

Is HLA-B27 Increased in Patients Diagnosed with Undifferentiated Arthritis? Results from the Leiden Early Arthritis Cohort

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ABSTRACT. Objective. Undifferentiated arthritis (UA) is a common form of arthritis. According to the Assessment of Spondyloarthritis international Society (ASAS) criteria for peripheral spondyloarthritis (pSpA), HLA-B27 can be used to help classify patients with pSpA. We tested whether HLA-B27 is increased in patients diagnosed with UA.

Methods. Prevalence of HLA-B27 was compared between healthy controls and patients with UA. SpA features were compared between HLA-B27-positive and -negative UA, and SpA.

Results. We found 10.1% of UA (38/375) versus 7.2% (403/5584) of controls were HLA-B27-positive (OR 1.5, 95% CI 1.0–2.1; $p = 0.037$). HLA-B27-positive patients with UA had more SpA features than HLA-B27-negative patients (mean 1.6, SD 1.0, and 0.9 SD 0.6; $p < 0.001$), but patients with SpA had significantly more SpA features (mean 4.5, SD 1.5; $p < 0.001$). Family history and preceding infection were features more common in HLA-B27-positive than in HLA-B27-negative UA (15.8% vs 1.3%, $p = 0.04$ and 15.8% vs 2.6%, $p = 0.04$). After HLA-B27 testing, 21 additional patients (5.6%) with UA could potentially have been classified with pSpA according to the ASAS criteria.

Conclusion. HLA-B27 is more common in patients with UA than in controls. However, the yield of HLA-B27 testing in UA is low. Our results suggest that HLA-B27 testing should be reserved for patients with additional SpA features. (First Release Aug 15 2014; J Rheumatol 2014; 41:1948–51; doi:10.3899/jrheum.131462)

Key Indexing Terms:

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Spondyloarthritis (SpA) refers to a group of common related rheumatic diseases, including ankylosing spondylitis, psoriatic arthritis, reactive arthritis, inflammatory bowel disease (IBD)-related spondylitis and arthritis, and undifferentiated SpA. The clinical presentation of SpA is heterogeneous, and no single distinguishing feature exists for all forms of SpA¹. However, various clinical laboratory and imaging features suggestive of SpA are known and form the building blocks for classification criteria for SpA and diseases in the SpA spectrum.

SpA can be distinguished by its clinical presentation as predominantly axial SpA (axSpA; e.g., inflammation of the spine and/or sacroiliac joints) or peripheral SpA (pSpA; e.g.,

arthritis, dactylitis, and/or enthesitis) and classified with 2 criteria sets developed by the Assessment of Spondyloarthritis international Society (ASAS). In patients with early arthritis, the ASAS pSpA criteria performed well with a good specificity at a reasonable sensitivity².

According to the ASAS criteria for pSpA, HLA-B27 can be used to help classify patients with pSpA³. While in adults HLA-B27 is better known for its association with axSpA rather than pSpA⁴, we recently showed that in a large cohort of patients presenting with arthritis, HLA-B27 was significantly more common in patients with SpA than in patients without SpA².

Undifferentiated arthritis (UA) is defined as an inflammatory arthritis in which no definitive diagnosis can be made. Inception cohorts from Europe and North America have shown that UA is common and may even be seen more frequently than rheumatoid arthritis (RA). Estimates of the percentage of patients diagnosed with UA range from 23% to 81% of early arthritis cohorts⁵. UA spans a wide spectrum of conditions with various natural courses. Some patients will have a benign, self-limiting disease course while others will experience disease persistence including development of destructive arthritis⁶. There are limited data on the preva-

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lence of HLA-B27 in UA but 1 study has reported an HLA-B27 prevalence of 16% in 75 patients with UA compared to 7% in controls⁷. This suggests that HLA-B27 is increased in UA and using the new ASAS pSpA criteria, HLA-B27 could be used to classify a proportion of UA as pSpA. Further, based on clinical experience, the 3e Initiative in Rheumatology recommends testing HLA-B27 in UA when SpA is suspected⁸. The aim of our study is to test the prevalence of HLA-B27 in a large cohort of patients with UA and to assess whether HLA-B27 testing is useful in patients with UA.

MATERIALS AND METHODS

Patients and data. Patients in our study were all participants of the Leiden Early Arthritis cohort (EAC). The EAC has been described extensively⁹. In short, the EAC is a population-based prospective cohort including patients with suspected arthritis with a symptom duration < 2 years referred by physicians to the rheumatology outpatient clinic of the Leiden University Medical Center to detect and treat rheumatic diseases in an early state⁹. Between February 1993 and February 2009, 2285 patients were included. All patients underwent an extensive analysis including but not limited to urinalysis, radiographs of affected joints and chest, and blood analysis of, among other things, acute-phase reactants, uric acid, creatinine, IgM-rheumatoid factor (RF), and anticitrullinated protein antibodies. Treating physicians were free to order any additional tests outside the protocol. Followup visits with standard clinical assessments were performed 3 months after the first presentation and yearly thereafter. Radiographs of the hands and feet were taken at baseline and yearly thereafter.

At the 1-year followup visit, according to the diagnosis of the treating rheumatologist, UA was found in 674 patients (29.5%). RA was diagnosed in 973 patients (42.6%) and SpA in 226 patients (9.9%). SpA features of the 226 patients with SpA have been reported². The remaining 412 patients (18%) had a large variety of diagnoses including systemic lupus erythematosus, osteoarthritis, gout, and infectious arthritis.

Data taken from the EAC database included age at inclusion, sex, localization of arthritis, the number and the pattern of affected joints (e.g., symmetric or asymmetric arthritis), erythrocyte sedimentation rate, C-reactive protein, and IgM-RF. HLA-B27 testing was not part of the study protocol. However, doctors were able to request it when they considered it necessary. Chart review showed that none of the patients with UA were typed for HLA-B27 prior to our study.

HLA-B27 testing. For our study, HLA-B27 was typed in all patients with UA using available DNA (n = 375). The remaining 299 patients with UA were not HLA-B27 typed: DNA samples of 89 patients were of insufficient quality or had been depleted and in 210 patients with UA, no samples had ever been stored because DNA sampling was only implemented a few years after the start of the cohort. HLA-B27 typing was performed with sequence-specific primers on genomic DNA with real-time polymerase chain reaction using SYBR Green. In a total volume of 7 μ l 12.5 ng, gDNA was mixed with 3.5 μ l iQ SYBR Green Supermix (Biorad Laboratories) and 100 nM of both forward primer (5'-GCT ACG TGG ACG ACA CGC T) and reverse primer (5'-GCG CCC GCG GCT CCT CT). Reactions were performed in a CFX-384 thermocycler (Biorad Laboratories) with the following protocol: 95.0°C for 3 min followed by 40 amplification cycles (95.0°C for 0:10; 71.0°C for 0:25); standard melting curves¹⁰. Results of HLA-B27 typing of 5584 blood donors who served as healthy controls were used for comparison¹¹.

SpA features. To compare SpA features between HLA-B27-positive and HLA-B27-negative patients with UA, a nested case-control analysis was performed. In total, 38 patients with UA were HLA-B27-positive. Each HLA-B27-positive patient with UA was matched to 2 HLA-B27-negative patients with UA (n = 76) based on age at inclusion and sex (p = 0.6 and

p = 1.00, respectively). Charts from these 114 patients (from baseline to 1-yr followup) were searched for additional SpA features as mentioned in the following criteria sets: Amor criteria, European Spondyloarthropathy Study Group criteria, ASAS pSpA criteria for pSpA, and CIASSification criteria for Psoriatic ARthritis (CASPAR) criteria^{3,12,13,14}. These features are family history for SpA, inflammatory back pain (IBP), psoriasis, dactylitis, enthesitis, uveitis, IBD, preceding infection (urethritis/cervicitis or diarrhea within 1 mo before the onset of arthritis/dactylitis/enthesitis), sacroiliitis on imaging (radiograph and/or MRI), a good response to nonsteroidal antiinflammatory drugs, RF negativity, and juxtaarticular new bone formations^{3,12,13,14}.

Statistical analysis. Crosstab analysis expressed in OR was used to study the frequency of HLA-B27 in patients with UA and SpA, and in controls. Throughout the study, chi-squared tests were used for dichotomous variables and Mann-Whitney U tests for continuous variables. Wilcoxon signed-rank test and McNemar's test were used for matched samples. SPSS 20.0 and Epi Info was used for analyses and graphs. P values < 0.05 were considered significant.

RESULTS

Of the 375 HLA-B27-typed patients with UA, 173 (46%) presented with a monoarthritis, 116 (31%) with oligoarthritis, and 86 (23%) with polyarthritis (Table 1). Of the patients with UA, 38/375 (10.1%) were HLA-B27-positive compared to 403/5584 Dutch blood donors (7.2%). This gave an OR of 1.5 (95% CI 1.0–2.1; p = 0.037). In comparison, 48/186 (25.8%) of patients with SpA (Table 2) were HLA-B27-positive (OR 4.5; 95% CI 3.2–6.3; p < 0.001 compared to healthy controls).

The 3 most frequently reported SpA features in the 114 patients with UA in the nested cases-control analysis were enthesitis (11.4%), psoriasis (8.8%), and a preceding infection (7%), in addition to RF negativity (72.8%). Family history (p = 0.04) and preceding infection (p = 0.04) were more common in HLA-B27-positive patients with UA than in HLA-B27-negative patients with UA. No significant differences were found for other SpA features, in the arthritis pattern (Table 2), or the number of affected joints (data not shown).

Overall, HLA-B27-positive patients with UA had more SpA features (mean 1.6; SD 1.0) than did

Table 1. Baseline characteristics of 375 HLA-B27 typed patients with undifferentiated arthritis.

Characteristic	
Age at inclusion, mean (SD)	50.5 (16.5)
Male, n (%)	147 (39.2)
Symptom duration, weeks (range)	16 (0–79)
ESR, mean (SD)	24.7 (24.1)
CRP, mean (SD)	17.0 (27.6)
Number of affected joints, mean (range)	2.5 (1–14)
Arthritis pattern	
Monoarthritis, n (%)	173 (46)
Oligoarthritis, n (%)	116 (31)
Polyarthritis, n (%)	86 (23)
HLA-B27 positivity, n (%)	38 (10.1)

ESR: erythrocyte sedimentation rate; CRP: C-reactive protein.

Table 2. Clinical characteristics of 38 HLA-B27-positive patients with undifferentiated arthritis (UA) and 76 age-matched and sex-matched HLA-B27-negative patients with UA, and 226 patients with spondyloarthritis (SpA). Data are n (%) unless otherwise indicated.

	HLA-B27+ Patients with UA, n = 38	HLA-B27- Patients with UA, n = 76	Patients with SpA, n = 226	p (patients with UA HLA-B27+ vs HLA-B27-)	p (patients with UA HLA-B27+ vs SpA)
Age, yrs, mean (SD)	48 (16.2)	48.2 (16.0)	43.7 (15.0)	0.6	0.11
Male	15 (39.5)	30 (39.5)	123 (54.4)	1.0	0.09
Arthritis pattern					
Monoarthritis	14 (36.8)	22 (28.9)	51 (24.9)		
Oligoarthritis	17 (44.7)	34 (44.7)	74 (36.1)	0.25 [#]	0.02 [#]
Polyarthritis	7 (18.4)	20 (26.3)	80 (39.0)		
Family history	6 (15.8)	1 (1.3)	169 (74.7)	0.04	< 0.001
Inflammatory back pain	2 (5.3)	0 (0)	35 (15.5)	0.25	0.09
Psoriasis	6 (15.8)	4 (5.3)	145 (64.2)	0.13	< 0.001
Dactylitis	2 (5.3)	1 (1.3)	60 (30.2)	0.13	< 0.001
Enthesitis	6 (15.8)	7 (9.2)	40 (17.7)	0.11	0.77
Asymmetric lower limb arthritis	7 (18.4)	12 (15.8)	64 (28.3)	0.31	0.20
Uveitis	2 (5.3)	0 (0)	8 (3.5)		0.61
Inflammatory bowel disease	0 (0)	0 (0)	12 (5.3)	—	0.26
History of preceding infection	6 (15.8)	2 (2.6)	38 (16.8)	0.04	0.88
Sacroiliitis on imaging	0 (0)*	0 (0)***	12 (20.6) [§]	—	0.38
RF-negative	30 (78.9)**	53 (69.7)	200 (91.7)	0.06	0.02
Good response to NSAID	1 (2.6)	0 (0)	7 (3.1)	0.32	0.88
Juxtaarticular bone formation	0 (0)	0 (0)	19 (8.4)		0.13
CRP, mg/l, mean (SD)	10.3 (11.4)	14.8 (24.5)	26.6 (35.6)	0.16	< 0.001
ESR, mm/h, mean (SD)	23.1 (23.7)	23.0 (20.6)	33.5 (29.7)	0.99	0.02
No. SpA features ^{##} , mean (SD)	1.6 (1.0)	0.9 (0.6)	4.5 (1.5)	< 0.001	< 0.001

[#] Comparing polyarthritis versus non-polyarthritis. ^{##} Not including HLA-B27. ^{***} Tested in 14 patients. ^{*} Tested in 6 patients. ^{**} Tested in 37 patients. [§] Tested in 58 patients. Numbers in bold face are statistically significant. ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; NSAID: nonsteroidal antiinflammatory drugs.

HLA-B27-negative patients with UA (mean 0.9; SD 0.6; $p < 0.001$; Table 2).

In the HLA-B27-positive UA group, 4/38 (10.5%) had no additional SpA features, 16/38 (42.1%) had 1 additional SpA feature, 10/38 (26.3%) had 2 SpA features, 7/38 (18.4%) had 3 SpA features, and 1/38 (2.6%) had 4 additional SpA features. In the HLA-B27-negative UA group, 16/76 (21.1%) had no SpA features, 53/76 (69.7%) had 1 SpA feature, 6/76 (7.9%) had 2 SpA features, and 1/76 (1.3%) had 3 SpA features.

Before HLA-B27 testing, 23/114 patients (20.2%) with UA fulfilled the ASAS pSpA criteria; 17 were HLA-B27-positive and 6 HLA-B27-negative. The most common SpA features resulting in a classification were psoriasis and a preceding infection. After HLA-B27 testing, 38 HLA-B27-positive patients with UA met these criteria, including the 17 patients that already met the ASAS pSpA criteria before testing. It is noteworthy that 7 HLA-B27-positive patients with UA did not have any other SpA features and had an arthritis pattern that was not asymmetric nor localized in the lower limb.

Given these results, we analyzed whether an asymmetric arthritis of the lower limb by itself would have given a higher percentage of HLA-B27 positivity in UA instead of testing all patients with UA. Sixty-four out of 375 patients

with UA had symmetric lower limb arthritis. Only 7/64 (10.1%) were HLA-B27-positive, the same percentage as in the entire group of patients with UA ($p = 0.7$).

Next, we compared the number of SpA features in the HLA-B27-positive patients with UA to the number of SpA features in patients with SpA (mean 4.5; SD 1.5). The result was statistically significantly higher in the latter group ($p < 0.001$). In patients with SpA, family history for SpA, IBP, psoriasis, and dactylitis were all more common than in HLA-B27-positive patients with UA ($p < 0.001$) as well as RF negativity ($p = 0.02$; Table 2). Age and sex were not different between the UA and SpA groups. Patients with SpA more often presented with a polyarthritis than did patients with UA [80/205 (39%) and 7/38 (18.4%) respectively; $p = 0.02$].

DISCUSSION

The prevalence of HLA-B27 in UA is higher than in controls. Although the results are statistically significant, the increase in HLA-B27 positivity in UA is modest at best (10.1% HLA-B27 positivity in UA compared to 7.2% in controls). This percentage is lower than the 16% (12/75 patients) reported in a previous study in patients with UA⁷. This could be because the EAC is a longstanding cohort and we have analyzed patients from 1993 to 2009. Physicians'

perceptions may change over time and this may be particularly relevant in diagnosing UA and SpA.

Classification criteria are not the same as diagnostic criteria. A clear example of the difference between diagnosis and classification is found in our cohort, where before HLA-B27 testing, 21 patients with UA met the ASAS classification criteria for pSpA but the clinical diagnosis was UA.

Classification criteria may influence clinical practice. This is one of the reasons we chose to study patients with UA included before 2010, because the new American College of Rheumatology/European League Against Rheumatism criteria for RA were published in 2010 and the ASAS pSpA criteria in 2011. Data from the Leiden EAC show that when the 2010 RA criteria are used as diagnostic criteria instead of the 1987 RA criteria, the number of patients with UA drops 33%, thereby potentially influencing what is considered as UA in daily practice¹⁵.

A limitation of this study was that not all patients with UA could be typed for HLA-B27. However, the reason for absence of DNA was not related to the outcome of the study and we found no differences in baseline characteristics between patients tested and not tested for HLA-B27 (data not shown).

Unfortunately, because the number of physicians working in the EAC over the years is large, it was not feasible to ask physicians if they would change their diagnosis based on the results of the HLA-B27 typing. Nevertheless, given the low number of HLA-B27-positive patients with UA, it is unlikely that this would have a substantial effect on a large number of patients.

The number of HLA-B27-positive patients with UA was low but selection of patients with UA on the pattern of the arthritis by itself to test for HLA-B27 (instead of testing all patients with UA) did not increase the percentage of HLA-B27 positivity. These results are in line with other studies that found no association between HLA-B27 and the location of the arthritis^{7,16}. The low yield of testing HLA-B27 in patients with UA as a group, but the observation that HLA-B27-positive patients with UA have more SpA features than HLA-B27-negative patients, raises the question of which patients with UA are suitable candidates for HLA-B27 testing.

To our knowledge there are no universally accepted diagnostic criteria for pSpA, but if the goal were to evaluate whether a patient with arthritis could be classified as pSpA, the first step would be to assess whether the criteria are already met with SpA features readily obtained from the patient's history and physical examination. For example, a patient with arthritis and a preceding infection already fulfills the ASAS pSpA criteria, but a patient with arthritis and a family history does not meet the criteria. But in the latter case, testing positive for HLA-B27 means the arthritis can be classified as pSpA. Thus, HLA-B27 testing in

patients with UA is useful only if a patient would meet criteria after testing positive.

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