Juvenile Idiopathic Arthritis: Towards Improving Clinical Care

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Anticarbamylated protein (anti-CarP) antibodies are present in sera of Juvenile Idiopathic Arthritis (JIA) patients

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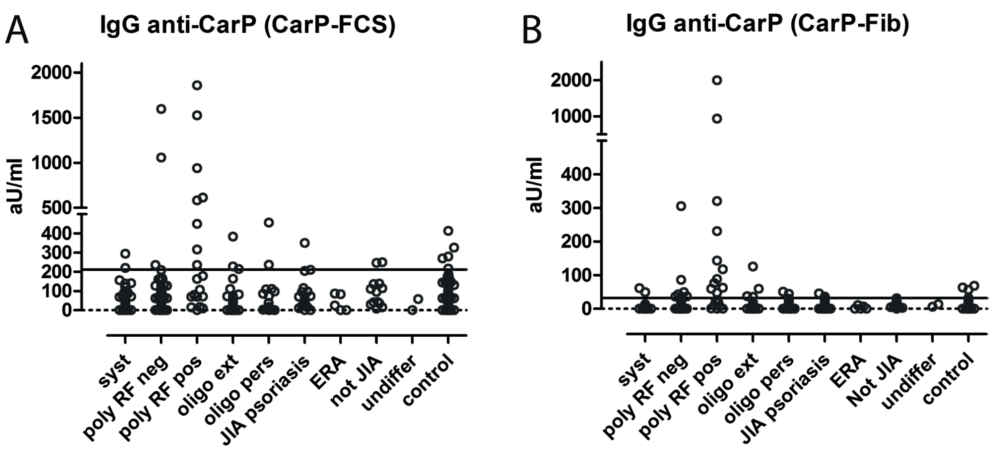
In Juvenile Idiopathic Arthritis (JIA) patients there is a lack of markers that predict severe disease. Although anti-citrullinated protein antibodies (ACPA) have contributed substantially to the understanding of Rheumatoid Arthritis (RA)[1], their detection in JIA has not been equally useful as incidence rates in JIA patients are low[2] and merely confined to the polyarticular IgM-RF positive category resembling RA. Recently, anti-carbamylated protein antibodies (anti-CarP) were detected in 45% of RA patients and importantly also in 16-20% ACPA-negative patients[3-5]. Within the ACPA-negative patients, anti-CarP antibodies were associated with more severe radiographic progression[3]. Since most JIA patients are ACPA-negative we investigated whether anti-CarP antibodies are present in sera of JIA patients and how they are related to ACPA and IgM-RF.

JIA patients from three Dutch sources were included: the *BeSt for Kids trial* (NTR 1574, a treatment strategy study) (n=33), a previously described cohort[6] (n=48) and the Arthritis and Biologicals in Children (ABC) Register[7] (n=153). Healthy controls (n=107) (mean age/ range 11/(2-20)) are stem-cell graft donors. Written informed consent was obtained from all patients and controls. Blood collection and storage are comparable among different cohorts. Cross-sectionally obtained sera from 234 JIA patients at variable time points in disease course were analyzed. All ILAR JIA categories were included[8] with polyarticular JIA overrepresented. Median disease duration at the time of serum collection was 2.3 years (IQR 0.7-6.8) (Table 1). Patients' disease characteristics were collected from patient files. Anti-CarP and ACPA antibodies were measured by ELISA as described previously.[3]

Table 1 | Disease characteristics of 234 JIA patients

Characteristics	**Number**
Gender m/f (%f)	76/158 (67,5%)
Median age (years) (IQR)	12.1 (8.4-16.2)
Median disease duration (IQR)	2.3 (0.7-6.8)
Median age at JIA onset (IQR)	8.8 (3.4-12.4)
ANA positive at disease onset	64 (27,4%)
Systemic JIA	35 (15,0%)
Poly-articular JIA RF negative	90 (38.5%)
Poly-articular JIA RF positive	19 (8,1%)
Oligo-articular JIA extended	41 (17,5%)
Oligo-articular JIA persistent	18 (7.7%)
Juvenile Psoriatic Arthritis	24 (10,3%)
Enthesitis Related Arthritis	5 (2.1%)
Undifferentiated	2 (0.8%)

We observed that 8.1% (19/234) of the JIA patients were positive for anti-Ca-FCS antibodies versus 4.7% (5/107) of controls (p=0.20); 13.2% (31/234) of patients vs 2.8% (3/107) of controls were positive for anti-Ca-Fib antibodies (p=0.003); 16.7% (39/234) of patients vs 8/107 (7,5%) of controls were positive for at least one anti-CarP antibody (p=0.028); 11/234 (4,7%) vs 0 of controls (p=0,017) were positive for both anti-CarP reactivities. Both anti-Ca-FCS and anti-Ca-Fib antibodies were predominantly present in polyarticular IgM-RF positive patients compared to other JIA categories (p<0.0001) (Figure 1). Additionally 53% (8/15) of ACPA-positive patients and 42.1% (8/19) of IgM-RF-positive patients were also



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	anti-CarP-FCS		anti-CarP-Fib	
	n/total (%)		n/total (%)	
All JIA in total	19/234 (8.1%)	NS	31/234(13.2%)	**
Systemic onset JIA	2/35 (5.7%)	NS	2/35(5.7%)	NS
Polyarticular RF negative	3/90 (3.3%)	NS	9/90(10%)	*
Polyarticular RF positive	8/19 (42.1%)	**	11/19(57.9%)	**
Oligo articular extended	3/41 (7.3%)	NS	4/41(9.8%)	NS
Oligo articular persistent	2/18 (11.1%)	NS	2/18(11.1%)	NS
Juvenile Psoriatic Arthritis	1/24 (4.2%)	NS	3/24(12.5%)	NS
Enthesitis related Arthritis	0	NS	0	NS
Undifferentiated	0	NS	0	NS
Controls	5/107 (4.7%)		3/107 (2.8%)	

Figure 1 | IgG anticarbamylated protein (anti-CarP) antibodies are present in juvenile idiopathic arthritis (JIA) sera. A cut-off for positivity(horizontal line) was determined using the mean plus two times the SD of the healthy controls. Antibodies against Ca-FCS (A) and Ca-Fib (B) in thesera of JIA patients and healthy controls are depicted in aU/mL. (C) Results of anti-CarP antibodies: positivity above cut-off per JIA category inabsolute number, percentage and significance (NS, not significant, *p<0.05, **p<0.01). FCS, fetal calfs serum; RF, rheumatoid factor.

positive for anti-CarP antibodies. Importantly, anti-CarP antibodies were also found in ACPA and IgM-RF-negative patients as 57,9% (11/19) of anti-CarP positive patients were negative for ACPA and 27,3% (3/11) were negative for IgM-RF. In total 9 JIA patients were positive for IgM-RF, ACPA and anti-CarP (Ca-FCS and/or Ca-Fib) antibodies. All triple positive patients were part of the ABC-register.

Disease duration at sample collection, ANA status or age were not associated with the presence of anti-CarP antibodies. In the second cohort[6] we did not find an association of anti-CarP positivity with disease activity measured by time-in-active-disease at the time of sampling. Within the ABC-register cohort no association was found between the presence of anti-CarP antibodies and ACR-Pedi 30 response[9] or reaching inactive disease[10] at 15 months after start of anti-TNF treatment. The cross-sectional nature of these three cohorts did not allow a more in depth analysis on association with clinical outcome.

This is the first study showing the presence of anti-CarP antibodies in JIA stimulating future studies on the diagnostic and prognostic value of anti-CarP antibodies in JIA.

Competing Interest: None declared.

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