Increased gut permeability in juvenile chronic arthritides. 
A multivariate analysis of the diagnostic parameters

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Abstract
Objective
This study was aimed at evaluating intestinal permeability (IP) in patients with oligoarticular juvenile idiopathic arthritis (o-JIA), spondyloarthropathy (SpA) associated with inflammatory bowel disease (IBD) and other forms of juvenile-onset chronic arthritidis (OIA) using the lactulose/mannitol (L/M) test in comparison with other non-invasive parameters of gut involvement.

Methods
A series of 26 children affected with o-JIA and 14 with either SpA/IBD or OIA were assessed for IP. The urinary L/M ratio was measured by gas chromatography. The erythrocyte sedimentation rate (ESR), C reactive protein (CRP), and faecal α1antitrypsin concentrations were also evaluated. Ten o-JIA patients displayed active arthritis while in 16 the disease was under control. Among the OIA patients, 11 were affected with psoriatic arthritis and the remaining 3 with chronic reactive arthritis. 14 patients with SpA-IBD had active synovitis or spine inflammation. 14 eo-pJCA and 22 OIA and SpA-IBD patients, respectively, were receiving NSAID therapy.

Results
The mean L/M ratios for the SpA-IBD (0.07 ± 0.02, mean ± SD), OIA (0.05 ± 0.02) and o-JIA (0.04 ± 0.02) patients were significantly higher (p < 0.001, p = 0.022 and p = 0.01, respectively) than those found in controls (0.02 ± 0.01). Logistic regression analysis disclosed a positive correlation between the L/M ratio and the presence of gastrointestinal manifestations (p = 0.011). The type of disease (p = 0.28), the disease activity in the JCA patient group (p = 0.24) and NSAID administration (p = 0.210) did not seem to significantly influence the L/M ratio.

Conclusions
All of the subtypes of juvenile chronic arthritides that we studied displayed an increased IP. Hence, gut wall inflammation (albeit asymptomatic) may also be present in o-JIA patients. The SpA-IBD patients with gastrointestinal symptoms displayed the highest mean L/M ratio values. The L/M test seemed to correlate with histopathological features of the gut mucosa. The L/M ratio was shown to be a highly sensitive but poorly specific test for predicting gut inflammatory disease compared to other non-invasive screening tests.

Key words
Early onset-pauciarticular juvenile chronic arthritis, late onset-pauciarticular juvenile chronic arthritis, juvenile spondyloarthropathy, intestinal permeability, lactulose/mannitol ratio.
Increased intestinal permeability (IP) to different substances has been documented in adult patients affected with juvenile spondyloarthritides (j-SpA) such as ankylosing spondylitis and reactive arthritis (1, 2). Nonetheless, it is uncertain whether this finding should be considered a primary feature of these patients or rather a consequence of inflammation and/or treatment with non-steroid anti-inflammatory drugs (NSAID) (3). So far little data are available about IP in children affected with chronic arthritis.

It is known that endoscopy and small bowel enema represent the gold standards for identifying gastrointestinal damage (4). However, these procedures are invasive and cannot be used as routine screening tests, particularly in children without gastrointestinal symptoms. IP may be assessed non-invasively in vivo by measuring the differential urinary excretion of different test substances administered orally (5). Recently, the usefulness of these procedures in clinical practice has been confirmed (6). Among them, the lactulose/mannitol (L/M) test has been demonstrated to be a reliable screening test in several childhood-onset gastrointestinal diseases (7). This latter test also seems to be useful for the detection of increased IP in rheumatic diseases, although it has not yet been statistically validated in children.

We investigated IP in children affected with oligoarticular, non-extended juvenile idiopathic arthritis (o-JIA) diagnosed according to the Duban criteria (8). Notably, o-JIA mainly affects very young girls, usually ANA+, with a higher risk of developing chronic uveitis. The results for o-JIA patients were compared with those for: (i) j-SpA patients with gastrointestinal involvement diagnosed according to the European Spondyloarthropathy Study Group (ESSG) criteria (9); and (ii) patients affected with other forms of idiopathic arthritides (OIA) without gastrointestinal involvement, whose median age was comparable to that of the j-SpA patients.

In particular, this study was aimed at: (i) evaluating IP in o-JIA, and comparing these patients with j-SpA and OIA patients; (ii) assessing the role of other disease-related factors (i.e. disease activity, NSAID medication) in increasing IP; and (iii) comparing the sensitivity and specificity of the L/M test with other non-invasive and non-instrumental parameters in patients who underwent gold standard procedures for the final diagnosis, in order to evaluate gut involvement.

Patients and Methods

Patients

Fifty-four children (21 males, 33 females) attending the Paediatric Rheumatology Unit of our Institute were prospectively studied during the period 1996-1998. The series was composed of: (i) patients affected with o-JIA; (ii) patients affected with j-SpA; and (iii) OIA patients without gastrointestinal involvement. The patients displayed different degrees of disease activity.

Patients with o-JIA. Twenty-six children (5 males, 21 females) were studied; their mean age was 3.9 yrs. (range 2-6 yrs.). Among them, 18 patients showed ANA positivity, whereas none of them was HLA B27+. No patient developed either gastrointestinal complaints or chronic uveitis. Ten patients showed active arthritis with the presence of swelling or, if swelling was absent, movement limitation or tenderness at the time of the clinical examination. The remaining 16 patients had achieved a good control of the disease since they did not show painful or swollen joints and had a normal range of motion.

Eleven o-JIA patients were off treatment, whereas 15 patients were being treated with naproxen (9 cases) or ibuprofen (6 cases); in 4 patients these drugs were given in combination with slow-acting anti-rheumatic drugs (SAARDs) (methotrexate in 3 cases and cyclosporin in 1 case).

Patients with juvenile SpA. Fourteen children and adolescents (9 males and 5 females) with j-SpA were studied. Their mean age was 11.2 yrs. (range 5-13 yrs.). Six patients in this subset were HLA B27 positive. Among them, 2 patients developed chronic uveitis. All of these patients complained of joint disease such as peripheral synovitis (9 cases), calcaneal and/or tarsal pain (4 cases) and dactylitis (1 case) associated with gastrointestinal symptoms such as abdominal pain,
chronic diarrhoea, and weight loss which raised the suspicion of inflammatory bowel disease (IBD) associated-SpA. All of the patients with gastrointestinal symptoms underwent gold standard procedures for the final diagnosis (i.e., ileosigmoidoscopy with multiple biopsies and/or small bowel enema) for IBD, as reported elsewhere (10). These latter examinations allowed us to diagnose Crohn’s disease in 6 patients and undifferentiated colitis (IC) in the remaining 8 patients.

### Patients with other idiopathic arthritides without gastrointestinal involvement (OIA)

This group was composed of 14 patients (7 males and 7 females): 11 patients affected with psoriatic arthritis and 3 patients with chronic reactive arthritis. Their mean age was 10.9 yrs. (range 6-14 yrs.). All of them displayed peripheral synovitis and enthesitic active disease without clinical signs of gastrointestinal involvement. Two patients developed chronic ulcerative ulcers. Only one patient was HLA B27+.

Six patients belonging both to the SpA and OIA groups were not receiving treatment, whereas 22 patients were receiving therapy: namely naproxen (14 cases), indomethacin (4 cases) and ibuprofen (4 case). Moreover, 4 patients with SpA/IBD were treated with sulfasalazine.

### Controls

Thirty-eight healthy, age-matched patients being followed by us, with no clinical evidence of inflammatory or infectious disease, were included as controls. Their parents’ informed written or oral consent was obtained. Among them, 15 children aged 3.1-5.8 years were compared with the o-JIA patients, whilst 23 children aged 9.8 - 14.0 years were compared with the SpA and oc-JIA patients.

### Examinations

The erythrocyte sedimentation rate (ESR, n.v. < 20 mm/1st hour), C reactive protein (CRP, n.v. 0.1-0.5 mg/dl) and haemoglobin concentrations were evaluated as disease activity indicators using standard laboratory techniques. Furthermore, we assayed serum class A immunoglobulins (IgA, n.v. for our laboratory: 2-6 years: 35-240 mg/dl, over 6 years: 70-350 mg/dl) as a sensitive marker of the mucosal immune response; and faecal occult blood (OB, monoclonal antibodies to human Hb: kit OC Haemodia, Eiken Chemical Co, Tokyo, Japan) and the faecal α1 antitrypsin level (Fα1-AT, n.v. < 250 µg/g of wet stool) as markers of gut disease.

The L/M test was performed in the whole patient series. After an overnight fast, patients received per os a solution containing 5 g lactulose and 2 g mannitol in 100 ml water, at a dosage of 2 ml/kg up to a maximum volume of 100 ml. Urine was collected over the following 6 hours and the L/M ratio was measured by gas chromatography. The methodology is based on the evaporation of urine samples in a stream of nitrogen, and the conversion of sugar into their methoximes by reaction with methoxyamine hydrochloride in pyridine, followed by silylation with BSTFA. The separation and quantitation were performed by capillary gas chromatography (Perkin Elmer Corporation, Norwalk, USA) on a DBI column at 200-290°C (at 3°C/min). Inositol was used as the internal standard. The final quantitative results were expressed as the ratio between the lactulose and mannitol concentrations (expressed as a percentage of the ingested dose). The results of the L/M test were compared with previously indicated non-invasive and non-instrumental parameters of gut inflammatory lesions.

ANA were detected by indirect immunofluorescence (IIF) on Hep2 cells; HLA B27 was screened by two-color direct immunofluorescence methods using a FACScan (Becton Dickinson, San Jose, CA, USA).

### Statistical analysis

Quantitative differences between patient groups for the L/M ratio, faecal α1 antitrypsin and IgA plasma concentrations were assessed by non-parametric statistical analysis using the Mann-Whitney U test. The relative importance of the factors which might influence gut permeability (gut inflammation, type of disease, disease activity, NSAID administration) were determined in a linear logistic regression. The sensitivity and specificity of each non-invasive parameter (IgA serum concentration, faecal occult blood, Fα1-AT concentration and L/M ratio) were calculated. Since our series was small, the analysis of subgroups of patients with different IP influencing factors could be somewhat biased.

### Results

The clinical characteristics of the whole patient series are summarised in Table I.

### Table I. Patients’ clinical characterization.

<table>
<thead>
<tr>
<th></th>
<th>j-SpA</th>
<th>OIA</th>
<th>Active o-JIA</th>
<th>Inactive o-JIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>14</td>
<td>14</td>
<td>10</td>
<td>16</td>
</tr>
<tr>
<td>Age (yr ± SD)</td>
<td>11.2 ± 3.9</td>
<td>11.6 ± 3.4</td>
<td>5.9 ± 2.2</td>
<td>6.2 ± 2.5</td>
</tr>
<tr>
<td>Uveitis</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>HLA B27+</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Patients on NSAID</td>
<td>10</td>
<td>12</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>Patients on SAARD</td>
<td>4</td>
<td>-</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Mean ESR value</td>
<td>64.5 ± 42.2</td>
<td>20.0 ± 17.1</td>
<td>29.0 ±18.1</td>
<td>15.1 ± 10</td>
</tr>
<tr>
<td>Mean CRP value</td>
<td>5.4 ± 4.2</td>
<td>0.4 ± 0.3</td>
<td>0.4 ± 0.2</td>
<td>0.5 ± 0.3</td>
</tr>
<tr>
<td>Mean IgA serum</td>
<td>251.3 ± 85.9</td>
<td>197.6 ± 69.5</td>
<td>196 ± 126.4</td>
<td>158.2 ± 80</td>
</tr>
<tr>
<td>Mean Fα1-AT µg/g</td>
<td>622.5 ± 496</td>
<td>164 ± 69.5</td>
<td>196 ± 126.4</td>
<td>158.2 ± 80</td>
</tr>
<tr>
<td>Patients with positive OB</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
The overall median L/M ratio for both the j-SpA and OIA patients was significantly higher (p < 0.001 and p = 0.022, respectively) than that for the controls. Moreover, the median L/M ratio for OIA was higher than that in o-JIA (p = 0.01) patients. As shown in Figure 1, the L/M ratio in j-SpA was significantly higher than that in OIA (p = 0.01).

When the median L/M ratios for the different groups of patients were statistically compared, j-SpA patients with gastrointestinal involvement showed the highest L/M ratio (p = 0.01). Conversely, the L/M ratios of children affected with active OIA and those with OIA in remission did not statistically differ from each other.

Both active and inactive o-JIA patients showed a median L/M ratio that was significantly increased compared to the controls (p = 0.015 and p = 0.03, respectively). No significant differences were detectable between the active and inactive o-JIA subgroups (p = 0.061), probably because of the limited number of patients studied.

Logistic regression analysis disclosed a positive correlation between the L/M ratio and the presence of gastrointestinal manifestations (abdominal pain in 8 cases, diarrhoea in 3 cases, haematochezia in 3 cases and weight loss in 2 cases) (p = 0.01). By contrast, the type of disease (o-JIA versus OIA without gastrointestinal involvement, p = 0.28), the disease activity (p = 0.24) and NSAID administration (p = 0.21) did not seem to significantly influence the L/M ratio. It is worth noting that there were significant associations between plasma IgA concentrations and Ftx1-AT and the presence of gastrointestinal involvement (p = 0.040 and 0.001, respectively), independent of the underlying disease and of the NSAID medication.

The sensitivity and specificity of the different non-invasive tests in discriminating patients with inflammatory gut disease were assessed only in j-SpA patients with gastrointestinal symptoms who underwent invasive procedures for a definitive diagnosis. The L/M ratio showed the highest degree of sensitivity (77%), followed by Ftx1-AT (60%), faecal occult blood (23%) and IgA plasma concentrations (7.7%). Conversely, the specificity of the L/M ratio was lower (56%) compared with the remaining non-invasive parameters tested (Ftx1-AT 91%, occult blood 97.5%, IgA plasma concentrations 97.5%).

**Discussion**

This study shows that children with either o-JIA, j-SpA with gastrointestinal involvement, or other idiopathic arthritides without gastrointestinal involvement have a higher IP than healthy controls. Notably, j-SpA patients with symptomatic gastrointestinal involvement had a significantly higher L/M ratio than those found in OIA patients without gastrointestinal involvement or in o-JIA patients with active or well controlled disease.

Increased IP is a common feature in children affected with several gastrointestinal diseases such as coeliac disease (11), IBD (12), intestinal infections (13) and food intolerance (14), as well as with systemic diseases or insults (e.g. extensive burns, septicemia shock, malnutrition, intensive chemotherapy) (15).

IP is assessed in vivo by measuring the excretion of probe molecules previously administered per os in timed urine samples. The using of single test substances [lactulose, polymers of polyethylene glycol PEG, or 51Cr-labeled ethylenediaminetetraacetic acid (EDTA)] may be significantly influenced by several pre-mucosal, mucosal and post-mucosal factors. The use of a mixture of a monosaccharide (L-rhamnose or mannitol) that pass transcellulary and a non-hydrolyzed disaccharide (i.e. lactulose) that likely pass by an intercellular route allows for a better assessment of intestinal adsorption. Moreover, the urinary disaccharide/monosaccharide excretion ratio remains unaffected by the above-mentioned variables (16).

Previous studies have validated the utility of the L/M ratio as a screening test in children with coeliac disease, as well as other malabsorption disorders (7). Furthermore, this test is harmless, inexpen-

**Fig. 1.** Comparison of L/M ratio in the different subsets of patients and controls (j-SpA vs C1 = 0.001; o-JIA vs C1 = 0.022; j-SpA vs OIA = 0.01).
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(21). In contrast, the finding of increased IP in ankylosing spondyloarthritis patients not on NSAID treatment and in their relatives led researchers to hypothesise that increased IP may be a "primary", genetically determined abnormality in SpA (22). A correlation between bowel permeability and CD45RO expression on circulating CD20+ T cells in patients with ankylosing spondylitis and their relatives has been documented (23).

Our study shows that an increased median L/M ratio is found in children affected with o-JIA or other idiopathic arthritis. As expected, we found the highest prevalence of IP in SpA children with gastrointestinal symptoms. Histopathological studies in adult SpA patients have confirmed the presence of inflammatory gut features (24).

In our experience the L/M ratio is a highly sensitive predictor of gut inflammatory diseases in children affected with another idiopathic arthritis without gastrointestinal involvement. In contrast, it has a lower specificity compared to other non-invasive tests (presence of faecal occult blood, increased Fox-1 AT excretion, increased IgA serum immunoglobulin concentration). It is of note that these tests lack diagnostic specificity and gastrointestinal involvement. In childhood as well as adult rheumatic diseases, such as o-JIA. This condition does not seem to be correlated with disease activity or NSAID medication.

A pathogenic role for increased IP has been proposed in patients with SpA. In particular, the enhanced transit of arthritis-inducing enterobacteria-derived antigens from the bowel lumen into the systemic circulation may act as a triggering phenomenon in SpA. The beneficial effect of sulfasalazine in SpA is likely due to its capacity to normalise IP and to prevent the entrance of antigens. Although further investigations are needed, our findings provide the first circumstantial evidence for increased IP in o-JIA patients.

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References


