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Clinical characteristics and long-term outcome of patients with refractory sprue diagnosed at a single institution

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Summary

Background: *Refractory sprue (RS) is a rare and severe celiac-like enteropathy not responding to a strict gluten-free diet. Although prognosis is generally poor, little is known about the long-term outcome of patients.* ***Aim:*** *to report baseline characteristics and long-term outcome of a series of patients diagnosed and treated in a single institution.* ***Materials:*** *We report a retrospective cohort of 25 consecutive patients (15 females; mean age 46 yr; range 28-71) diagnosed with RS based on the presence of a non-responsive celiac-like enteropathy. All patients were intensively treated with a gluten-free diet, steroids, nutritional support and immunosupression.* ***Results:*** *Clinical and biological characteristics of patients suggest that, at least, 24 patients had clear evidences of celiac disease. HLA DQ2/DQ8 genes were present in all the 24 patients typed and autoimmune enteropathy was excluded in all. According to the genotyping, 12 patients had a polyclonal lymphocyte population (RS type I) and 13 exhibited monoclonal TCR-γ gene rearrangements (RS type II). Sixteen patients had evidence of ulcerative jejunitis (UJ) (7 in RS type I and 9 in type II). Overall median follow-up time after diagnosis of RS was 29 mo/patient (range 7 to 204) (45 mo for type I and 24 mo for type II). Overall mortality was 48% (12 patients), 6 in each type. Eight patients with UJ (50%), 3 with lymphoma (two T-cell and one B-cell type) and 4 (44%) without ulcers died during follow-up. The causes of death were sepsis in the context of a progressive deterioration but without overt malignancies (n=5), vascular causes (n=3) and severe malnutrition (n=1). Three- and 5-yr survival rate after diagnosis of RS for the overall population was 60% and 56%. There was no differences between type I (67%, 58%) and type II RS patients (54% for both periods). Patients with UJ had lower but non-significant 3- and 5-yr survival rates (56% and 50%, respectively) compared with patients without ulcers (78% and 66%). Survivors had a favorable outcome. While 11 patients persists asymptomatic, two other cases still have mild diarrhea and one low body weight.* ***Conclusions:*** *We confirm that RS is a severe celiac disease-related disorder with very high mortality. Diagnosis of overt lymphoma (12%) in our long-term follow-up was not as frequent as was reported by other groups. A proportion of patients persist in good health for a long time irrespective of the nature of the IEL infiltration or the presence of UJ.*

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Resumen

Características clínicas y evolución a largo plazo de una serie de pacientes con sprue refractario diagnosticados en una sola institución

Introducción: *El sprue refractario (SR) es una rara y severa entidad consistente en una enteropatía tipo celíaca que no responde a una estricta dieta libre de gluten. Aún cuando el pronóstico es generalmente pobre, poco es conocido acerca de la evolución de los pacientes a largo plazo.* ***Objetivo:*** *reportar las características clínicas y la evolución a largo plazo de una serie de pacientes diagnosticados y tratados en una sola*

institución. ***Materiales:*** *Reportamos una cohorte retrospectiva de 25 pacientes consecutivos (15 mujeres; edad media 46 años; rango 28-71) diagnosticados como SR sobre la base de una enteropatía tipo celíaca que no respondió a la dieta libre de gluten. Todos los pacientes recibieron un tratamiento intensivo consistente en dieta libre de gluten, alimentación enteral o parenteral, corticosteroides e inmunosupresión.* ***Resultados:*** *Los elementos clínicos y biológicos sugieren que 24 pacientes exhibían claras evidencias de enfermedad celíaca. Los genes HLA DQ2/DQ8 estuvieron presentes en los 24 pacientes estudiados y se excluyó la enteropatía autoinmune en todos los casos. De acuerdo al genotipo, 12 pacientes presentaron una población linfocitaria intraepitelial policlonal (SR tipo I) y 13 exhibieron un rearreglo genético monoclonal del TCR-γ (SR tipo II). Dieciséis pacientes presentaron evidencias de yeyunitis ulcerativa (YU) (7 en SR tipo I y 9 en el tipo II). El tiempo promedio de seguimiento luego del diagnóstico de SR fue 29 meses/paciente (rango 7 - 204) (45 y 24 meses para tipo I y tipo II, respectivamente). La mortalidad global fue del 48% (12 pacientes), 6 en cada tipo de SR. Ocho pacientes con YU (50%) murieron durante el seguimiento, 3 con linfoma (dos de células T y uno de células B) y cuatro (44%) individuos sin úlceras también fallecieron. Las causas de muerte fueron vasculares (n=3), sepsis en el marco de deterioro progresivo sin desarrollo de malignidad (n=5) y desnutrición progresiva (n=1). La tasa de sobrevida a 3 y 5 años luego del diagnóstico de SR fue de 60% y 56%, respectivamente, sin observarse diferencias entre pacientes con SR tipo I (67%, y 58%) y tipo II (54% para ambos períodos). Los pacientes con YU tuvieron tasas menores de sobrevida a 3 y 5 años (56% y 50%, respectivamente) comparadas con las exhibidas por pacientes sin úlceras (78% y 66%). Once pacientes evolucionaron favorablemente encontrándose asintomáticos, dos pacientes persistieron con diarrea y otro permaneció con bajo peso.* ***Conclusiones:*** *Nuestros resultados confirman que el SR es una patología grave relacionada con la enfermedad celíaca presentando una muy elevada mortalidad. El diagnóstico de linfoma (12%) en el largo plazo de seguimiento de nuestro estudio no fue tan frecuente como ha sido reportado por otros grupos. Una proporción de pacientes persisten en buen estado de salud en el largo plazo independientemente de la naturaleza de la infiltración de LIE ó la presencia de YU.*

Celiac disease (CD) is an autoimmune enteropathy produced by intolerance to dietary gluten in genetically predisposed individuals where clinical and mucosal abnormalities improve or normalize by exclusion of gluten from the diet.[1] The disease is characterized by an increased risk for complications and associated disorders.[2] Refractory sprue (RS) is a rare condition characterized by a severe malabsorption syndrome as a consequence of a celiac-like enteropathy not responding to a gluten-free diet (GFD).[2,3] Non-responsiveness may be secondary, when patients have proved CD and previously responded to gluten withdrawal but subsequently develop symptoms despite strict dietary compliance, or primary, when the patient initially fails to respond to strict avoidance of gluten following diagnosis.[4,5] In every case, other causes of refractoriness should be excluded such as continued gluten intake (the most common cause),[6] the presence of small bowel bacterial overgrowth, exocrine pancreatic insufficiency or dietary intolerance to food proteins other than gluten (soy or cows' milk protein).[5,6] In other circumstances, a different diagnosis such as collagenous enteritis or colitis, autoimmune enteropathy or enteropathy-associated T-cell lymphoma (EATL) must be explored.[5-8]

Clinical, laboratory and immunopathological findings have suggested that a substantial proportion of these patients show clear evidence of having CD before the diagnosis of RS.[5] In contrast, despite the existence of the typical celiac HLA genotype, diagnosis of CD in other patients cannot be reasonably supported on the bases of serology, clinical features and/or histology.[5] Very elegant studies from. Cellier et al.[8,9] have shown that most RS patients (>75%) are characterized by the presence of a non-lymphomatous (normal cytological appearance) but abnormal subset of intraepithelial lymphocyte (IEL) population containing CD3ε and restricted rearrangement of the TCRγ chain but lacking the surface expression of T-cell receptors. Interestingly, this immunophenotype is identical to that of EATL lymphocytes and suggests that the aberrant IEL population present in ulcerative jejunitis (UJ) and RS constitutes an evidence of a neoplastic T-cell disorder (RS type II).[8-11] Studies have shown that a minority of patients with RS patients have a polyclonal, phenotypically not aberrant repertoire of IEL (RS type I).[12]

Most series agree that the very poor prognosis of

patients with type II RS is due to EATL with a very aggressive clinical course.[9-13] In contrast, despite refractoriness to a GFD, most authors suggest that patients with a polyclonal IEL population (type I) often respond to additional therapeutics such as the use of dietary measures (enteral and/or parenteral nutrition) and aggressive pharmacological approaches (steroids and immunosuppressive agents) (9,14,15). Knowledge about the long-term outcome in patients with RS is hindered for several reasons. Mostly relevant is the scanty number of patients reported in different series treated with very different therapeutic schedules. In addition, the potential for selection bias exists in retrospective series. Our aims in this study are to evaluate clinical presentation, baseline characteristics and long-term clinical course of a non-selected series of patients with RS assessed and followed-up using a very strict diagnostic and treatment protocol in a single tertiary referral center.

Patients and methods

Patients. The present report is a retrospective analysis of the baseline characteristics and the outcome of a series of consecutive patients diagnosed with RS in a tertiary referral centre. Between 1987 and 1999 we diagnosed RS in 25 patients (15 female and 10 male; median age 46 yr, range 28-71 yr). Diagnosis of RS was based on the presence of an initial (primary RS) or subsequent (secondary RS) failure of a strict GFD to restore normal intestinal structure and function in patients who have a celiac-like enteropathy.[2] We arbitrarily consider a 6-months GFD period as reasonable to await clinical and histological improvement before making a diagnosis of RS. Patients with a primary RS were put on intensive treatment before the rule of 6-months on a GFD due to the profound clinical deterioration, lack of response to a GFD and the presence of UJ. After suspicion of RS, patients were exhaustively explored to determine the presence of lymphoma and a 6-month period was employed in order to await for a malignant complication before a definitive diagnosis. We estimated the onset of refractoriness from the time of initiation of an alternative therapy (steroids as a first line) due to lack of response to a GFD.

Suspicion of CD was based on the presence of clinical features, a characteristic celiac enteropathy,[1] the presence of a positive CD-related serology and the response to strictly monitored GFD. The onset of symptoms of CD was estimated based on the age reported for appearance of symptoms. At the initial diagnostic work-up for a potential RS, patients were exhaustively explored for any significant disorder associated with refractoriness (e.g. associated endocrine disorder, pancreatic insufficiency, small bowel bacteria overgrowth, etc.). Patients were systematically explored for CD-related serology (antigliadin antibodies –AGA- type IgA and IgG), antiendomysial antibodies (EmA), anti-tissue transglutaminase antibodies (a-tTG). The presence of autoimmune enteropathy was excluded by the absence of serum evidence of anti-enterocyte autoantibodies.[16] Finally, before definitively categorizing patients as refractory to dietary measurements, alimentary behaviors were strictly monitored by expert physicians and nutritionists.

Endoscopic procedures and histological assessment. Histological diagnosis of CD in 24 patients was performed based on the analysis of intestinal biopsy samples obtained through endoscopic procedures. The initial diagnosis of patient # 25 was done by jejunal biopsy samples obtained using a multipurpose biopsy capsule (Bolt, French, Pollard). Duodenoscopy and push enteroscopy were performed using commercial videoendoscopes (Pentax, Japan) and several small intestinal biopsy samples (n>6) were obtained in the same procedures with endoscopic forceps. Samples were either fixed in 10% formalin for conventional histological and immunophenotypic assessment or snap-frozen in liquid nitrogen. Characterization of histological findings was performed using the modified Marsh´s classification which categorizes mucosal abnormalities as: type 0 (normal), type I (intraepithelial lymphocytosis), type II (lymphocytosis and moderate crypt hyperplasia) or type III (IIIb subtotal atrophy; IIIc total villous atrophy).[1] Intraepithelial lymphocyte count was expressed as percentage of lymphocytes per 100 epithelial cells.

Patients exhibited symptoms for a median of 48 mo. (range 3-560) before suspicion of CD. All patients had classical symptoms of GI compromise before suspicion of CD and most relevant symptoms were: chronic diarrhea in 20 and malnutrition in 5 cases. Due to the severity of symptoms, 21 patients

had required diagnostic procedures and intensive treatments on an inpatient basis before diagnosis of CD. When RS was suspected, all patients underwent small bowel double-contrast radiological examinations and CT scans to determine structural abnormalities of the small intestine. If these examinations suggested the presence of masses, enlarged lymph nodes or ulcers, an enteroscopy and/or a laparotomy were subsequently performed. Thus, 12 enteroscopies and 13 laparotomic procedures (which included partial resection of affected segments or systematic resections of apparently non-affected jejunal and ileal segments) were done. While 19 patients had at least one of these procedures performed, 6 other with no evidence of morphological abnormalities did not require additional studies.

Specialized determinations and procedures

Serology determinations. Determinations of antigliadin (AGA) (IgA and IgG subtypes) and anti-tissue transglutaminase antibodies were performed by an ELISA method, as previously reported, using commercial kits (INOVA Diagnostics Inc.; CA; USA).[17] Endomysial antibodies were determined by indirect immunofluorescence on monkey esophagus substrate (INOVA Diagnostics Inc.; CA; USA).[17] Antienterocyte antibodies were determined by a homemade indirect immunofluorescence method using human small intestinal substrate.[16] Fecal α1-antitrypsin clearance was determined using radial immunodifussion, as previously reported.[18]

Molecular analysis. DNA was extracted from paraffin-embedded or frozen samples by proteinase K digestion and phenol-chloroform purification, precipitated with ethanol and resuspended in 10 mmol/L Tris and 1 mmol/L EDTA. All samples gave a detectable product of 274 pb from DRβ gene after amplification with primers PSP-49 and Amp-A.[20] We amplified rearranged TCR genes by polymerase chain reaction (PCR) as a useful strategy to establish clonality in biopsy material.[21] Amplification reactions were performed with 250 ng of DNA in a 50μL reaction that contained 16.6 mM ammonium sulfate, 67 nM Tris-HCl pH 8.8, 10 mM β-mercaptoethanol, 2.0 mM MgCl2, 200 μg/mL gelatin, 200 μM each dNTP, 25 pmol of each primer and 1 U Taq polymerase. Temperature conditions were 90 sec at 94º C, and 120 sec at 72º C for mixes I plus III, during 45 cycles. Staining was performed with ethidum bromide and was visualized under ultraviolet light. Pooled primers in mixes I and III and the respective sequences were as previously published.[21] Genomic DNA was also extracted from whole blood and amplified with the allele-specific primer sets and an internal control primer set specific for human G3PDH gene using a MJ research PT100 thermal cycler (MJ Research Inc. Watertown, MA, USA), followed by the high-resolution PCR-SSP typing procedure above referred.

HLA class II typing. Alleles of either DQ2 or DQ8 molecules primary associated with CD susceptibility were typed in DNA samples from 24 patients following protocols from a commercial DQB1* "high resolution" PCR-sequence specific primers typing kit (Fastype System, Bio Synthesis).[22]

Outcome assessment and treatment

All patients were followed-up by physicians and nutritionists expert in malabsorption, CD and RS. They were periodically re-evaluated and clinical, laboratory, and tests assessing the morphology of abdominal organs (small bowel double-contrast studies, ultrasound and CT scan) were considered according to demand. Treatment consisted in a strict GFD periodically monitored including serology and intestinal biopsy. After the diagnosis of RS patients continued on a GFD and all except one case received oral and/or parenteral steroids (oral prednisone: 1 mg/kg body weight/day [n=23]; i.v. hydrocortisone: 4.5 mg/kg body weight/day [n=12] followed by oral prednisone at equivalent doses). Based on a 2 month demonstrated lack of response of these therapeutic measures, 13 patients were started on azathioprine (2 mg/kg body weight/day p.o., administered b.i.d.), two on cyclosporine (5 mg/kg body weight) and one on 6-mercaptopurine (1.5 mg/kg body weight/day) (due to secondary effects by the use of azathioprine). Doses of steroids were tapered after 2 months on azathioprine, cyclosporine or 6-mercaptopurine administration according to the theoretical onset of the effect of the immunosupression drug. A small group of these cases (n=7) have been reported in an open-label trial assessing the use of azathioprine in RS.[23]

Statistics

Results are expressed as mean ± standard error of the mean (SEM) or median and range; and analyzed with t test (paired and unpaired), Fisher´s exact,

Mann-Whitney ranked sum test and Wilcoxon signed rank test as appropriate. A p value <0.05 was considered significant. Three- and five-yr survival rates were calculated according to conventional formula. Survival for patients with and without ulcers was calculated from the time of diagnosis of UJ. Survival for patients with and without the aberrant IEL infiltration was calculated from the estimated onset of overt symptoms of CD, the time of diagnosis of CD and the estimated onset of refractoriness. The actuarial percentage of survival was expressed by Kaplan-Meier curves and the differences assessed by the Mantel-Haenzel log-rank test. The proportion of patients with complications was compared by calculation of odds ratios and 95% CI. Statistical analysis was performed using Statistix 7.0 (Analytical Software, Tallahassee, FL, USA).

Results

Baseline data of patients

Clinical aspects. Table 1 shows clinical and biochemical characteristics of RS patients at diagnosis. Presence of weight loss (n=25), chronic diarrhea (n=24), abdominal pain (n=20) and fever (n=10) were the most prevalent findings. According to the clinical assessment, 13 patients were categorized as having a primary RS and 12 as secondary RS. Three patients had a first-degree relative with CD. At diagnosis of RS, patients had a low mean hemoglobin level (11.2 mg/dL, range 8.5 to 16.5) (17 patients had values below 12 g/dL), low mean serum albumin (2.4 g/dL, range 0.9 to 4.3) (21 patients had values below 3.5 g/dL), low body mass index (19.4 kg/m2, range 14.9 to 27.1) and increased fecal α1-antitrypsin clearance 105 mL/day, range 12 to 652).

At diagnosis, all patients underwent duodenos-

Table 1. *Some clinical, laboratory and genetic characteristics of refractory sprue patients assessed at diagnosis. Patients 1 to 13 were molecularly characterized as having a monoclonal TCR_ gene rearrangement (type II RS) and patients #14 to #25 had a polyclonal intraepithelial lymphocyte population (RS type I). Hb: hemoglobin levels; Alb: serum albumin; α1AT CL: fecal α1 antitrypsin clearance; AGA-A and AGA-G: antigliadin antibodies type IgA and IgG, respectively; EmA: Antiendomysial antibodies type IgA; BMI: body mass index; UJ: diagnosis of ulcerative jejunitis. ND: not determined.*

Patient	Age/ sex	Hb g/dL	Alb g/dL	α1 AT CL mL/day	BMI Kg/m2	AGA -A	AGA -G	Ema	U J	Surgery	Entero scopy	HLA DQ2-DQ8
1 GS	29 F	9,5	1,49	169	15,6	-	+	+	Yes	Yes	Yes	B1 201/501
2 TP	50 F	11,5	1,77	66,2	18,9	-	+	-	Yes	—	Yes	B201/302
3 MA	46 F	9,5	3,3	80,5	15,3	-	+	-	Yes	Yes	—	B1604/501
4 LD	31 M	12,3	4,02	79	14,9	-	+	+	Yes	Yes	—	B1201/203
5 JA	58 F	9,3	0,96	71,4	18,9	-	-	-	Yes	—	Yes	B1201/501
6 SR	38 F	8,5	2,48	97,2	18,5	+	+	-	Yes	Yes	Yes	B1303-6/302-7-8
7 MS	53 F	—	1.29	102	19.4	+	+	-	Yes	—	Yes	ND
8 JC	54 M	12,6	1,79	386	27,1	-	-	+	Yes	—	Yes	B1201/202-302
9 AS	45 F	11,6	2,4	109	18.0	+	-	-	Yes	—	Yes	B1201/202-302-A501
10 CE	45 M	14,7	4,3	105	21,1	-	-	-	—	—	Yes	B1201/202-302-A501
11 ZF	63 F	11	1,75	85,9	18.0	+	+	-	—	—	—	B1201/202
12 RG	50 M	16,5	4	130	19,4	-	-	+	—	—	—	B1201/202-302-A201
13 JL	29 M	9,8	2,44	131,6	17,2	+	+	-	—	Yes	Yes	B1201/201
14 EP	54 F	11,2	1,29	332,5	21,5	-	-		Yes	Yes	Yes	B1201/201
15 AM	44 M	9,5	0,9	460	19,3	+	+	-	Yes	Yes	—	B1201/201
16 AI	41 F	10	3,2	234	17,3	+	+	+	Yes	Yes	—	B1201/202
17 MDP	52 M	12,7	1,19	652	19,5	+	+	+	Yes	Yes	—	B1201/202 -A501
18 MEC	54 F	8,5	3,3	12	20,9	+	+	-	Yes	Yes	Yes	B1201/02-203- A501
19 MC	60 M	12,9	3,35	108,8	22,3	+	+	+	Yes	Yes	Yes	B1201/02-203- A501
20 MEM	53 F	9	2	20	14,9	+	+	-	Yes	Yes	—	B1201/02- A201-501
21 MCH	43 F	10	2,06	13,8	22,8	+	+	+	—	—	—	B1201/202
22 JV	53 F	12	3,4	349,7	19,4	-	+	+	—	—	—	B1302-306
23 JB	50 M	15,7	3,7	103,3	23,4	+	-	+	—	—	—	B1201/202
24 RO	61 M	10,7	2	122,9	19,3	-	+	+	—	Yes	—	B1202/301
25 FL	57 F	11	2,9	33,2	20,6	+	+	-	—	—	—	B1202/201

copy for diagnostic and biopsy purposes.[19] Twelve patients (48%) underwent enteroscopy for assessment and biopsy of ulcers suspected by radiology. Overall, the combined analysis detected intestinal ulcers in 16 patients. While, duodenoscopy evidenced the presence of ulcers in six (36%), enteroscopy evidenced ulcers in other five and surgery in five additional cases. This analysis emphasizes the necessity of a combined analysis for detection of ulcers. According to Marsh classification, baseline assessment of intestinal mucosal morphology showed that 13 patients had type IIIc enteropathy, 11 had a type IIIb lesion and one type IIIa. The median value of intraepithelial lymphocyte count for RS patients was 35%.(range 18%-55%).

Thirteen patients underwent exploratory laparotomy based on the suspicion of anatomic abnormalities (thickness of the intestinal wall, masses, enlargement of lymphoid nodules, ulcers, etc.): 8 of the RS type I subgroup and 5 of the RS type II subgroup.

Baseline data from patients with RS type I and type II (table 2) did not show significant differences in terms of symptoms, laboratory tests (hemoglo-

Table 2. *Data of refractory sprue patients categorized according to the existence of a polyclonal (RS type I) or monoclonal (RS type II) TCRγ rearrangement of intraepithelial lymphocytes (IELs) in intestinal mucosa. EmA: endomysial antibodies; Histological classification (see methods); Fecal α1AT Cl: fecal α1 antitrypsin clearance.*

Parameters	RS type I (n=12)	RS type II (n=13)
Time from the estimated onset of symptoms (mo.)		
Median (range)	168 (27-642)	75 (45-345)
Time from diagnosis of CD (mo.) Median (range)	73 (15-363)	48 (9-111)
Diarrhea (n of patients)	9	11
Fever (n of patients)	4	6
Ulcerative jejunitis (n of cases)	7	9
Body mass index (kg/m2) Median (range)	20.1 (15.0-23.4)	18.3 (15.0-27.1)
Hemoglobin (g/dL) Median (range)	10.8 (8.5-15.7)	11.2 (8.5-16.5)
Serum albumin (g/dL) Median (range)	2.5 (0.9-3.7)	2.4 (1.0-4.3)
Fecal α1AT Cl (mL/day) Median (range)	116 (12-652)	102 (66-386)
EmA (n of positive cases)	7	4
Histology (Marsh) (n of cases)		
IIIa	1	-
IIIb	7	4
IIIc	4	9
LIEs count (%) Median (range)	25 (18-48)	30 (20-55)
3-yr Survival rate (%)	67	54
5-yr Survival rate (%)	58	54

bin, serum albumin, fecal α1-antitrypsin clearance, number of patients positive for EmA, histological classification according to Marsh, % of IELs, and number of patients with UJ. Patients with type II RS had a lower BMI (p<0.06). Table 3 compares baseline clinical, laboratory and histological data of patients categorized as having or not diagnosis of UJ. Once again, no differences were shown in most assessed parameters. Compared with patients without evidence of ulcers, those with UJ exhibited a trend toward a lower body weight at diagnosis (p<0.06) and mean serum albumin (1.9 g/dL vs. 2.9 g/dL; p<0.06).

Specialized laboratory studies. At the time of diagnosis of CD and/or when a refractory enteropathy was suspected, 11 patients had a positive EmA test and/or a-tTG, 13 had positive IgA type AGA and 17 type IgG. Overall, 19 patients had IgA type EmA and/or AGA and 22 had one or more positive tests.

Thirteen patients had a monoclonal TCR_ gene rearrangement (type II RS) and 12 cases had a polyclonal lymphocyte population (RS type I). (table 1) Interestingly, when findings from duodenal and je-

Table 3*. Clinical and biochemical parameters of patients at baseline (diagnosis of RS) and outcome of patients grouped according to the presence or absence of ulcerative jejunitis. EmA: endomysial antibodies; Histological classification (see methods); Fecal α1AT Cl: fecal α1 antitrypsin clearance.*

Parameters	With ulcers (n=16)	Without ulcers (n=9)
Time from the estimated onset of symptoms (mo.) Median (range)	74 (39-642)	170 (27-616)
Time from diagnosis of CD (mo.) Median (range)	38 (9-363)	83 (15-288)
Diarrhea (n of patients)	12	8
Fever (n of patients)	8	2
Abdominal pain (n of patients)	16	3
Monoclonal TCR γ (n of cases)	9	4
Body mass index (kg/m2) Median (range)	19.0 (14.9-27.1)	19.5 (17.2-23.4)
Hemoglobin (g/dL) Median (range)	10.0 (8.5-12.9)	11.0 (9.8-16.5)
Serum albumin (g/dL) Median (range)	1.9 (0.9-4.0)	2.9 (1.7-4.3)
Fecal α1AT Cl (mL/day) Median (range)	105 (12-652)	105 (14-350)
EmA (n of positive cases)	6	5
Histology (Marsh) (n of cases)		
IIIa	1	-
IIIb	8	3
IIIc	7	6
LIEs count (%) Median (range)	45 (18-55)	25 (20-44)
3-yr Survival rate (%)	56	78
5-yr Survival rate (%)	50	66

junal samples were compared in the same patient, both molecular genotypic characteristics concurred in all cases.

Based on radiological, endoscopic and pathological findings, 16 (64%) patients had multiple ulcers in the small intestine, indicative ulcerative jejunitis. Morphological characteristics of ulcer were widely variable, ranging from an aphthous appearance to big and profound lesions with sharp edges and dense fibrin. Interestingly, while 9 of 13 (69%) patients with a monoclonal TCRγ receptor rearrangement had evidence of UJ, 7 of 12 (58%) with polyclonal lymphocytes also had ulcers.

Considerations about diagnosis of celiac disease. Based on the combined clinical information (familial history, former response to a GFD, histological findings, etc.) and serology features, we estimate that all patients had evidence of gluten sensitivity whether or not they had evidence of response to a GFD. If we exclude those cases only positive for AGA type IgG (low specificity for CD) but without former response to a GFD or familial features, we conclude that 24 patients have concrete features indicative of CD.

Outcome

General aspects. The initial clinical outcome of most patients was characterized by a profound deterioration. This was evidenced by the inability to perform daily activities at the time of diagnosis which required strict monitoring, support and treatment on the inpatient basis in 20 patients. Surgical approach in patients whe underwent laparotomy did not avoid of complications with 4 patients requiring re-explorations for abdominal sepsis or leakage.

Median follow-up after diagnosis of RS for the overall population was 29 mo/patient (range 7 to 204 no); 45 mo. (range: 8-204 no) for type I and 24 mo. (range: 7-108 no) for type II. Overall mortality reached 48% (12/25 patients) with 6 cases in each subtype of RS. On the other hand, while 8 patients with UJ (50%) (3 with lymphoma 37%) radical during follow-up 4 of 9 (44%) without ulcers, also died. Up to now, 3 patients (12%) developed overt lymphoma (two T-cell and one B-cell type). One patient (#17) previously characterized as a type I RS with UJ, who had been exhaustively explored for malignant complications, developed an extra-abdominal B-cell lymphoma localized in lymph nodes. It was diagnosed one year after being successfully treated with TPN, steroids and immunosupression (cyclosporin). After a short period on treatment, the patient regained his original body weight and was free of symptoms for eight months. The patient did not respond to chemotherapy and died shortly after diagnosis of lymphoma. Two other patients developed an intestinal T-cell lymphoma phenotypically defined as EATL. One of these cases (#2) has been previously characterized as type I RS and the other (#18) as type II. A common finding for the three patients who developed overt lymphoma was the association with UJ. The causes of death were lymphoma (n=3), sepsis in the context of a progressive deterioration without overt malignancies and a severe refractoriness to other treatments including steroids and immunosupression (n=5), due to vascular causes (n=3) and severe malnutrition (n1). Causes of death and characteristics of these patients are reported in table 4. The median time from diagnosis of RS to death was 17mo (range 7 to 204 no)

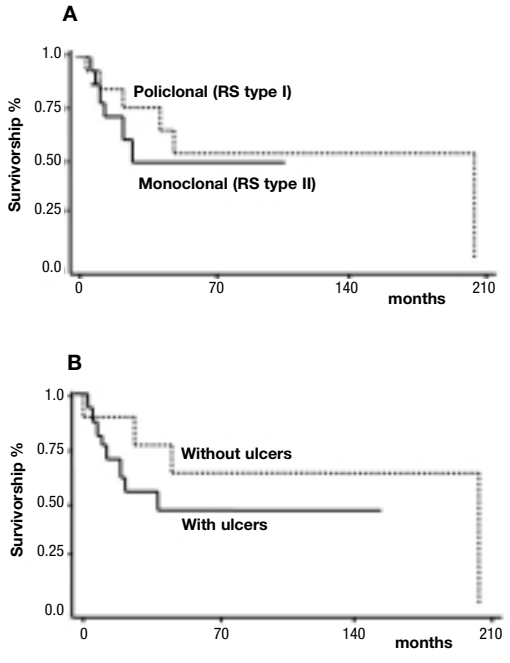
Three- and five-yr survival rate for the overall population was 60% and 56%, respectively, and there was no difference between RS type I and type II patients (table 2). Interestingly, deaths in patients with monoclonality (type II) seem to occur earlier with respect to diagnosis of RS than those with type I RS. In RS type II, all 6 patients died in the first 3 yr-period of follow-up compared with 3 of 6 in the RS type I group. Patients with UJ had a lower but non-significant 3- and 5-yr survival rate compared with patient without ulcers (table 3). (fig. 1)

Therapeutic modalities. A monitored GFD was given to all patients and maintained during follow-up despite a lack of initial response. Steroids were systematically administered in 24 of 25 patients after refractoriness to GFD was determined. Only one patient (#4) was not treated with steroids after an estimated complete resection of the jejunum containing ulcers. The patient is still alive only on a GFD and the molecular assessment of the excised segment showed aberrant IEL infiltration. Sixteen patients received nutritional support (4 only received enteral nutrition and 11 total parenteral nutrition and/or enteral support) in conjunction with a GFD and steroids. Finally, in 16 patients we added immunomodulation (13 cases were treated with azathioprine, 2 with cyclosporine and 1 case intolerant

Table 4. *Clinical characteristics of 12 RS patients as assessed at the time of death. Patients #2 to #11 have had an aberrant intraepithelial lymphocyte infiltration determined by molecular biology. Patients #15 to #25 had a polyclonal population. M and F represent male and female gender. S: steroids; AZP: azathioprine; TPN: total parenteral nutrition; EN: enteral nutrition, DIC: disseminated intravascular coagulation; PTE: Pulmonary thromboembolism.*

Patient	Age/ gender (yr)	Time on follow-up after RS dx. (mo.)	Cause of death	Treatment
2 TP	50 F	6	Lymphoma	S+TPN
3 MA	46 F	11	Sepsis	S+AZP+TPN+EN
5 JA	58 F	13	Intestinal ischemia	S+AZP+TPN+EN
7 MS	53 F	12	Sepsis-Pneumonia	S+AZP+EN
8 JC	54 M	24	Stroke	S
11 ZF	63 F	6	PTE	S+AZP+EN
15 AM	44 M	42	Sepsis	S+AZP+TPN+EN
17 MDP	52 M	22	Lymphoma-Sepsis	S+Cy+TPN+EN
18 MEC	54 F	10	Lymphoma-DIC-Sepsis	S+EN
21 MCH	43 F	3	Sepsis	S+TPN+EN
22 JV	53 F	48	Progressive deterioration	S
25 FL	71 F	204	Sepsis	S+AZP

Figure 1. *Actuarial survival (Kaplan-Meier curves) of refractory sprue patients assessed from the time of suspicion of the complication. A: patients categorized by presence or absence of monoclonal TCR_ gene rearrangement. B: patients categorized by the presence or absence of ulcers. p: NS (Mantel-Haenzel log-rank test).*



to azathioprine was treated with 6-mercaptopurine). Three cases did not receive immunomodulators because of sepsis (two of them developed lymphoma), 2 other because of improvement after steroids and another due to the complete resection of ulcers and a positive response to a GFD. Finally, one case had a progressive nutritional impairment and died.

Clinical status of survival patients. At December 2004, 13 patients were still alive for a median follow-up of 66 mo. (range 15 to 156 no) (table 5). Six of these patients were categorized as RS type I and seven were RS type II. Molecular analysis of biopsy samples obtained at the follow-up (n=8) detected persistence of the characteristics assessed in samples from diagnosis. Histological characteristics of post-treatment biopsy samples showed marked improvement in all, with almost complete recovery in eight. While the clinical condition was considered good or excellent in 11 patients, in 2 other cases diarrhea still persists 15 and 57 months after diagnosis. They required new series of immunosuppressive drugs (6-MP and azathioprine). Another patient (#9) persists with a low BMI 19 mo after diagnosis of RS and 9 mo after immunosuppression was stopped. The patient is not having GI symptoms.

Table 5. *Clinical characteristics of 13 surviving RS patients as they were assessed in December of 2004. Patients #1 to #13 have an aberrant intraepithelial lymphocyte infiltration determined by molecular biology. Patients #14 to #24 have a polyclonal population. M and F represent male and female gender. S: steroids; AZP: azathioprine; TPN: total parenteral nutrition; EN: enteral nutrition.*

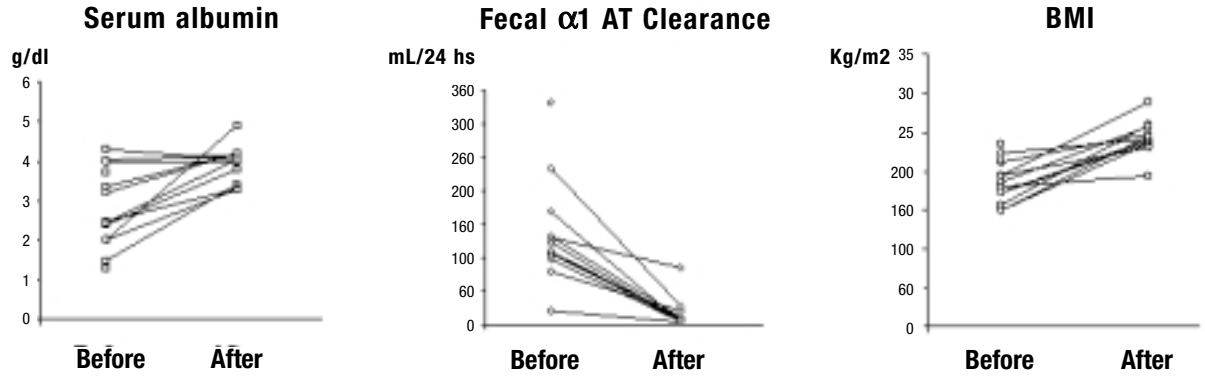
Patient	Age/ gender (yr)	Time on follow-upafter RS dx. (mo.)	Actual clinical Status	Las BMI (Kg/m2)	Treatment
1 GS	30 F	65	Good	27.4	S+AZP+TPN
4 LD	40 M	108	Good	23.2	(only surgery)
6 SR	43 F	66	Good	22.2	S+AZT+EN
9 AS	47 F	20	Good	19.5	S+AZT
10 CE	49 M	57	Diarrhea	22.5	S+AZP
12 RG	51 M	15	Mild diarrhea	23.0	S+6-MP
13 JL	35 M	70	Good	25.4	S+AZP+TPN+EN
14 EP	60 F	75	Good	-	S+Cy+TPN+EN
16 AI	53 F	156	Good	26.7	S+EN
19 MC	61 M	19	Fair	17.5	S+AZP+TPN+EN
20 MM	56 F	31	Good	23.9	S+AZP+TPN+EN
23 JB	59 M	107	Good	27.1	S
24 RO	68 M	105	Excellent	28.9	S

All patients follow strict gluten avoidance (table 5). Comparing final vs. baseline (at diagnosis of RS) determinations, survival patients significantly improved BMI (18.8±0.8 vs. 24.3±0.7 Kg/m2, respectively; p<0.0001), serum albumin (2.8±0.3 vs. 3.9±0.1 g/dL, respectively; p<0.03) fecal a1 antitrypsin clearance (134±21 vs. 16±7 mL/day, respectively; p<0.004), and the number of cases with positive EmA type IgA (8/12 vs. 0/10; p<0.0004) and AGA type IgA (6/12 vs. 1/11, respectively) decreased. (figure 2)

Discussion

Refractory sprue is a rare, poorly understood malabsorption syndrome which is difficult to diagnose, is lacking a well-established therapy and is characterized by a poorly understood outcome.[14] Several authors agree that there is enough evidence for conside-

Figure 2*. Biochemical features (serum albumin and fecal* α*1 antitrypsin clearance) and body mass index (BMI) in survivor patients as they were assessed at diagnosis of refractory sprue (Before) and at December of 2004 (After). For statistical significance see the Results Section.*



ring RS as a manifestation of IEL lymphoma (cryptic lymphoma) that in some cases may progress to overt EATL.[8-14] One of the most relevant, still unsolved, topics is whether it represents an evolutionary state of a pre-existing CD or constitutes a common final event of a group of heterogeneous conditions characterized by a celiac-like enteropathy with similar clinical features and poor prognosis.[10,24] In general, the prognosis of most patients is severe with a progressive clinical and nutritional deterioration.[9] Information regarding these patients is limited by the scanty number of cases reported, the difficulties for diagnosing RS, the diversity of pathobiologic findings, and the fact that most series are based on selected patients diagnosed in different centers.

Herein, we report 25 consecutive patients diagnosed at the same institution in whom pre-and post-diagnostic assessments were performed using a similar diagnostic and therapeutic approach by the same physician team. All patients enrolled were exhaustively investigated (with non-invasive and invasive procedures including laparotomy) in order to exclude the presence of overt lymphoma at the time of diagnosis of RS. In our opinion, the most relevant features of the present report were mostly related to some obscure characteristics of the disorder. Firstly, one of the most daunting challenges facing clinician and experienced people is establishing the true pre-existence of CD in RS patients. In keeping with a prior experience of French researchers,[8,9] we confirmed that most, if not all patients diagnosed with RS in this study had evidence of CD, based on histological findings, clinical features (response to a GFD, family history of CD and the presence of CD-specific antibodies) and the characteristic HLA genotype. This evidence firmly suggests CD in 22 patients (IgA EmA and AGA in 19 cases and clinical and histological response to a gluten-free diet in other three patients). HLA DQ2 and/or DQ8 were detected in all the 24 patients typed.

According to the TCRγ analysis, 12 patients had a polyclonal lymphocyte population (RS type I) and 13 exhibited monoclonal TCR-γ gene rearrangement characteristic of a RS type II. The almost similar proportion of patients with polyclonal or aberrant monoclonal IEL populations contrasts with prior experience from other authors who reported that most well-defined RS patients (>75%) have an IEL infiltration characterized by morphologically normal cells expressing intracellular CD3_ receptors but lacking surface CD3, CD4 and CD8 and having a restricted rearrangement of the gene.[8] This discrepancy between studies seems to be intriguing, but we suggest that it simply may reflect a selection bias with an incorrect estimation of the magnitude of subgroups due to the retrospective nature of studies. In such context, our study was based on a robust clinical identification of patients and the subsequent characterization of subgroups using the molecular analysis of biopsy samples for the clonal intestinal TCR_ gene rearrangement. Furthermore, exclusion criteria in our study were limited to the identification of overt lymphoma at the time of diagnosis of RS and the lack of CD serology or genotyping. In contrast, prior studies seem to include patients collected in other centers with potential clinical and diagnostic differences.

Another interesting feature of the study population was the presence of intestinal ulcerations in a substantial number of patients with RS. Based on findings from small bowel barium studies, enteroscopy and laparotomy, 16 patients (64%) had multiple ulcers in the small bowel. In contrast to a prior report,[9] UJ patients were similarly represented in both types of RS patients (seven in RS type I and nine in type II). All patients having the suspicion of intestinal ulcers were exhaustively explored using either laparotomy (n=6) or enteroscopy (n=5) or both procedures (n=5) in order to obtain histological sampling for establishing the presence of lymphoma and genotyping. Our findings are not in agreement with those reported by Cellier et al.[9] where UJ was found only in patients with the characteristic abnormal clonal T-cell proliferation. We estimate that a possible explanation for such difference with respect to the French study might be at least partially related to the relatively increased representation of type I RS in our population.

Our long-term evaluation confirms that mortality of patients with RS is high approaching 50%. Mortality was similar in RS type I (50%) and RS type II (46%). All deaths in RS type II patients occurred in the first 3-year of follow-up and 3 of 6 patients in RS type I died during that period. Patients with UJ had a higher three- and five-yr mortality rates compared with patients without evidence of intestinal ulcerations, but this was not statistically significant. Once again, these observations differ from those

previously reported by Cellier et al.[9] who have shown a low mortality in the subgroup of patients without ulcers. Finally, all deaths among patients without ulcers (n=4) were due to complications induced by the severe clinical deterioration and infectious complications but not to malignancies. In this context, lymphomas only occurred in patients with UJ. The fact that one patient with molecular evidences of a type I form of RS developed an EATL strongly suggests that the malignancy could be generated by aberrant IEL focally localized in the small intestine, missed by the exhaustive exploration performed. Of note is the relatively short interval between suspicion of RS and diagnosis of overt lymphoma (ranging 6 mo. to 12 mo.) in the 3 cases diagnosed. This fact suggests that, despite our intensive approach to diagnose complications, malignancies cannot be ruled out in early phases. Another very interesting aspect assessed in the study population is related to the high mortality associated with infections which were detected in 7 cases. While in 4 cases sepsis was related with a profound clinical deterioration and its consequences, in other 2 cases we suspected that infection could be associated with the use of immunosupression.

Our study also explored the long-term clinical outcome of survivors, an aspect of the disorder which has not been revised by former studies. Very interestingly, we have shown that most survival patients are in good or excellent clinical status after one-year of treatment with immunomodulators and that, they remain active and without symptoms only on a GFD. This outcome was evidenced irrespective of the molecular characteristics of IEL infiltration and the presence or not of UJ. Two patients relapsed and required new series of immunossupression. However, their clinical status was significantly better than that assessed at diagnosis of RS.

In conclusion, we report a unique series of RS patients diagnosed and followed-up by a single professional team using an intensive diagnostic and therapeutic protocol. Our data confirm that RS is an evolutionary state of CD with most patients having a compatible past clinical history and a positive CD-related serology in the context of the well-established HLA-DQ2/DQ-8 genetic predisposition. In an important proportion of cases suspicion and diagnosis of RS requires profound and detailed analysis requiring specialized centers. Very interestingly, our population was constituted by an unusual equivalent proportion of RS types I and II which allowed us to compare both subsets. In this context, we have shown that both subgroups share a similar clinical behavior and outcome. Our study confirms that RS is a severe disorder with very high mortality, mainly during the first three-year period after diagnosis of RS. Diagnosis of overt lymphoma in our long-term follow-up was not as frequent as reported by other groups. Finally, a proportion of patients still persist alive for a long time independently of the nature of the IEL infiltration.

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