritis: a population based incidence study from the Nordic countries. J Rheumatol 2001;28: 2737–43.
13. Berntson L, Fasth A, Andersson-Gare B, Herlin T, Kristinsson J, Lahdenne P, et al. The influence of heredity for psoriasis on the ILAR classification of juvenile idiopathic arthritis. J Rheumatol 2002;29:2454–8.
14. Burgos-Vargas R, Rudwaleit M, Sieper J. The place of juvenile onset spondyloarthropathies in the Durban 1997 ILAR classification criteria of juvenile idiopathic arthritis [editorial]. J Rheumatol 2002;29:869–74.
15. Martini A. Are the number of joints involved or the presence of psoriasis still useful tools to identify homogeneous entities in juvenile idiopathic arthritis? J Rheumatol 2003;30:1900–3.
16. Ravelli A, Felici E, Magni-Manzoni S, Pistorio A, Novarini C, Bozzola E, et al. Patients with antinuclear antibody–positive juvenile idiopathic arthritis constitute a homogeneous subgroup irrespective of the course of joint disease. Arthritis Rheum 2005; 52:826–32.
17. Helliwell PS, Hetthen J, Sokoll K, Green M, Marchesoni A, Lubrano E, et al. Joint symmetry in early and late rheumatoid and psoriatic arthritis: comparison with a mathematical model. Arthritis Rheum 2000;43:865–71.
18. Greenacre M. Correspondence analysis in practice. 2nd ed. Boca Raton (FL.): Chapman & Hall/CRC; 2007.
19. Guzman J, Burgos-Vargas R, Duarte-Salazar C, Gomez-Mora P. Reliability of the articular examination in children with juvenile rheumatoid arthritis: interobserver agreement and sources of disagreement. J Rheumatol 1995;22:2331–6.
20. Magni-Manzoni S, Epis O, Ravelli A, Klersy C, Visconti C, Lanni S, et al. Comparison of clinical versus ultrasound-determined synovitis in juvenile idiopathic arthritis. Arthritis Rheum 2009;61: 1497–504.
21. Griffin TA, Barnes MG, Ilowite NT, Olson JC, Sherry DD, Gottlieb BS, et al. Gene expression signatures in polyarticular juvenile idiopathic arthritis demonstrate disease heterogeneity and offer a molecular classification of disease subsets. Arthritis Rheum 2009;60:2113–23.
22. Stoll ML, Zurakowski D, Nigrovic LE, Nichols DP, Sundel RP, Nigrovic PA. Patients with juvenile psoriatic arthritis comprise two distinct populations. Arthritis Rheum 2006;54:3564–72.
23. Southwood TR, Petty RE, Malleson PN, Delgado EA, Hunt DW, Wood B, et al. Psoriatic arthritis in children. Arthritis Rheum 1989;32:1007–13.
24. Petty RE. Growing pains: the ILAR classification of juvenile idiopathic arthritis. J Rheumatol 2001;28:927–8.
25. Tsitsami E, Bozzola E, Magni-Manzoni S, Viola S, Pistorio A, Ruperto N, et al. Positive family history of psoriasis does not affect the clinical expression and course of juvenile idiopathic arthritis patients with oligoarthritis. Arthritis Rheum 2003;49:488–93.
26. Saurenmann RK, Levin AV, Feldman BM, Rose JB, Laxer RM, Schneider R, et al. Prevalence, risk factors, and outcome of uveitis in juvenile idiopathic arthritis: a long-term followup study. Arthritis Rheum 2007;56:647–57.
27. Barnes MG, Thompson SD, Griffin TA, Grom AA, Glass DN, Colbert RA. B-cell signature in patients with JIA is associated with age at onset suggesting biologically relevant classification criteria [abstract]. Arthritis Rheum 2009;60 Suppl:S232.

4.3 Conclusioni

I risultati ottenuti confermano che le categorie dell'attuale classificazione ILAR: oligoartrite persistente, oligoartrite estesa, poliartrite FR-negativa, artrite psoriasica e artrite indifferenziata individuano gruppi non omogenei di pazienti.

È stato inoltre dimostrato che i pazienti ANA-positivi costituiscono un gruppo molto omogeneo, e che pertanto questo criterio si dimostra essere migliore per classificare i pazienti affetti da Artrite Idiopatica Giovanile, rispetto al sistema attualmente in uso basato principalmente sull'interessamento articolare nei primi sei mesi di malattia.

REFERENZE

1. Ravelli A, Martini A. Juvenile idiopathic arthritis. Lancet 2007;369:767–78.
2. JRA Criteria Subcommittee of the Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. Current proposed revision of the JRA criteria. Arthritis Rheum 1977;20 Suppl 2:195–9.
3. Wood P. Special meeting on: nomenclature and classification of arthritis in children. In: Munthe E, editor. The care of rheumatic children. Basel: EULAR; 1987. p. 47–50.
4. Petty RE, Southwood TR, Manners P, Baum J, Glass DN, Goldenberg J, et al. International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. J Rheumatol 2004;31:390–2.
5. Ramsey SE, Bolaria RK, Cabral DA, Malleson PN, Petty RE. Comparison of criteria for the classification of childhood arthritis. J Rheumatol 2000;27:1283–6

6. Foeldvari I, Bidde M. Validation of the proposed ILAR classification criteria for juvenile idiopathic arthritis. J Rheumatol 2000;27:1069–72.
7. Fantini F. Classification of chronic arthritides of childhood (juvenile idiopathic arthritis): criticisms and suggestions to improve the efficacy of the Santiago-Durban criteria. J Rheumatol 2001;28:456–9.
8. Hofer MF, Mouy R, Prieur AM. Juvenile idiopathic arthritides evaluated prospectively in a single center according to the Durban criteria. J Rheumatol 2001;28:1083–90.
9. Krumrey-Langkammerer M, Hafner R. Evaluation of the ILAR criteria for juvenile idiopathic arthritis. J Rheumatol 2001;28:2544–7.
10. Merino R, De Inocencio J, Garcia-Consuegra J. Evaluation of ILAR classification criteria for juvenile idiopathic arthritis in Spanish children. J Rheumatol 2002;28:2731–6.
11. Berntson L, Fasth A, Andersson-Gare B, Kristinsson J, Lahdenne P, Marhaug G, et al. Construct validity of ILAR and EULAR criteria in juvenile idiopathic

arthritis: a population based incidence study from the Nordic countries. J Rheumatol 2001;28:2737–43.

12. Berntson L, Fasth A, Andersson-Gare B, Herlin T, Kristinsson J, Lahdenne P, et al. The influence of heredity for psoriasis on the ILAR classification of juvenile idiopathic arthritis. J Rheumatol 2002;29:2454–8.

13. Burgos-Vargas R, Rudwaleit M, Sieper J. The place of juvenile onset spondyloarthropathies in the Durban 1997 ILAR classification criteria of juvenile idiopathic arthritis [editorial]. J Rheumatol 2002;29:869–74.

14. Martini A. Are the number of joints involved or the presence of psoriasis still useful tools to identify homogeneous entities in juvenile idiopathic arthritis? J Rheumatol 2003;30:1900–3.

15. Ravelli A, Felici E, Magni-Manzoni S, Pistorio A, Novarini C, Bozzola E, et al. Patients with antinuclear antibody–positive juvenile idiopathic arthritis constitute a homogeneous subgroup irrespective of the course of joint disease. Arthritis Rheum 2005; 52:826–32.

CAPITOLO 5

5.1 Efficacia di micofenolato mofetil in pazienti con vasculiti sistemiche (panarterite nodosa)

La Poliarterite nodosa (PAN) è una vasculite necrotizzante che interessa arterie di piccolo e medio calibro con il coinvolgimento multiorgano, che colpisce i pazienti di tutte le età, ma estremamente rara in soggetti di età pediatrica. Negli adulti, l'incidenza annua è stimata tra 2,0 e 9.0 casi /milione [1]. I dati epidemiologici durante l'infanzia mancano a causa dell'esiguo numero di soggetti affetti, anche se in diversi lavori sembra essere la più frequente vasculite sistemica, dopo la vasculite di Schönlein-Henoch (HSP) e la malattia di Kawasaki [2]. La PAN è più frequente nelle popolazioni asiatiche ed è stata riportata in tutti i gruppi etnici [3]. Colpisce entrambi i sessi, con un picco all'età di 10 anni, e i casi familiari sono scarsi [4]. Le principali manifestazioni cliniche sono malessere, febbre, calo ponderale, livedo reticularis, porpora, ulcere e

cancrena. I sintomi muscolo-scheletrici sono rappresentati da artralgia e mialgia, le manifestazioni gastrointestinali si presentano come forti dolori addominali e sono piuttosto comuni; durante il corso della malattia si possono verificare eventi ischemici a livello degli organi interessati, segni neurologici quali emiplegia, convulsioni, mononeurite e polineurite, perdita della vista e difetti focali, infine, il coinvolgimento renale è importante in diversi pazienti e si presenta con proteinuria, ematuria e ipertensione [1-3].

Nonostante il trattamento aggressivo con la combinazione di corticosteroidi e agenti citotossici, la prognosi complessiva della PAN è severa nella maggior parte dei pazienti sia quod vitam sia quod valitudinem, considerati gli effetti collaterali legati ai farmaci [5, 6].

Il Micofenolato Mofetile (MMF) è un inibitore reversibile non competitivo della inosina monofosfato deidrogenasi, un enzima che svolge un ruolo chiave nella sintesi degli acidi nucleici e nella proliferazione cellulare, ma inibisce selettivamente la proliferazione dei linfociti e la produzione di anticorpi. La maggior esperienza con l'uso di MMF è stata raggiunta nel trattamento della glomerulonefrite

proliferativa lupica, ma alcuni risultati incoraggianti sono stati ottenuti nel trattamento delle vasculiti sistemiche [7, 8].

REFERENZE

1. Dillon MJ, Ozen S (2010) Polyarteritis nodosa and cutaneous polyarteritis in textbook of pediatric rheumatology, 6th edn. Elsevier Saunders, Amsterdam, pp 498–502
2. Ozen S, Bakkaloglu A, Dusunsel R, Soylemezoglu O, Ozaltin F, Poyrazoglu H, Turkish Pediatric Vasculitis Study Group et al (2007) Childhood vasculitis in Turkey: a nationwide survey. Clin Rheumatol 26:196–200
3. Ozen S, Anton J, Arisoy N, Bakkaloglu A, Besbas N, Brogan P et al (2004) Juvenile polyarteritis: results of a multicenter survey of 110 children. J Pediatr 145:517–522
4. Mason JC, Cowie MR, Davies KA, SchoWeld JB, Cambridge J, Jackson J et al (1994) Familial polyarteritis nodosa. Arthritis Rheum 37:1249–1253
5. Dillon MJ (2006) Vasculitis treatment-new therapeutic approach. Eur J Pediatr 165:351–357
6. Tullus K, Marks SD (2009) Vasculitis in children and adolescents: clinical presentation, etiopathogenesis, and treatment. Paediatr Drugs 11:375–380

7. Wright E, Dillon MJ, Tullus K (2007) Childhood vasculitis and plasma exchange. Eur J Pediatr 166:145–151

8. Iaccarino L, Rampudda M, Canova M, Della Libera S, Sarzi-Puttinic P, Doria A (2007) Mycophenolate mofetil: what is its place in the treatment of autoimmune rheumatic diseases? Autoimmun Rev 6:190–195

5.2 Mycophenolate mofetil treatment in two children with severe polyarteritis nodosa refractory to immunosuppressant drugs

Rheumatol Int
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SHORT COMMUNICATION

Mycophenolate mofetil treatment in two children with severe polyarteritis nodosa refractory to immunosuppressant drugs

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Abstract Polyarteritis nodosa (PAN) is a necrotizing vasculitis of small- and medium-sized arteries with multiorgan involvement, rarely reported in childhood. Despite aggressive treatment with the combination of corticosteroids and cytotoxic agents, the overall prognosis is poor in most patients. We report on two siblings, now 15- and 14-year-old, affected with childhood onset PAN, refractory to multiple therapies, who showed rapid clinical and laboratory improvement when mycophenolate mofetil was introduced. The relationship between the administration of this immunosuppressant agent and the reduced disease activity is confirmed by the sustained absence of disease flares over 4 years of treatment.

Keywords Polyarteritis nodosa · Mycophenolate mofetil · Childhood vasculitis

Introduction

Polyarteritis nodosa (PAN) is a necrotizing vasculitis of small- and medium-sized arteries with multiorgan involvement affecting patients of all ages, but extremely rare in children and adolescents. In adults, its estimated annual incidence ranges from 2.0 to 9.0/million cases [1]. Epidemiologic data in childhood are lacking due to the small number of affected subjects, though in several series it seems to be the most common systemic vasculitis, after Henoch–Schönlein purpura (HSP) and Kawasaki disease [2]. PAN is more frequent in Asian populations and has been reported in all ethnic groups [3]. Boys and girls seem equally affected, with a peak at the age of 10 years, and familial cases are scanty [4]. The main clinical manifestations are malaise, fever, weight loss, livedo reticularis, purpura, ulcers and gangrene. Musculoskeletal symptoms in terms of arthralgia or myalgia and gastrointestinal manifestations in terms of severe abdominal pain are rather common; during the disease course, ischemic symptoms of the affected organs, neurological signs such as hemiplegia, seizures, mononeuritis and polyneuritis, visual loss and focal defects can occur; at last, renal involvement is prominent in several patients with proteinuria, hematuria and hypertension [1–3].

Despite aggressive treatment with the combination of corticosteroids and cytotoxic agents, the overall prognosis of PAN is poor in most patients and survivors frequently display severe side effects related to medications [5, 6]. Mycophenolate mofetil (MMF) is a noncompetitive and reversible inhibitor of inosine monophosphate dehydrogenase, an enzyme that plays a key role in nucleic acid synthesis and cell proliferation; it inhibits selectively lymphocyte proliferation and antibody formation. The greatest experience with the use of MMF has been achieved in the treatment for proliferative lupus glomerulonephritis, but some encouraging results have also been obtained in the treatment for systemic vasculitis [7, 8].

We describe two patients, now aged, respectively, 15 and 14 years, who are siblings, brother and sister, the only

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sons of their family, who both developed PAN first manifestations in infancy with neurological symptoms; diagnosis of PAN was delayed due to the initial nonspecific clinical picture that appeared refractory to multiple drugs. MMF has been administered to these patients who surprisingly responded to the drug and remained in stable remission over a 4-year period.

Case reports

Case 1

Case 1 was a boy, born after uneventful pregnancy and delivery; his psychomotor development was normal. At 2 years, he suddenly presented vertigo and vomiting, being referred to a regional Hospital. As routine laboratory tests, electroencephalogram (EEG) and brain computed tomographic (CT) scan resulted normal, he was discharged without any therapy, with the provisional diagnosis of flu-like illness. At 5 years of age, he abruptly developed relapsing episodes of ear lobe acrocianosis. Extended laboratory investigations, including clotting tests and antibody profile, antinuclear (ANA) and anti-ENA (extractable nuclear antigens) antibodies, resulted normal. Two years later, HSP was diagnosed due to the presence of palpable purpuric rash at the lower extremities and migrant arthralgia with no joint swelling. No abnormalities on full blood tests were detected. In November 2003, he was referred to our Pediatric Unit with a one-month history of intermittent fever (38°C) and arthralgia. Physical and neurological examination was unremarkable. Blood tests showed erythrocyte sedimentation rate (ESR) 30 mm/h, hemoglobin (Hb) 8.9 mg/dl (thalassemic trait), white blood count (WBC) 9.34/mm^3 with 54.6% of neutrophils, platelets 3.85/mm^3 and C-reactive protein (CRP) 6.2 mg/dl (n.v. < 0.5 mg/dl). Blood, stool and urine cultures were negative, as were serological tests for the most common viral and bacterial infections. Indomethacin (3 mg/kg/day) was effective on the pain. At the age of 8 years, he developed painful erythematosus nodules on the anterior face of legs, aphthous stomatitis and neurosensorial bilateral hypoacusia, associated with raised parameters of inflammation. Six months later, vertigo, tinnitus and diplopia were complained. Owing to the hypothesis of a side effect to indomethacin, the drug was stopped with a prompt relapse of musculoskeletal pain. Neurosensorial hypoacusia and left central facial palsy were detected by otolaryngologist evaluation. Blood tests showed increased ESR (48 mm/h), CRP (8, 6 mg/dl) and fibrinogen (786 mg/dl). All others laboratory results were within normal limits, including hepatitis C screening, liver function tests, ANA, ANCA (antineutrophil cytoplasmic antibodies), anticardiolipin (ACL) antibodies and urinalysis. Brain magnetic resonance imaging (MRI) with gadolinium excluded focal alterations and pathological lesions, while brain scintigraphy showed hypoperfusion in the right inferior frontal region. Skin lesion biopsy revealed the presence of necrotizing vasculitis of medium-sized arteries. Total body bone scintigraphy resulted normal. In this period, hypertension was first detected (140/90 mmHg). Central nervous system MRI with angiographic sequences revealed ischemic abnormalities of Willis' circle. The association of myalgia, pain, cutaneous lesions, hypertension and neurological signs prompted to diagnose PAN and oral prednisone (1.5 mg/kg/day) was introduced. Angiography of cerebral vessels was proposed but refused by parents. Despite aggressive therapy (pulsed corticosteroids, azathioprine and antihypertensive drug), the boy developed ischemic lesions of three digits of both hands and iloprost intravenous infusion (0.5 ng/kg/min) for 10 days was successfully administered. Over 2 months, multiple ulcerative cutaneous lesions appeared on the legs Fig. 1a. Due to persistent disease activity, in March 2006, after azathioprine washout, MMF was introduced (2 g/day), with the aim of achieving a drug serum range of 3–5 mg/dl), followed by clinical and laboratory improvement. Over 6 months, corticosteroid was progressively tapered to 5 mg/day dose maintained. Up to now, the clinical symptoms are stable, blood pressure within the normal range for age and laboratory examinations all normalized. His neurological outcome is normal, except for a limited area of paresthesia on the medium face of his left thigh. The dose of prednisone (5 mg/day) and MMF (2 g/day) is unchanged at the last follow-up.

Case 2

Case 2 was a girl, born after a normal pregnancy and delivery; her psychomotor development was regular. At 30 months of age, she was referred to our Emergency Unit due to left hemiplegia and bilateral optical neuritis. Laboratory workups including functional clotting tests and autoantibodies were all normal, as brain CT scan and EEG. Over time, similar episodes recurred, and periodic ataxia was the temporary diagnosis. After 6 months, cerebral MRI revealed areas of hyperintensity on the thalamus in three consecutive evaluations. At 7 years, maculopapular rash on the face and upper extremities, arthralgia and hypertension worsened the disease course. She was referred to our Paediatric Unit in March 2004, with a history of myalgia, neuropathy and cutaneous nodules Fig. 1b. Physical and neurological examination was normal, except for blood pressure of 150/92 mm/Hg. Abnormal laboratory results included raised ESR (50 mm/h), anemia (Hb 8.9 mg/dl, thalassemic trait), leukocytosis (WBC 14.1/mm^3 with 61.4% of neutrophils), platelets 2.97/mm^3 and increased

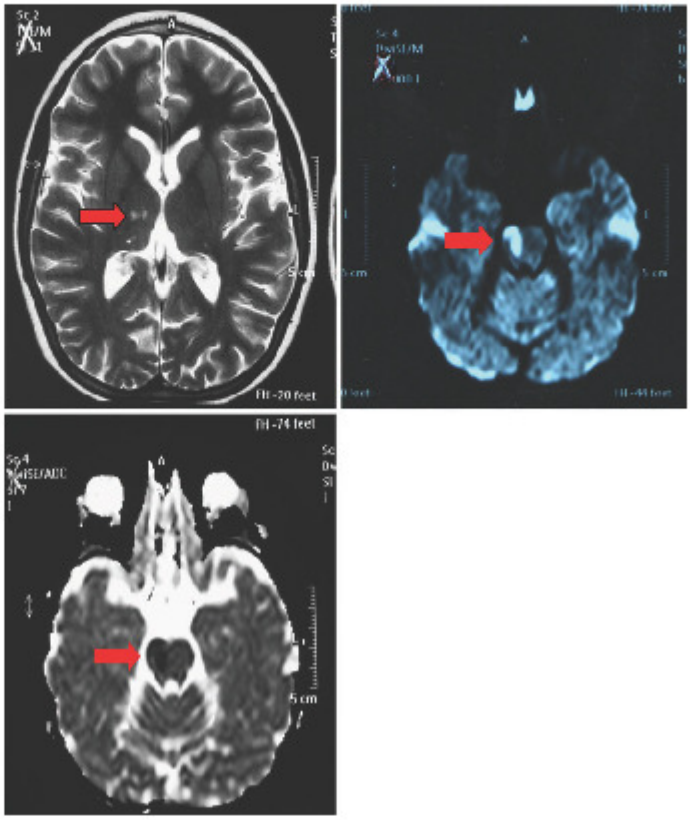

Fig. 1 *Right side* of pons: hyperintense lesion on diffusion imaging that inverted on the apparent diffusion coefficient maps related to the presence of a cytotoxic edema secondary to an acute stroke on the side of *right* perforants of the basilar trunk. Flair and T2-weighted images: two little hyperintense areas at the right thalamus not positive on the diffusion imaging, therefore related to previous lacunar infarction

CRP (2.64 mg/dl). Blood culture, urine analysis and liver enzymes were normal. Serological tests for HIV, hepatitis A and C, varicella-zoster herpes virus and Epstein–Barr virus resulted negative. ANA, ACL and ANCA antibodies were undetected. A skin biopsy showed necrotizing vasculitis of medium-sized vessels. According to the recent classification criteria of vasculitis, PAN was diagnosed. Angiography was refused by relatives. The constellation of hypertension, polyneuropathy and ischemic brain lesions in a patient with necrotizing vasculitis prompted to hypothesize the same disease of her brother. Oral corticosteroids and azathioprine were introduced. In December 2005, deep cutaneous ulcers appeared on the legs and pulsed methylprednisolone (30 mg/kg), intravenous iloprost (0.5 ng/kg/min) for 10 days and oral cyclophosphamide (2 g/kg) were added. Brain scintigraphy showed hypoperfusion in the right thalamic region. In January 2006, after a severe stroke with left facial-brachial-crural hemiparesis, MRI ischemic lesions were confirmed at the right pons area (Fig. 1). MMF was introduced at the same dose of her brother. After 6 months, corticosteroid was discontinued and reduced to 5 mg/day. Her general condition progressively improved. Blood pressure became normal following antihypertensive drugs. Since then, under MMF, no recurrences of neurological symptoms have been observed and MRI lesions are stable (Fig. 2). At December 2010 follow-up, both children were in good general condition with complete healing of skin lesions. They are attending regularly high school with good performance, and no side effects related to MMF have been observed.

Discussion

To the best of our knowledge, these described patients are the first cases of severe PAN who dramatically responded to MMF and achieved sustained remission over a 4-year follow-up. High dose of corticosteroids induced only a partial

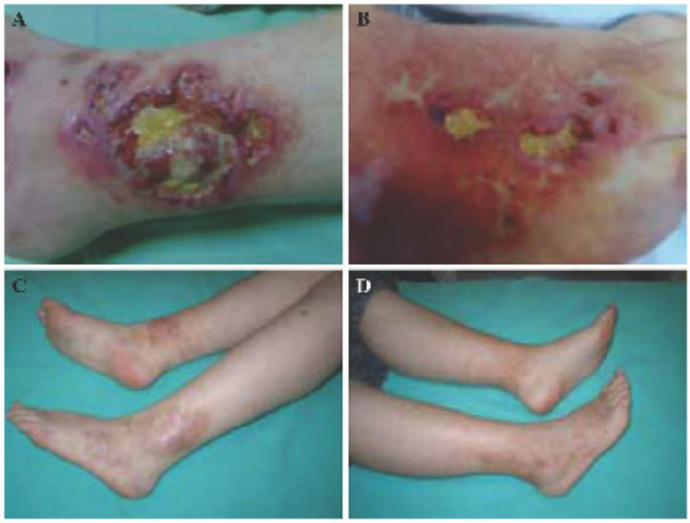

Fig. 2 a–d Deep cutaneous ulcers in brother and sister before MMF and at last follow-up

control of their illnesses. Azathioprine and cyclophosphamide were ineffective. Conversely, MMF provided prompt, impressive and persistent improvement in both clinical symptoms and laboratory parameters regardless of a progressive tapering of steroid dose.

According to the recent classification criteria for childhood, PAN may be diagnosed in the presence of a systemic inflammatory disease with evidence of necrotizing vasculitis or angiographic abnormalities of medium/small-sized arteries (mandatory criterion) plus one of five criteria: (1) skin involvement; (2) myalgia/muscle tenderness; (3) hypertension; (4) peripheral neuropathy; and (5) renal involvement [9]. Although the disease is usually systemic with multiorgan involvement, it is not uncommon to encounter patients at early stages of the disease with only a single organ affected and nonspecific skin lesions. Different drugs have been shown to be unsuccessful in controlling the disease activity, and long-term prognosis is poor in most cases. Despite aggressive medical management, the rate of mortality is still high and the drug-induced morbidity is frequent [1–3]. At onset, the diagnosis is challenging as the first symptoms are common to other vasculitis.

In our patients, the presenting manifestations were characterized by neurological symptoms and then associated with severe and awful skin lesions. The proper diagnosis was delayed as the initial neurological disease was not regarded as brain ischemia and cutaneous features in the boy were misdiagnosed as HSP. In several cases, the disease may be fatal as visceral involvement is aggressive [10–14]. Recently, tumor necrosis factor alpha blockade has been proposed in patients refractory to other therapies [15]. In our patients, HLA typing showed a common HLA haplotype (BW4/BW6, DR7, DR11, DR52, DR53, DQ2 and DQ7) supporting a genetic predisposition, though no other cases have been observed in their family. Despite its rarity, PAN should be assumed in young children with vasculitis, even when not all criteria for diagnosis are satisfied. Pending further controlled studies to confirm our observation and based on the clinical and MRI outcome at a 4-year follow-up, MMF should be considered as either alternative or adjunctive therapy in those forms of refractory PAN which are particularly difficult to control. However, further studies are needed to determine MMF real place in the treatment for PAN, and we would also suggest that a long-term follow-up is observed to ascertain its real therapeutic efficacy.

Conflict of interest None.

References

1. Dillon MJ, Ozen S (2010) Polyarteritis nodosa and cutaneous polyarteritis in textbook of pediatric rheumatology, 6th edn. Elsevier Saunders, Amsterdam, pp 498–502
2. Ozen S, Bakkaloglu A, Dusunsel R, Soylemezoglu O, Ozaltin F, Poyrazoglu H, Turkish Pediatric Vasculitis Study Group et al (2007) Childhood vasculitis in Turkey: a nationwide survey. Clin Rheumatol 26:196–200
3. Ozen S, Anton J, Arisoy N, Bakkaloglu A, Besbas N, Brogan P et al (2004) Juvenile polyarteritis: results of a multicenter survey of 110 children. J Pediatr 145:517–522
4. Mason JC, Cowie MR, Davies KA, Schofield JB, Cambridge J, Jackson J et al (1994) Familial polyarteritis nodosa. Arthritis Rheum 37:1249–1253

5. Dillon MJ (2006) Vasculitis treatment-new therapeutic approach. Eur J Pediatr 165:351–357
6. Tullus K, Marks SD (2009) Vasculitis in children and adolescents: clinical presentation, etiopathogenesis, and treatment. Paediatr Drugs 11:375–380
7. Wright E, Dillon MJ, Tullus K (2007) Childhood vasculitis and plasma exchange. Eur J Pediatr 166:145–151
8. Iaccarino L, Rampudda M, Canova M, Della Libera S, Sarzi-Puttinic P, Doria A (2007) Mycophenolate mofetil: what is its place in the treatment of autoimmune rheumatic diseases? Autoimmun Rev 6:190–195
9. Ozen S, Pistorio A, Iusan SM, Bakkaloglu A, Herlin T, Brik R, Paediatric Rheumatology International Trials Organisation (PRINTO) et al (2010) EULAR/PRINTO/PRES criteria for Henoch-Schönlein purpura, childhood polyarteritis nodosa, childhood Wegener granulomatosis and childhood Takayasu arteritis: Ankara 2008. Part II: final classification criteria. Ann Rheum Dis 69:798–806
10. Appenzeller S, Faria AV, Zanardi DA, Fernandes SR, Costallat LT, Cendes F (2008) Vascular involvement of the central nervous system and systemic diseases: etiologies an MRI findings. Rheumatol Int 28:1229–1237
11. Topaloglu R, Kazik M, Saatci I, Kalyoncu M, Cil BE, Akalan N (2005) An unusual presentation of classic polyarteritis nodosa in a child. Pediatr Nephrol 20:1011–1015
12. Kendirli T, Yüksel S, Oral M, Unal N, Tulunay M, Dilek US, Yalçnkaya F (2006) Fatal polyarteritis nodosa with gastrointestinal involvement in a child. Pediatr Emerg Care 22:810–812
13. Engel DG, Gospe SM Jr, Tracy KA, Ellis WG, Lie JT (1995) Fatal polyarterits nodosa with predominant central nervous system involvement. Stroke 26:699–701
14. Buonuomo PS, El Hachem M, Callea F, Bracaglia C, Diociaiuti A, Pardeo M, Campana A, Insalaco A, Cortis E, Ugazio AG (2010) Necrosis of the tongue as first symptom of Polyarteritis Nodosa (PAN): unusual presentation of a rare disease in children. Rheumatol Int [Epub ahead of print]
15. Feinstein J, Arroyo R (2005) Successful treatment of childhood onset refractory Polyarteritis nodosa with tumor necrosis factor alpha blockade. J Clin Rheumatol 11:219–222

CAPITOLO 6

6.1 Ialinosi Sistemica Infantile

Le fibromatosi ialine sono rare malattie autosomiche recessive caratterizzate da accumulo di una grande quantità di tessuto fibroso ialino nella cute e organi interni. Sono state descritte due sindromi apparentemente distinte: la Fibromatosi Ialina Giovanile (JHF) e la Ialinosi Sistemica Infantile (ISH) [1]. Recenti studi genetici hanno rivelato che mutazioni nel gene che codifica per la proteina morfogenesi capillare 2 (CMG2) potrebbe essere la causa di entrambe le patologie [2]. CMG2 è una proteina transmembrana, che viene indotta durante la morfogenesi capillare che lega la laminina e il collagene tipo IV, attraverso il dominio di Von Willebrand A (vWA), suggerendo che la modificazione dell'assemblaggio della matrice della membrana basale sia la causa della deposizione ialina perivascolare, caratteristica di queste condizioni.

Le caratteristiche cliniche principali comprendono noduli sottocutanei multipli, iperplasia gengivale, noduli perianali, ridotta elasticità cutanea, generalizzato ispessimento della

cute con aree di iperpigmentazione, contratture articolari, osteoporosi, bassa statura. ISH caratteristicamente mostra coinvolgimento viscerale, diarrea persistente o infezioni, che possono portare ad exitus nei primi 2 anni di vita [3, 4].

Si descrive una bambina di 3 anni d'età affetta da ISH confermata dal quadro clinico, istopatologoico e mediante analisi molecolare.

REFERENZE

1. Urbina F, Sazunic I, Murray G. Infantile systemic hyalinosis or juvenile hyaline fibromatosis? Pediatr Dermatol 2004; 21: 154-9.
2. Hanks S, Adams S, Douglas J, Arbour L, Atherton DJ, Balci S, et al. Mutations in the gene encoding capillary morphogenesis protein 2 cause juvenile hyaline fibromatosis and infantile systemic hyalinosis. Am J Hum Genet 2003; 73: 791-800.
3. Lindvall LE, Kormeili T, Chen E, et al. Infantile systemic hyalinosis: Case report and review of the literature. J Am Acad Dermatol 2008;58: 303-7.
4. Shin HT, Paller A, Hoganson G, Willner JP, Chang MW, Orlow SJ. Infantile systemic hyalinosis. J Am Acad Dermatol 2004; 50(2 Suppl): S61-4.

6.2 Infantile systemic hyalinosis: an atypical milder form

and suggest referring to both as "hyaline fibromatosis syndrome" [5].

There is no specific treatment for the hyalinoses. The recommended treatment for JHF is surgical removal of the lesions, but local recurrences are common. There is a limited response to intralesional steroid injection in the early stages. Capsulotomy, corticosteroids, adrenocorticotropic hormone (ACTH) and physiotherapy have produced some improvement in the treatment of the joint contractures and gingivectomy has been tried for the gingival hypertrophy. Therapeutic trials with dimethylsulfoxide, ketotifen, calcitriol, and D-penicillamine have been attempted in some cases [6].

Juvenile hyaline fibromatosis and ISH remain stigmatizing, incapacitating, and sometimes fatal disorders, with no satisfactory treatment. The discovery of the responsible mutations provides some hope for gene therapy in the future. ■

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1. Enzinger FM & Weiss SW. *Soft Tissue Tumors*, 3rd edn. Mosby, St. Louis,1995 (Year Book).
2. Miyake I, Takumaru H, Sugino H, *et al.* Juvenile hyaline fibromatosis: case report with five years' follow-up. *Am J Dermatopathol* 1995; 17: 584-90.
3. Katagiri K, Takasaki S, Fujiwara S, *et al.* Purification and structural analysis of extracellular matrix of a skin tumor from a patient with juvenile hyaline fibromatosis. *J Dermatol Sci* 1996; 13: 37-48.
4. Glover MT, Lake BD, Atherton DJ. Infantile systemic hyalinosis: newly recognized disorder of collagen? *Pediatrics* 1991; 87: 228-34.
5. Nofal A, Sanad M, Assaf M, *et al.* Juvenile hyaline fibromatosis and infantile systemic hyalinosis: a unifying term and a proposed grading system. *J Am Acad Dermatol* 2009; 61: 695-700.
6. Urbina F, Sazunic I, Murray G. Infantile systemic hyalinosis or juvenile hyaline fibromatosis? *Pediatr Dermatol* 2004; 21: 154-9.

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Infantile systemic hyalinosis: an atypical milder form

The hyaline fibromatoses are rare autosomal recessive disorders characterized by a large quantity of hyalinized fibrous tissue in the skin and internal organs. Two apparently distinct syndromes have been described: juvenile hyaline fibromatosis (JHF) and infantile systemic hyalinosis (ISH) [1]. Recent genetic studies revealed that mutations in the gene encoding capillary morphogenesis protein 2 (CMG2) might be the cause of both conditions [2]. Their main clinical features include multiple subcutaneous skin nodules, gingival hyperplasia, perianal nodules, joint contractures, osteoporosis. ISH characteristically shows visceral involvement, persistent diarrhea or infections, leading to death in the first 2 years of life [3, 4].

We describe a 3-year-old girl with ISH confirmed by clinical and histopathological findings and DNA sequence analysis. The patient was born at full term to unrelated parents after a normal pregnancy and delivery. Other members of the family were healthy and no symptoms or signs of disease were present at birth. At age 2 weeks a right upper limb contracture with inflected attitude was noticed and, by the age of 4 weeks, it involved both upper and lower limbs. At 4 months she had upper and lower limb mobility reduction, with crying crises during passive mobilizations. Moreover the patient presented with severe hand contractures, especially involving metacarpal-phalangeal joints, and inflected attitudes on the fingers. Neurological examination was normal. Results of hematologic and biochemical studies were within normal limits as well as instrumental investigations (electroencephalography, cranial ultrasonography, hip ultrasonography, radiographs of the lumbosacral spine). At 11 months she presented with worsened joint contractures that led to assumption of the frog leg position *(figures 1A, B)*. She had red colored, raised lesions on her perioral area and multiple, pearly, grouped papules on the dorsum of the neck and behind the ears; the skin showed hyperpigmented areas over the malleoli. Examination of the oral cavity revealed gingival hyperplasia *(figure 1C)*. Multiple coalescent hyperpigmented papules, sparing the perineum, were visible on the perianal area *(figure 1D)*. The patient demonstrated physical developmental delay, while social development appeared to be age-appropriate. Ophthalmologic examination did not reveal any abnormalities.

Figure 1. A) Contractures of joints result in "frog-leg" positioning. **B)** Upper limb and hand contractures, particularly of the metacarpal-phalangeal and proximal interphalangeal joints. **C)** Gingival hyperplasia and red colored, raised lesions on the perioral area. **D)** Fleshy nodules in the perianal region. **E)** Beneath a hyperparakeratotic epidermis there is extensive deposition of amorphous eosinophilic material in the papillary dermis, with focal deposits in the deeper dermis. **F)** The thick homogeneous eosinophilic bands of hyalinized material surround the capillaries and are also interspersed in the interstitium with scattered spindle cells.

Electrocardiography and 2D-echocardiography were normal. Organic acids, amino acids, mucopolysaccharides, oligosaccharides, sialic acid, and lysosomal enzymes were normal. Skeletal plain radiographs revealed generalized osteopenia with underdevelopment of the epiphyses. A skin biopsy from the posterior auricular region demonstrated hyalinization of the papillary and reticular dermis, with thickened collagen bundles reminiscent of scleroderma, and amorphous eosinophilic hyaline material in the papillary dermis *(figures 1E, F)*. ISH was suspected and the DNA analysis revealed on gene encoding CMG2 two heterozygous mutations: C39F on Exon 1 and P357delT on Exon 13.

The patient has an atypically mild form of ISH with early clinical onset and progressive joint contractures and absence of visceral involvement, persistent diarrhea or infections [1, 3-5]. According to Hanks *et al.* the mutation in the cytoplasmic domain could explain this atypically mild form of ISH. The clinical overlapping of both ISH and JHF suggests that they may represent different variants of the same disease spectrum [1]. Different mutations in the gene encoding CMG2 were found, although only a few of them are described as compound heterozygotes in the literature [2, 3].

Recently, Nofal *et al.* proposed, in order to unify the wide spectrum of clinical findings, a single designation of "hyaline fibromatosis syndrome", which would encompass the different presentations and cases with infantile or juvenile onset. They also suggested dividing this syndrome into mild, moderate, and severe subtypes, according to the severity of organ involvement. Understanding a clear genotype-phenotype correlation is important to explain better the phenotypical variability of the disease [6]. ■

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1. Urbina F, Sazunic I, Murray G. Infantile systemic hyalinosis or juvenile hyaline fibromatosis? *Pediatr Dermatol* 2004; 21: 1549.
2. Hanks S, Adams S, Douglas J, Arbour L, Atherton DJ, Balci S, *et al.* Mutations in the gene encoding capillary morphogenesis protein 2 cause juvenile hyaline fibromatosis and infantile systemic hyalinosis. *Am J Hum Genet* 2003; 73: 791-800.
3. Lindvall LE, Kormeili T, Chen E, *et al.* Infantile systemic hyalinosis: Case report and review of the literature. *J Am Acad Dermatol* 2008; 58: 303-7.
4. Shin HT, Paller A, Hoganson G, Willner JP, Chang MW, Orlow SJ. Infantile systemic hyalinosis. *J Am Acad Dermatol* 2004; 50 (2 Suppl): S61-4.
5. ElKamah GhY, Mostafa IM. Heterogeneity and atypical presentation in infantile systemic hyalinosis with severe labio-gingival enlargement: First Egyptian report. *Dermatology Online Journal* 2009; 15: 6.
6. Nofal A, Sanad M, Assaf M, *et al.* Juvenile hyaline fibromatosis and infantile systemic hyalinosis: a unifying term and a proposed grading system. *J Am Acad Dermatol* 2009; 61: 695-700.

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Idiopathic eruptive macular pigmentation in a 50-year-old man

Idiopathic eruptive macular pigmentation (IEMP) is an uncommon dermatosis characterized by multiple, asymptomatic, grey to dark brown, 3 mm to 30 mm, non-confluent, fixed macules involving the neck, trunk and proximal extremities [1].

Galdeano *et al.* [2] suggested five diagnostic criteria for IEMP: i) eruption of brownish, non-confluent macules located on the trunk, neck and proximal extremities in children/young adults; ii) absence of preceding inflammatory skin disease; iii) no previous medications; iv) basal cell layer hyperpigmentation and occasionally dermal melanophages without visible damage of the basal layer or lichenoid inflammatory infiltrate; v) normal mast cell counts.

The disease resolves gradually and treatment is not necessary. Previous reports are almost exclusively in children and adolescents [2, 3]; only two cases in young adults have been described [4, 5]. We report an unusual "late-onset" case in a 50-year-old man.

A 50-year-old Caucasian man was examined for persistent, asymptomatic, multiple macules which developed 2 years previously on his trunk, axillary folds and proximal extremities of his arms *(figure 1A)*. The grey-blue, quadrangular macules ranged from 3 mm to 8 mm, and were non-migrating. The Darier sign was negative. No symptoms, signs or drug intake preceded the appearance of hyperpigmentation. No laboratory abnormalities were detected.

A skin biopsy from a pigmented lesion showed increased pigmentation of the basal layer in an otherwise normal epidermis, many melanophages and a mild, perivascular, lymphohistiocytic infiltrate in the upper dermis *(figure 1B)*. As the clinical and histological features fulfilled the criteria for IEMP, except the age of onset, IEMP was diagnosed. No therapy was prescribed because spontaneous resolution was expected. The dermatitis remained unchanged at six months follow up.

The aetiology and the pathogenesis of IEMP are unknown. While hereditary factors do not seem to be significant, hormonal factors may be involved as the disease primarily occurs during childhood and adolescence [3]. Milobratovic *et al.* [4] reported IEMP during pregnancy in a woman with a history of thyroid adenoma, autoimmune Hashimoto's thyroiditis and alopecia areata, and they hypothesized that complex hormonal changes and autoimmunity may have a role in the etiopathogenesis of IEMP. Sunlight does not seem to be a factor as lesions typically involve areas unexposed to the sun, do not change after sun exposure and patients have no history of photosensitivity [4].

Various diseases should be considered in the differential diagnosis; erythema dyschromicum perstans (EDP) [6], urticaria pigmentosa, lentigines and multiple café-au-lait macules, post-inflammatory macular hyperpigmentation (PIH). EDP (ashy dermatosis) is an acquired dermatosis characterized by the rapid onset of small, asymptomatic, ash-coloured macules, with identical morphology and distribution to IEMP lesions [6]. Furthermore, both

CAPITOLO 7

7.1 Utilità della teletermografia computerizzata nel follow up di pazienti con Spondiloartropatia Giovanile (SpAG)

La Spondiloartropatia ad esordio giovanile (SpAG) è un termine che si riferisce a un gruppo di malattie infiammatorie croniche caratterizzate da entesopatia e artropatia che generalmente colpisce gli arti inferiori. I bambini di solito presentano SpA indifferenziata che evolve in forme differenziate nel tempo. Il segno distintivo di queste malattie è la dimostrazione della flogosi delle articolazioni sacroiliache (sacro ileite) [1-2]. I segni radiografici della sacroileite si vedono raramente nei bambini, la risonanza magnetica potrebbe identificare alterazioni acute e croniche delle articolazioni sacro-iliache di bambini con SpA indifferenziata e definitiva, che potrebbero non essere evidenti alla radiografia [3]. La risonanza magnetica, essendo un'indagine costosa, non può essere eseguita in tutti i pazienti con dolore al rachide lombosacrale. Inoltre, i risultati non specifici della risonanza magnetica possono complicare ulteriormente il problema

diagnostico, e pertanto non è raccomandato per l'approfondimento diagnostico di routine del dolore al rachide [4].

La Termografia a infrarossi (IRT) è una tecnica non invasiva che permette di misurare la radiazione infrarossa emessa dal corpo umano, che, come peraltro tutti i corpi ad una temperatura diversa dallo zero assoluto, irraggia energia termica sotto forma di onde elettromagnetiche infrarosse; avendo a disposizione un dispositivo sensibile alla lunghezza d'onda di tale radiazione (3-5 μm) è possibile rilevare e misurare la distribuzione della temperatura nei vari distretti del corpo umano.

In ambito medico il razionale di questa tecnica parte dall'assunto che la distribuzione della temperatura cutanea del corpo umano dipende dall'insieme dei processi di scambio di calore tra la cute, i tessuti profondi, la vascolarizzazione locale e l'attività metabolica, e la regolazione dell'attività simpatica e parasimpatica deputata al mantenimento dell'omeostasi. Quello che ne risulta è un'immagine termografica costituita da due componenti di base: il calore di fondo e il disegno vasale [5]. Il **calore di fondo** costituisce la componente non focale ed è tanto

minore quanto più estesa è la superficie cutanea in rapporto al volume corporeo che riveste, e viceversa. Tipicamente ipotermiche sono le mani, i piedi, le orecchie, i capezzoli, i testicoli; sono ipertermici il tronco e più o meno tutte le aree nelle quali è difficoltosa la cessione di calore all'ambiente: solco sottomammario, ascelle, inguini, pieghe glutee. Nella distribuzione del calore di fondo vige una certa simmetria corporea. Al **disegno vasale**, prevalentemente venoso, possono contribuire tronchi arteriosi superficiali, come i peduncoli arterovenosi della mammella.

La presenza di una patologia determina un'alterazione a livello dell'attività termogenetica delle strutture sottostanti e della operatività dei meccanismi di smaltimento del calore. Quello che ne risulta, quindi, è un sovvertimento della quantità di calore emesso dalla cute sovrastante la patologia, che non si estende alle regioni limitrofe e controlaterali e che può esprimersi sia con aumento che come diminuzione, a seconda del tipo di danno.

Classico è il paragone tra l'infiammazione, che determina un aumento della temperatura grazie all'aumento dell'afflusso sanguigno locale, e la cicatrizzazione, che è caratterizzata dalla sostituzione del normale tessuto con

tessuto connettivo, ricco di matrice extracellulare e povero di capillari, che da luogo ad un abbassamento della temperatura [6-8]. Ovviamente il grado di alterazione dipenderà da una serie di fattori: la profondità della lesione, il grado di intensità della patologia stessa, le caratteristiche della cute, la quantità di tessuto adiposo sottostante [9].

L'eventuale presenza, quindi, di asimmetrie nella distribuzione del calore e/o di alterazioni del controllo della temperatura può essere correlata a possibili quadri patologici.

In reumatologia la IRT è già stata applicata per la valutazione della flogosi articolare [10, 11], per lo studio del fenomeno di Raynaud [12-14] e per la valutazione della temperatura cutanea in corso di morbo di Paget [15] e di algodistrofia [16, 17].

L'obiettivo dello studio è verificare l'efficacia e l'applicabilità dell' IRT nel rilevare l'infiammazione articolare nei bambini affetti da SpAG, e, in particolare, l'attendibilità diagnostica dell'IRT nel follow up di pazienti affetti da sacro ileite ed il possibile ruolo di questa tecnica nella rilevazione dell'attività di malattia nei pazienti affetti da SpAG.

REFERENZE

1. Gensler L, Davis JC Jr. Recognition and treatment of juvenile-onset spondyloarthritis. Curr Opin Rheumatol 2006;18:507-11.

2. Burgos-Vargas R. The juvenile-onset spondyloarthropathies. Rheum Dis Clin North Am 2002;28: 531–560

3. Dougados M, Hochberg MC. Why is the concept of spondyloarthropathies important? Best Pract Res Clin Rheumatol 2002;16:495–505

4. Bollow M, Biedermann T, Braun J, et al. Use of dynamic magnetic resonance imaging to detect sacroiliitis in HLA B-27 positive and negative children with juvenile arthritides. J Rheumatol 1998;25:556–64.

5. A. Merla and G.L. Romani. “Functional Infrared Imaging in Clinical Applications,” in The Biomedical Engineering Handbook, CRC Press,JD Bronzino, 32.1 – 32.13, Nov. 2005.

6. Kastberger G, Stachl R. Infrared imaging technology and biological applications. Behav Res Methods Instrum Comput. 2003;35:429-39.

7. Merla A, Romani G. Biomedical Applications of Functional Infrared Imaging. Conf Proc IEEE Eng Med Biol Soc 2005;1:690-3.
8. Merla A, Romani GL. Functional infrared imaging in medicine: a quantitative diagnostic approach. Conf Proc IEEE Eng Med Biol Soc 2006;1:224-7.
9. Aweruch, M.S., Thermography: its current diagnostic status in muscular-skeletal medicine, Med. J. Aust, 1991;154,441.
10. Collins AJ, Ring F, Bacon PA, Brookshaw JD. Thermography and radiology: complementary methods for the study of inflammatory diseases. Clin Radiol 1976;27:237–43.
11. Bacon PA, Ring EJF, Collins AJ. Thermography in the assessment of anti-rheumatic agents. In: Gordon G, Hazleman B, eds. Rheumatoid arthritis. Amsterdam: Elsevier, 1977:463–75.
12. European Association of Thermology. Raynaud's phenomenon: assessment by thermography. Thermology 1988;3:69–73.
13. Darton K, Black CM. The use of infrared thermography in a rheumatology unit. Br J Rheumatol 1990;29:291–2.

14. Howell KJ, Smith RE, Knight CJ *et al*. Thermography in rheumatology (abstract). Thermol Int 2000;10:36.

15. Ring EFJ, Davies J. Thermal monitoring of Paget's disease of bone. Thermology 1990;3:167–72.

16. Will RK, Ring EFJ, Clarke AK, Maddison PJ. Infrared thermography: what is its place in rheumatology in the 1990s? Br J Rheumatol 1992;31:337–44.

17. Cooke ED, Glick EN, Bowcock SA et al. Reflex sympathetic dystrophy (algoneurodystrophy): temperature studies in the upper limb. Br J Rheumatol 1989;28:399–403.

7.2 Infrared thermography could be a detection tool for sacroiliac joint inflammation in juvenile spondyloarthropathies.

Accettato con revisione.

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Abstract

Juvenile-onset spondyloarthropathies (JSpA) refers to a group of pediatric disorders characterized by enthesopathy and arthropathy. The hallmark of JSpA is sacroiliitis. Aim: to define the clinical utility of infrared thermography (IRT) in detection of disease activity in JSpA. Methods: we studied 30 children (20M, 10F, mean age 13 yrs, range 8-20 yrs) with active sacroiliitis, diagnosed by bone scintigraphy

and confirmed by magnetic resonance imaging (MRI) and 10 children with scoliosis. The thermographic analysis were performed with Digital Infrared Camera Land FTI 6. The measurements were performed in a temperature and humidity controlled room. The subjects observed 15 minutes of acclimation in the measurement room before thermographic observation. Results: At enrolment, patients showed an higher mean ΔA-gradients on IRT, if compared to those recorded in controls. None of the 10 children with scoliosis had increased sacroiliac activity on thermography. Conclusions: IRT could help in the diagnosis of sacroiliitis and might be helpful in the objective serial assessment of sacroiliitis in patients with active disease.

Background

Juvenile-onset spondyloarthropathies (JSpA) is a term that refers to a group of pediatric disorders characterized by enthesopathy and arthropathy. Children usually present with undifferentiated SpA and progress to differentiated forms over time. The hallmark of these diseases is sacroiliitis. [1]. Radiographic sacroiliitis is rarely seen in children; MRI

might identify acute and chronic changes in the sacroiliac joints of children with undifferentiated and definite SpA that might not be evident on radiography [2]. MRI, being an expensive investigation, may not be practical in every patient with back pain. Moreover, non-specific findings on spinal MRI may further compound the diagnostic problem, and therefore, not recommended for the work-up of routine back pain.

Infrared thermography (IRT) is a non-invasive technique that detects infrared radiation to provide an image of the temperature distribution across the body surface [3]. The physiological fundament of IRT is that the human body skin temperature distribution depends on the complex relationships defining the heat exchange processes between skin tissue, inner tissues, local vascularization, metabolic activity, and the regulating of the sympathetic and parasympathetic activity to maintain the homeostasis. The presence of a disease, then, locally interferes with the heat balance resulting in an increase or in a decrease of the skin temperature, both with respect to the surrounding regions or the unaffected contra lateral region [4-5]. Skin temperature, under controlled environmental conditions, is a function that

correlates with blood flow; the presence of an inflammatory disease interferes with the local heat balance and causes an abnormal increase of the upper skin temperature [6-8]. The aim of this study was to define the efficacy and applicability of IRT in detecting joint inflammation in children with JSpA.

Patients and Methods

We studied 30 children (20M, 10F, mean age 13yrs, range 8-20yrs) with active sacroiliitis, diagnosed by MRI with gadolinium enhancement and 10 age-matched children with scoliosis as controls. The thermographic analysis were performed with Digital Infrared Camera Land FTI 6 (plane array 256x256, HgTeCd sensor). The spectral band was within 3-5 μm. The time resolution was 0.05 s, and the temperature sensitivity wass 0.1 K. The temperature measurement noise was reduced to about 0.03 K by averaging each time 32 images with a delay of 30 ms. Emissivity of the skin was estimated as $\varepsilon = 0.95$. The measurements were performed in a temperature and

humidity controlled room (24°±1 °C and 60% of humidity, respectively, no direct ventilation).

The subjects were undressed and thermoequilibrated for 15 minutes [9], than thermographic images were taken in lumbar region. Considering the individual skin temperature variability and all conditions that can modify it, all thermal gradients ≥1°C between the studied area and the adjacent skin were considered as positives. Three thermal gradients were calculated: ΔA, ΔB, ΔC (between SI and lateral, medial and median areas, respectively). ΔA was considered the most reliable, being both medial and median areas excessively close to the studied joint and, mostly, comprised into the inflammatory zone.

All patient's parents signed informed consent form prior to inclusion in the study.

Results

Among almost 250 children who contacted our department because of suspected rheumatologic disorders in the period between February 2008 to September 2009, 30 (8,3%) were eligible for the study.

MRI performed in all children with SI pain associated with enthesopathy and/or peripheral arthritis at admission, confirmed diagnosis of JSpA showing edema in the upper quadrant of the sacrum and in the iliac bone, irregularities of the joint surface and even erosions and cysts.

At enrolment, before specific therapy onset (T0), patients showed an higher mean ΔA-gradients on IRT, if compared to those recorded in controls, both on right (1.7±1.2 vs 0.1±0.1, $p<0.0001$) and left joints (1.8±1.0 vs 0.2±0.1, $p<0.00001$).

A ΔA-gradient ≥1°C was recorded on the right SI joint in 73.3% of children affected by JSpA and on the left SI joint in 86.6%. Sacroiliac inflammation on IRT was not detectable in controls. The difference between cases and controls was statistically significant (right SI $p<0.001$; left SI $p<0.0001$); no difference was observed between right and left SI joints (Fig.1, Fig.2).

After 6 months of specific treatment (T1), infrared thermography was performed in 15 out 30 patients (50%). A significant reduction of ΔA-gradient, in respect to baseline, has been demonstrated in patients who underwent to the second evaluation after the introduction of treatment

(Fig.1c). After therapy onset, the percentage of patients who demonstrated a ΔA-gradient ≥1°C on IRT was significantly decreased when compared to the baseline evaluation (right 33 vs 73.3%, p=0.02, and left 40 vs 86.6%, p=0.003).

Discussion

To the best of our knowledge, this is the first study that provides information on the possible clinical implication of infrared functional imaging on pediatric patients with sacroiliitis.

The human body is a perfect emitter of infrared radiation with maximal emission around 8–10-μm wavelengths, and infrared thermography is defined as the recording of temperatures by means of infrared radiation at wave lengths between 0.8 μm and 1 mm [10].

Although the body transfers heat to the surrounding using conduction, convection, evaporation and radiation, under stable ambient conditions of 18–25°C, the principal mechanism to achieve equilibrium between the body and its environment is radiation [11] : this makes IRT suitable to record the temperature differences in clinical practice by a

non- invasively approach and without any radiation-like side effects.

IRT has been proposed in several pediatric fields of application, such as studying and following hemangiomas, vascular malformation, varicoceles, abscess [12].

Some of the clinical applications of thermography in rheumatology reported so far are the assessment of inflamed joints [13], the response to cold challenge of the hands in Raynaud's phenomenon [14] and algodystrophy [15].

The clinical application of IRT has a long history in musculoskeletal disorders.

After the first report of thermographic evaluation of pain by Albert et al1 in 1964, there have been several studies in this field. High sensitivity of IRT, correlations between thermographic and other findings, such as computed tomography, myelography, and magnetic resonance imaging, and thermographic abnormalities associated with lumbar radiculopathy have been demonstrated [16-20]. The different termographic pattern of the sacro-iliac joints in health and in active (clinically and radiographically) inflammation has been established in various studies and it

has been showed an increased heat over the sacro-iliac joints [21].

A number of imaging technologies have been studied in an effort to improve the assessment of articular inflammation activity. However, all of the current technologies have limitations. For instance, plain radiographs are insensitive to early changes and ultrasound can quantify changes in effusion and synovitis, but it is highly user-dependent. MRI has proven to be more sensitive and reliable than clinical examination in the detection of bone edema and has the ability to quantify changes in synovial volumes and erosions [22,23]. However, MRI involves substantial time and cost, exposure to contrast agents, and the need for sedation in young children [24].

Other imaging modalities, such as bone scintigraphy, have been proposed as tools to improve reproducibility and quantify changes in SI joints. Unlike thermal surface imaging, which collect exterior joint data, these other modalities examine structures below the joint surface.

IRT can be performed in the pediatric age group, is non-invasive, without any biological side effects, requires no sedation or anesthesia and can be repeated as desired for

follow-up, with objective results that can demonstrated as colored images, stored in the computer memory. Periodic thermographic studies to follow progression of lesions seem to be a useful and reproducible method for repeated and long-term examination [12].

Another characteristic of IRT studies is that this method can get additional information about the status and disturbances of the sympathetic vasomotor tone. It gives a possibility to evaluate the vasomotor activity of the sympathetic nerve fibers and to detect possible sympathetic dysfunction, which cannot be shown by present conventional methods [25].

The ability of the IRT equipment to produce color images is of further advantage in explaining to the older or school-age child or the parents the results of the examination without the use of complicated medical terminology. This procedure has found an excellent acceptance by the children and their parents as a primary or a follow-up procedure.

Conclusion

The findings from this study suggest that surface imaging could be used to improve the assessment of disease activity

in sacroiliitis. Although the number of subjects we analyzed was small and will require further validation, our results demonstrate that this approach is feasible. The IRT measures described in this study were accurate and sensitive to small changes in joint volume and shape (inflammation). Thermal gradients values greater than 1°C could be used to identify patients with active sacroiliitis.

Our data suggest that IRT is an effective and useful tool for diagnosis of early sacroiliitis but might be more helpful in the objective serial assessment of SI in patients with active disease. A long term follow up and more large clinical studies are necessary to validate IRT as a diagnostic tool applicable in daily clinical practice.

References

Gensler L, Davis JC Jr (2006) Recognition and treatment of juvenile-onset spondyloarthritis. Curr Opin Rheumatol 18:507-11.

Dougados M, Hochberg MC (2002) Why is the concept of spondyloarthropathies important? Best Pract Res Clin Rheumatol 16:495–505

Burgos-Vargas R (2002) The juvenile-onset spondyloarthropathies. Rheum Dis Clin North Am 28: 531–560

Sulpice M, Deslandre CJ, Quartier P (2009) Efficacy and safety of TNFalpha antagonist therapy in patients with juvenile spondyloarthropathies. Joint Bone Spine 76:24-7.

Bollow M, Biedermann T, Braun J, et al (1998) Use of dynamic magnetic resonance imaging to detect sacroiliitis in HLA B-27 positive and negative children with juvenile arthritides. J Rheumatol 25:556–64.

Kastberger G, Stachl R (2003) Infrared imaging technology and biological applications. Behav Res Methods Instrum Comput. 35:429-39.

Merla A, Romani G (2005) Biomedical Applications of Functional Infrared Imaging. Conf Proc IEEE Eng Med Biol Soc 1:690-3.

Merla A, Romani GL (2006) Functional infrared imaging in medicine: a quantitative diagnostic approach. Conf Proc IEEE Eng Med Biol Soc 1:224-7.

Grennam DM, Caygill L (1982) Infra-red thermography in the assessment of sacro-iliac inflammation. Rheumatol Rehabil 21:81-7.

Engels JM, Flesch U, Stuttgen G (1985) Thermological methods: Thermological terminology. In: Engel JM, Ring EFJ (eds) Applied thermology. VCH, Weinheim, pp 269–272

Stuttgen G, Flesch U (1985) Dermatological thermography thermography of the skin. In: Engel JM, Ring EFJ (eds) Applied thermology. VCH, Weinheim, pp 13–20

Saxena AK, Willital GH. Infrared thermography: experience from a decade of pediatric imaging. Eur J Pediatr. 2008 Jul;167(7):757-64

Iwase S, Cui J, Wallin BG, Kamiya A, Mano T. Effects of increased ambient temperature on skin sympathetic nerve activity and core temperature in humans. Neurosci Lett. 2002;327:37–40. doi: 10.1016/S0304-3940(02)00374-9.

Wasner G, Schattschneider J, Heckmann K, Maier C, Baron R. Vascular abnormalities in reflex sympathetic dystrophy (CRPS I): mechanisms and diagnostic value. Brain. 2001;124:587–599. doi: 10.1093/brain/124.3.587.

Huygen FJ, Niehof S, Klein J, Zijlstra FJ. Computer-assisted skin videothermography is a highly sensitive quality

tool in the diagnosis and monitoring of complex regional pain syndrome type I. Eur J Appl Physiol. 2004

Albert SM, Glickman M, Kallish M 1964. thermography in orthopedics. Ann N Y Acad Sci. 121:157-70

Sherman RA, Barja RH. Thermographic correlates of chronic pain: analysis of 125 patients incorporating evaluations by a blind panel. Arch Phys Med Rehabil 1987;68:273-9.

LaBorde TC. Thermography in diagnosis of radiculopathies. Clin J Pain 1989;5:249-53.

Thomas D, Cullum D, Siahamis G, Langlois S. Infrared thermographic imaging, magnetic resonance imaging, CT scan and myelography in low back pain. Br J Rheumatol 1990;29:268-73.

Takahashi Y, Takahashi K, Moriya H. Thermal deficit in lumbar radiculopathy. Correlations with pain and neurologic

signs and its value for assessing symptomatic severity. Spine 1994;19:2443-50.

Jacobsson H, Vestersköld . The thermographic pattern of the lower back with special reference to the sacro-iliac joints in health and inflammation. Clin Rheumatol. 1985 Dec;4(4):426-32.

L.Bird P, Lassere M, Shnier R, Edmonds J. Computerized measurement of magnetic resonance imaging erosion volumes in patients with rheumatoid arthritis: a comparison with existing magnetic resonance imaging scoring systems and standard clinical outcome measures. Arthritis Rheum. 2003;48:614–624. doi: 10.1002/art.10820.

Goupille P, Roulot B, Akoka S, Avimadje AM, Garaud P, Naccache L, Le Pape A, Valat JP. Magnetic resonance imaging: a valuable method for the detection of synovial inflammation in rheumatoid arthritis. J Rheumatol. 2001;28:35–40

Spalding SJ, Kwoh CK, Boudreau R et al. Three-dimensional and thermal surface imaging produces reliable measures of joint shape and temperature: a potential tool for quantifying arthritis. Arthritis Res Ther. 2008;10(1):R10.

Zaproudina N, Ming Z, Hanninen OO (2006) Plantar infrared thermography measurements and low back pain intensity. J Manipulative Physiol Ther 29:219-23.

CONCLUSIONI

Le malattie auto infiammatorie e le malattie autoimmuni sono dovute ad un'alterazione nell'omeostasi del sistema immunitario, le prime dovute a un' alterazione dell'immunità innata e le seconde sia di quella innata che di quella adattativa.

Le malttie auto infiammatorie e le malattie autoimmuni sono attualmente suddivise in due gruppi distinti, ma considerando le similitudini nel quadro clinico e bioumorale e la risposta terapeutica, potrebbero essere collocate in un continuum di malattia, quindi considerate come un unico gruppo di patologie con un ampio spettro di anomalie immunologiche e cliniche che comprendono ad un'estremità le malttie autoimmuni ed all'altra le malattie autoinfiammatorie.

Ulteriori studi sul ruolo potenziale dell'inflammasoma nell'autoimmunità, potrebbero comportare l'identificazione di nuovi target terapeutici per il trattamento delle diverse malattie autoimmuni.