Frequency of triggering bacteria in patients with reactive arthritis and undifferentiated oligoarthritis and the relative importance of the tests used for diagnosis

C Fendler, S Laitko, H Sörensen, C Gripenberg-Lerche, A Groh, J Uksila, K Granfors, J Braun, J Sieper

Abstract

Objectives—Reactive arthritis (ReA) triggered by Chlamydia trachomatis or enteric bacteria such as yersinia, salmonella, Campylobacter jejuni, or shigella is an important differential diagnosis in patients presenting with the clinical picture of an undifferentiated oligoarthritis (UOA). This study was undertaken to evaluate the best diagnostic approach.

Patients and methods—52 patients with ReA, defined by arthritis and a symmetrical preceding infection of the gut or the urogenital tract, and 74 patients with possible ReA, defined by oligoarthritis without a preceding symptomatic infection and after exclusion of other diagnoses (UOA), were studied. The following diagnostic tests were applied for the identification of the triggering bacterium: for yersinia induced ReA—stool culture, enzyme immunoassay (EIA), and Widal's agglutination test for detection of antibodies to yersinia; for salmonella or campylobacter induced ReA—stool culture, EIA for the detection of antibodies to salmonella, and stool culture, enzyme immunoassay (EIA), and Widal's agglutination test for detection of antibodies to yersinia; for infections with shigella—stool culture; for infections with Chlamydia trachomatis—culture of the urogenital tract, micro-immunofluorescence and immunoperoxidase assay for the detection of antibodies to Chlamydia trachomatis.

Results—A causative pathogen was identified in 29/52 (56%) of all patients with ReA. In 17 (52%) of the patients with enteric ReA one of the enteric bacteria was identified: salmonella in 11/33 (33%) and yersinia in 8/33 (24%). Chlamydia trachomatis was the causative pathogen in 12/19 (63%) of the patients with urogenic ReA. In patients with the clinical picture of UOA a specific triggering bacterium was also identified in 35/74 (47%) patients: yersinia in 14/74 (19%), salmonella in 9/74 (12%), and Chlamydia trachomatis in 12/74 (16%).

Conclusions—Chlamydia trachomatis, yersinia, and salmonella can be identified as the causative pathogen in about 50% of patients with probable or possible ReA if the appropriate tests are used.

Reactive arthritis (ReA) is a well-known complication of enteric infections caused by yersinia, salmonella, shigella, and campylobacter, or of urogenital tract infections caused by Chlamydia trachomatis. This form of ReA is regarded as part of the spondyloarthropathies and HLA-B27 is positive in about 50% of these patients. The arthritis has a typical joint pattern which is also characteristic for the whole group of spondyloarthropathies: an asymmetrical arthritis predominantly of the legs. In most patients an oligoarthritis or monoarthritis is present. Patients with such a joint pattern constitute up to 50% of patients in clinics for early arthritis. Therefore ReA is an important differential diagnosis. Despite the clinical relevance there are no established criteria available for the diagnosis of ReA. Earlier criteria relied almost exclusively on clinical indicators of a symptomatic preceding infection, such as urethritis/cervicitis, or on symptoms characteristic for the whole group of spondyloarthropathies. However, it is likely that in a substantial proportion of patients with ReA the preceding infection is asymptomatic or associated with only a few symptoms, often labelled as undifferentiated arthritis or undifferentiated oligoarthritis (UOA). Better laboratory tests identifying the triggering bacteria are now available and are increasingly used for the diagnosis of ReA. Especially in patients with possible ReA or UOA.

In this study we used all available tests (except polymerase chain reaction (PCR) for the detection of bacteria in the joint) for the identification of the bacteria causing ReA in a large number of patients with ReA (having a symptomatic preceding infection of the gut or the urogenital tract) or possible ReA (UOA with a joint pattern compatible with ReA). We report the frequency of single bacteria in these two subgroups and the relative importance of the different tests used.

Patients and methods

Patients’ selection and characteristics
In this study 126 patients from different rheumatology clinics in Berlin, Germany, with a clinical diagnosis of ReA (n=52) or UOA (n=74) were included. A diagnosis of ReA was made if patients presented with the clinical picture of an asymmetrical arthritis and a preceding symptomatic urethritis or enteritis no longer than four weeks before the onset of arthritis. A diagnosis of UOA was made after...
Table 1 Characteristics of all patients, patients with reactive arthritis (ReA), and patients with undifferentiated oligoarthritis (UDA).  

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>ReA</th>
<th>UDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>126</td>
<td>52</td>
<td>74</td>
</tr>
<tr>
<td>Age (years)</td>
<td>36.6</td>
<td>36.8</td>
<td>37.4</td>
</tr>
<tr>
<td>Range</td>
<td>18–65</td>
<td>18–60</td>
<td>18–65</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>66/60</td>
<td>28/24</td>
<td>38/36</td>
</tr>
<tr>
<td>Disease duration (week)</td>
<td>14</td>
<td>8</td>
<td>30</td>
</tr>
<tr>
<td>Range</td>
<td>1–394</td>
<td>1–260</td>
<td>2–354</td>
</tr>
<tr>
<td>HLA-B27+ (%)</td>
<td>45.2</td>
<td>57.7</td>
<td>35.1</td>
</tr>
<tr>
<td>Patients with arthritis (%)</td>
<td>42</td>
<td>39</td>
<td>47</td>
</tr>
</tbody>
</table>

*Mean; † median.  
Pa (time of inclusion into the study)

exclusion of other diagnoses if an oligoarthritis (fewer than five joints affected), either asymmetrically or predominantly of the legs, was present and the criteria for ReA were not fulfilled. Other diagnoses were excluded by appropriate tests. Table 1 shows the patients’ characteristics. Some of these patients were later included in a treatment trial with antibiotics.  

SEROLOGY  
In all serological tests used (except the Widal test) antibodies of different immunoglobulin classes, IgM, IgG, and IgA, were investigated. Antibodies to Yersinia enterocolitica O:3 and O:9 and Yersinia pseudotuberculosis I and III were studied by enzyme immunoassay (EIA) and agglutination test (Widal), and to Salmonella enteritidis and Salmonella typhimurium by EIA. More than 90% of cases with salmonellatriggered ReA are caused by salmonellae that have O antigens in common with these two species (serotypes B and D). The remaining salmonellae with different O-specific polysaccharide have a lipid A core and other components that are similar to the corresponding components of serotypes B and D, and are also detected in this EIA, though at a lower efficiency. Antibodies to Campylobacter jejuni were also measured by EIA. For this the antigen was prepared from Campylobacter jejuni using an acid glycine method as described by Kosunen et al. Antibody titres of at least two standard deviations (SD) above the mean of a healthy control population from the Berlin area for at least IgG plus IgA or IgM were regarded as positive in the EIA and a titre of ≥ 1/320 for the Widal test. Antibodies against Shigella flexneri were not looked for because a reliable test is not available. Antibodies against Chlamydia trachomatis were determined by a micro-immunofluorescence test (MIF). An IgG titre > 1/64 with a positive IgA or a positive IgM were regarded as positive. Chlamydia-specific antibodies were also analysed by a commercially available immunoperoxidase assay (IPA) (Iapzyme Chlamydia, Medac GmbH, Hamburg, Germany).  

BACTERIA IN STOOL AND UROGENITAL SWAB  
Stool samples of each patient were studied for the presence of yersinia, salmonellae, shigella, and Campylobacter jejuni using established culture methods. For testing urogenital swabs for the presence of Chlamydia trachomatis samples were cultured on McCoy cell monolayers and inclusion bodies identified by immunofluorescence labelled antibodies to chlamydia (Kallestad/Pathfinder, Kallestad Diagnostics, Texas, USA).  

RESULTS  
Frequencies of causative bacteria in patients with reactive arthritis  
A causative pathogen was identified in 29/32 (91%) of all patients with ReA (with a preceding symptomatic infection). Patients with ReA were further divided into enteric ReA (preceding enteritis, n=35) and urogenic ReA (preceding urethritis/cervicitis, n=19). In 17/33 (52%) of the patients with enteric ReA one of the enteric bacteria was identified, salmonella in 11/33 (33%) and yersinia in 6/33 (18%) (fig 1). Chlamydia trachomatis was the causative pathogen in 12/19 (63%) of the patients with urogenic ReA (fig 1).  

Table 2 Criteria used for the identification of the triggering bacterium as a probable or possible cause of reactive arthritis (ReA); always in the presence of an arthritis either asymmetrically or predominantly of the legs.  

<table>
<thead>
<tr>
<th>Chlamydia</th>
<th>Yersinia</th>
<th>Salmonella</th>
<th>Campylobacter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probable ReA</td>
<td>Chlamydia positive in urogenital smear or symptomatic urethritis</td>
<td>Antibody titres of 1 SD above normal for IgG plus IgA or IgM</td>
<td>Antibody titres of 3 SD above normal for IgG plus IgA or IgM</td>
</tr>
<tr>
<td></td>
<td>Yersinia positive in stool</td>
<td>Antibody titres of 2 SD above normal for IgG plus IgA or IgM</td>
<td>Antibody titres of 2 SD above normal for IgG plus IgA or IgM</td>
</tr>
<tr>
<td>Possible ReA</td>
<td>Chlamydia positive in urogenital smear or IgG &gt; 1/64 plus positive IgA or IgM</td>
<td>Antibody titres of 2 SD above normal for IgG plus IgA or IgM</td>
<td>Antibody titres of 2 SD above normal for IgG plus IgA or IgM</td>
</tr>
</tbody>
</table>

*Only one of the criteria has to be fulfilled.  
†As defined in the “Patients and methods” section; SD = standard deviation.  

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In patients with the clinical picture of uOA a specific triggering bacterium was identified in 35/74 (47%) of patients, yersinia in 14/74 (19%), salmonella in 9/74 (12%), and Chlamydia trachomatis in 12/74 (16%) (fig 1). There was no evidence for campylobacter or shigella infection in any of the patients with ReA or uOA. Interestingly, 17/34 (50%) of the patients with uOA in whom a triggering bacterium could be identified were positive for HLA-B27 compared with 30/92 (33%) among the clinically defined ReA group, 20/74 (35%) in the whole group of patients with uOA, and 9/40 (23%) in the UOA group without identification of a triggering bacterium (table 1).

In 6% of patients with salmonella induced arthritis, 4% with yersinia induced arthritis, and 10% with chlamydia induced arthritis there was laboratory evidence of infection with one of the other bacteria. None the less, a decision about the triggering infectious agent was made in these patients on the basis of the inhibition with relevant bacterial antigens in corresponding EIAs or based on the experts’ opinion, or both. For these cases the experts’ opinion was based on the following evidence: two patients with the diagnosis of salmonella induced arthritis presented both with a preceding diarrhoea and the presence of salmonella-specific antibodies, and in one of them salmonella was detected in the stool; both patients also had a positive chlamydia-specific serology. One patient with the diagnosis of yersinia induced arthritis presented with a preceding diarrhoea and yersinia-specific antibodies; in this patient Chlamydia trachomatis was also detected in the urogenital tract. Two patients with chlamydia induced arthritis presented both with a preceding symptomatic urethritis and detection of chlamydia in the urogenital tract; in both patients salmonella-specific antibodies were also present. However, in 7/126 (6%) patients in whom there was evidence of infections with two of the ReA-triggering bacteria no clear decision was possible. Three of these patients fulfilled the criteria for a positive anti-chlamydiyal MIF test and had a preceding enteritis, three patients had a positive anti-chlamydiyal MIF test and a positive yersinia serology, and one patient had a preceding urethritis, detection of Chlamydia trachomatis in a urogenital swab, a positive anti-chlamydiyal MIF test, preceding enteritis, and a positive yersinia serology. These patients, certainly the last one, probably had infections by two different pathogens as has been reported before. For the analysis, these patients were not included in any of the groups in which a causing bacterium was identified.

COMPARISON OF TESTS AND CLINICAL SYMPTOMS IN PATIENTS WITH YERSINIA OR SALMONELLA INDUCED ARTHRITIS

In 20 patients yersinia and in 20 patients salmonella was identified as the causative bacterium for arthritis. A positive serology showed a sensitivity of 90% for patients with yersinia induced arthritis and of 95% for patients with salmonella induced arthritis (fig 2). This indicates that in most of the patients the diagnosis was based on serology because—in contrast with chlamydia induced ReA—cultures for these bacteria were rarely positive (fig 2). In less than 5% of a local healthy control population a positive serology for yersinia or salmonella was found, resulting in a good specificity of about 95% for these tests. When a cut off point of 3SD above the mean of a control population was used there was only a small difference for the salmonella EIA but a difference of 30% for the yersinia EIA if compared with a cut off point of 2SD. However, the agglutination test (Widal) for the detection of yersinia antibodies (almost all IgM antibodies) had a low sensitivity of less than 20%. A preceding diarrhoea was only reported in 35% of patients with yersinia induced arthritis but in 60% of patients with salmonella induced arthritis (fig 2), indicating that triggering yersinia infections are associated more often with no symptoms or only minor symptoms compared with patients with salmonella infections. In patients with chlamydia induced arthritis Chlamydia trachomatis was identified as the trigger for arthritis in 24 patients. In 12/24 (50%) of these patients a preceding symptomatic urethritis/cervicitis was present (fig 2). In 10/24 (42%) of all patients with chlamydia...
induced ReA Chlamydia trachomatis was detectable in the urogenital tract (fig 2). In 17/24 (71%) serology was positive if MIF was used, but only in 10/24 (42%) of patients if the IPA was used (fig 2). Antibodies specific for Chlamydia trachomatis measured by MIF were positive according to the criteria outlined in the “Patients and methods” section in less than 5% of a local healthy population. In patients with preceding urethritis/cervicitis the detection of Chlamydia trachomatis in the urogenital tract and the MIF had a
similar sensitivity of 50–60% for the diagnosis of chlamydia induced arthritis, whereas the sensitivity was clearly lower for the IPA (33%) (fig 3A). The detection of Chlamydia trachoma-
tis in the urogenital tract correlated best with a preceding urethritis (66%) and a positive MIF (50%) but not so well with the IPA (30%) (fig 3B). A positive MIF (detecting chlamydia-
specific antibodies) did not correlate well with a preceding urethritis (35%), a positive culture (24%), or with a positive IPA test (59%), con-
sidering that the IPA test detects the same anti-
tibodies (fig 3C). Interestingly, of all the variables used in the diagnostic investigation for chlamydia induced arthritis, correlation of the variables with each other was only 60% or less (fig 3).

Discussion
We followed the recommendations made by the “Third International Workshop on React-
tive Arthritis”13 and attempted to identify trig-
erging bacteria in patients with ReA, which was
defined by the presence of a symptomatic enteric or urogenic infection, and in patients with possible ReA, with the typical joint pattern but without a symptomatic preceding infection (UOA).

We identified the causative infection in 29/52 (56%) patients with ReA. When the ReA was split into enteric and urogenic ReA, yersinia or salmonella were identified as the causative pathogen in 17/33 (52%) patients with enteric ReA, salmonella being more common than yersinia. Patients with salmonella induced ReA more often had a symptomatic enteritis than patients with yersinia induced ReA. This is in line with previous reports showing that the gastrointestinal symptoms are absent or mild in ReA triggered by yersinia, whereas such symptoms are more severe and of longer dura-
tion in patients with salmonella induced ReA compared with salmonella infection without arthritis.26 27

However, none of the patients was positive for campylobacter or shigella. Surprisingly, we
did not have a single case of arthritis induced by Campylobacter jejuni, even though it is a
common cause of enteritis in Berlin. It remains to be determined whether this reflects a very
low prevalence of campylobacter induced arthritis or, rather, a low sensitivity of the sero-
logical test used. In contrast, shigella is not a likely cause of enteritis and ReA in Germany except among people travelling to areas with a poor standard of hygiene. Furthermore, no good serology is available for the diagnosis of shigella infections. Therefore, we might have missed single cases because we had to rely only on positive stool cultures.

Even more interestingly, a triggering bacte-
rion was also identified in 35/74 (47%) patients with UOA, yersinia being slightly more common (19%) than salmonella (12%) or Chlamydia trachomatis (16%). Fifty per cent of these patients were positive for HLA-B27, a similar number as for the ReA group, indicat-
ing that these patients had the same disease.

There have been two comparable studies in the past. Kvien et al identified a triggering bacterium in 40–50% of patients with probable or possible ReA.28 Chlamydia trachomatis was the most common (22% of patients), followed by yersinia and salmonella.29 Hannu et al identi-
died a triggering bacterium in 29/60 (45%) patients with possible ReA: yersinia in 10 patients, Chlamydia trachomatis in eight, Yersinia enterocolitica in three, and Campylobacter jejuni in two pa-
tients.30 However, in both these studies the laboratory results were not clearly correlated with the presence or absence of a symptomatic preceding infection. Furthermore, in the study by Kvien et al no chlamydia-specific serology was used and in the study by Hannu et al the only serology used for the diagnosis of salmonella or yersinia induced arthritis was the
Widal agglutination test. Thus in both studies the frequency of the triggering bacterium might have been underestimated. Neverthe-
less, all these studies, including the present one, suggest that Chlamydia trachomatis, yers-
ine, and salmonella are the most common bacteria causing ReA and that these bacteria are also relevant in patients with the clinical picture of undifferentiated arthritides. Although yersinia is a relevant pathogen in continental Europe it seems to be rather rare in the United Kingdom and America.

There is currently no gold standard for the diagnosis of ReA such as histology for malig-
nancies. None of the criteria used are generally accepted or have been evaluated properly.43 44

However, the criteria used in the study by Hannu et al44 (and not only healthy controls as in this study) only serology used for the diagnosis of salmonella and the MIF for Chlamydia pneumoniae were used and in the study by Hannu et al44 the only serology used for the diagnosis of salmonella or yersinia. Therefore, we might have been underestimated. Neverthe-
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off point of 2 or 3 SD should be used. Obviously, a higher cut off point improves specificity but loses sensitivity. Not surprisingly, the Widal test for the detection of Yersinia-specific antibodies showed only a low sensitivity. It has been known for a long time that if only IgG and IgA class antibodies are present the agglutination test may remain negative. 13 This has been shown to be true also for antibodies against yersinia. 12 In this earlier study only 22 out of 53 serum samples which were strongly positive for *Yersinia enterocolitica* as determined by measurement of IgG and IgA class antibodies in ELISA showed a positive reaction in the bacterial agglutination tests. Because of this, the agglutination test is only useful in acute infection but not in more chronic disease. We did not include the Widal test for salmonella-specific antibodies because a low sensitivity for more chronic cases had been previously reported.

The MIF seems to be still the best serological test for chlamydia-specific antibodies. 14 This is also supported by the high specificity found in our study. However, because it is laborious and therefore expensive it is not in general use. Various other tests, mainly ELISA, using whole Chlamydia trachomatis antigen or chlamydia-specific major outer membrane protein or lipopolysaccharide are used instead. Unfortunately, these tests have a relatively low specificity and sensitivity for the diagnosis of chlamydia induced arthritis of about 76% and 73%, respectively. 15 In our study one of the commercially available immunoperoxidase asays also had a low sensitivity for the diagnosis of chlamydia induced arthritis. Only 59% of patients with a positive MIF were also positive when the IFA was applied. This discrepancy shows that there is a need for an improved serological test which can be applied for the diagnosis of chlamydia induced arthritis in daily clinical practice.

For the diagnosis of enteric ReA, a search for the triggering bacterium in stool cultures seems to be useful only in patients with a preceding symptomatic enteritis but not in patients with the clinical picture of UOA. Yersinia or salmonella was detected in the stool in 9% of patients who had diarrhoea in the preceding four weeks but in none of the patients without preceding gut symptoms. In an earlier report salmonella was found in 4% of patients with possible ReA, but again it was not differentiated in this study between patients with and without diarrhoea. 16

The situation is different for Chlamydia trachomatis. In 63% of patients with a urogenetic ReA Chlamydia trachomatis could be identified as the triggering bacterium, a figure similar to that of earlier reports. 17 18 However, the detection of Chlamydia trachomatis contributed to diagnosis also in four patients with UOA. Thus in a diagnostic investigation of patients with ReA a search for *Chlamydia trachomatis* in the urogenital tract should be included. This is now easier and cheaper because *Chlamydia trachomatis* has been cultivated in the first portion of the morning urine with a sensitivity close to that of other methods. 19 20

In chlamydia induced arthritis diagnosis was based on the presence of a preceding symptomatic urethritis/cervicitis, a positive MIF for the detection of chlamydia-specific antibodies, and/or on the detection of *Chlamydia trachomatis* in the urogenital tract. However, there was only a poor overlap between these variables (fig 3), indicating that all three should be used in a diagnostic investigation for chlamydia induced arthritis.

In this study we did not look for *Chlamydia pneumoniae* as a causative agent of arthritis. *Chlamydia pneumoniae* can also induce ReA, 21 though clearly less often than *Chlamydia trachomatis*. 22 23

The PCR method was not used in this study because testing for enteric bacteria in the joint is normally negative and the possible value of PCR testing for chlamydia was not clear at the start of this study (discussed by Sieper et al 24). However, *Chlamydia trachomatis* has been detected in synovial fluid or synovial membrane by PCR in up to 30% of patients with undifferentiated arthritis 25 26 27 and is thus a promising diagnostic tool for the future, though there is currently no agreement on the optimal technique for using the chlamydia-specific PCR. 28 However, it must be emphasised that the specificity of this method has to be further clarified because *Chlamydia trachomatis* was also found by PCR in the joints of healthy controls. 29

In conclusion, the data presented here underline the fact that ReA is an important differential diagnosis in patients with undifferentiated arthritis and that in most cases the triggering bacterium can be identified. Thus a prospective study for the definition and evaluation of generally accepted criteria for the diagnosis of ReA is urgently needed. In the meantime, the data presented here on the frequency of single bacteria associated with ReA and the discussion about the tests used for diagnosis may be helpful.

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Triggering bacteria in patients with reactive arthritis


Frequency of triggering bacteria in patients with reactive arthritis and undifferentiated oligoarthritis and the relative importance of the tests used for diagnosis

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