Negative association between smoking and anti-saccharomyces cerevisiae antibodies in Crohn's disease

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Abstract

Background : **Crohn's disease (CD) is a polygenic multifactorial heterogeneous disease. Anti-Saccharomyces Cerevisiae antibodies (ASCA) correlate highly with CD and are present in 50-80% of patients. The reason for ASCA positivity or negativity in CD is unknown. The aim of our work was to analyse clinical, epidemiological and genetic characteristics in ASCA + or ASCA- CD patients.**

Methods : **113 patients with CD were tested for ASCA (IgA and IgG) by using a commercial kit (Medipan Diagnostica). Age, gender, systemic manifestations, familial form of disease, age at diagnosis, location and behaviour of the disease, smoking habit as well as genotyping for –308 TNF gene polymorphisms were determined.**

Results : **38.9% CD patients were negative for both IgA and IgG ASCA while 61.1% were ASCA positive (respectively IgA and IgG : 31.9% ; IgA only : 9.7% ; IgG only : 19.5%). The only significant difference between ASCA+ and ASCA- patients was for smoking habit : there were 29% smokers in ASCA+ versus 50% in ASCA – CD patients (P = 0.03). This low proportion of smokers was more prominent in ASCA IgA+ patients than in isolated ASCA IgG+ patients (25.6% versus 45.5%) and was minimal in patients with high titers of ASCA IgA (0/8). Logistic regression showed smoking habit still borderline for significance (P = 0.057).**

Conclusions : **Our results suggest a negative association between smoking and ASCA positivity in CD. This association was more prominent for ASCA IgA+. It indicates that smoking habit should be taken into account when analysing ASCA status in CD patients and may suggest an influence of smoking on immunization against intestinal material.** (Acta gastroenterol. belg., **2003**, 66, **1-6**).

Key words : ASCA, Crohn's disease, smoking.

Introduction

Crohn's disease (CD) is a polygenic multifactorial heterogeneous entity (1). Recently new genetic discoveries have focused attention on NOD2 leucine-rich repeat variants (2-4). NOD2 gene variants may confer susceptibility to CD by altering the recognition of bacterial components and/or by over-activating NF-κB in monocytes. Beside NOD2, other genes and environmental factors are involved. Among these, smoking has been associated with CD in general (5,6) and particularly with severe forms of the disease (7-13) while TNF gene has been suggested to predispose to CD (14,15) or to influence its phenotype (16). The fact that these genetic and environmental factors are not relevant to all CD patients emphasizes the heterogeneity of the disease (17). Subclinical markers may help to better identify homogeneous subgroups of patients (18).

Antibodies to oligomannosidic epitopes of the yeast *Saccharomyces Cerevisiae* (ASCA) have been described since 1988 in sera from patients with CD (19). The response was specific for *Saccharomyces Cerevisiae* and did not include other yeast such as Candida Albicans suggesting that it is not simply the result of a generalized increase in intestinal permeability (20). Furthermore, ASCA are highly correlated to CD being found in 50 to 80% of the patients while rarely positive in ulcerative colitis or healthy controls (18-28).

Reasons for ASCA positivity in CD are unknown. *Saccharomyces Cerevisiae* may be considered as a relevant dietary antigen with a possible role in the etiopathogenesis of CD or ASCA could sign a cross reaction between *Saccharomyces* antigens and an unknown infectious agent.

So far, no clinical and epidemiological characteristic and no genetic or environmental factor has been universally associated with ASCA positivity in CD.

The aim of this study was to analyse clinical, epidemiological, particularly smoking habit, and genetic characteristics in ASCA positive and ASCA negative CD patients.

Patients and methods

113 patients followed up in our institution with a diagnosis of CD based on standard criteria were retrospectively studied. These patients were selected according to the availability of a serum sample in our serum bank. No other particular criteria was used to select patients. They had given their informed consent for this study. Demographic and clinical characteristics were collected from our computerized database for IBD patients.

Clinical characteristics

Gender, age, presence of systemic manifestations and existence of familial form of inflammatory bowel disease were determined. For age at diagnosis, disease location and behaviour, patients were characterized according to Vienna classification of CD (29). Age at

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Table 1. — **Patients characteristics in total population and in ASCA+ or ASCA- patients (*P = 0.03) (Vienna Classification : Age at diagnosis A1 = less than 40 years, A2 = greater than or equal to 40 years ; Location L1 = terminal ileum, L2 = colon, L3 = ileocolon, L4 = upper GI ; Behavior B1 = non-stricturing non-penetrating, B2 = structuring, B3 = penetrating)**

	Total Population (n = 113)	ASCA+ (n = 69)	ASCA- (n = 44)
Gender	79 women / 34 men	47 women / 22 men	32 women / 12 men
Age (years)	41.63 ± 14.71	41.55 ± 15.55	41.75 ± 13.47
Age at diagnostic (%) (available in 107 patients)	A1 : 85 A2 : 15	A1 : 86.6 A2 : 13.4	A1 : 82.5 A2 : 17.5
Location at 3 years (%) (in 94 patients)	L1 : 39.4 L2 : 29.7 L3 : 26.6 L4 : 4.3	L1 : 39 L2 : 25.4 L3 : 30.5 L4 : 5.1	L1 : 40 L2 : 37.1 L3 : 20 L4 : 2.9
Behavior at 3 years (%) (in 92 patients)	B1 : 56.5 B2 : 14.1 B3 : 29.4	B1 : 49.1 B2 : 15.8 B3 : 35.1	B1 : 68.6 B2 : 11.4 B3 : 20
Extra-intestinal manifestations (in 112 patients) (%) Family history of IBD (in 112 patients) (%)	Yes : 34.8 No : 65.2 Yes : 17 No : 83	Yes : 34.8 No : 65.2 Yes : 19.1 No : 80.9	Yes : 34.9 No : 65.1 Yes : 13.6 No : 86.4
Smoking habit (%) (in 113 patients)	Smoker : 37.2 Non-smoker : 62.8	**Smoker : 29*** Non-smoker : 71	**Smoker : 50*** Non-smoker : 50
Genotyping for –308 TNF gene polymorphism (available in 88 patients) (%)	Genotype frequencies : – 11 : 73.9 – 12 : 22.7 – 22 : 3.4 Allelic frequencies : – 1 : 85.2 – 2 : 14.8	Genotype frequencies : – 11 : 74.1 – 12 : 20.4 – 22 : 5.5 Allelic frequencies : – 1 : 84.3 – 2 : 15.7	Genotype frequencies : – 11 : 73.5 – 12 : 26.5 – 22 : 0 Allelic frequencies : – 1 : 86.8 – 2 : 13.2

diagnosis was defined as A1 (less than 40 years) or A2 (greater than or equal to 40 years), location as L1 (terminal ileum), L2 (colon), L3 (ileocolon) or L4 (upper GI), behaviour as B1 (non-stricturing non-penetrating), B2 (stricturing) or B3 (penetrating). For location and behaviour of disease, status was determined after 3 years of evolution in every patient to avoid influence of disease duration on the classification (30).

Smoking habit

Smoking habit was defined as follows : patients were smokers if they smoked more than 7 cigarettes per week and non-smokers if they never smoked, or smoked less than 7 cigarettes per week. Patients with ancient smoking habit but who had stopped smoking before diagnosis of CD (ex-smokers) were considered as non-smoker. No patient in our series has stopped smoking after the diagnosis of CD.

Polymorphism for α-308 TNF gene

The -308 single base pair polymorphism located in the promoter region of TNF gene was studied by allele specific PCR as previously described (16).

ASCA status

Blood was taken by venipuncture. Sera were separated after clotting by centrifugation. Serum samples were stored at –20° before use. ASCA IgA and IgG were determined by two commercial enzyme immunoassays (Medizym®ASCA IgA and Medizym®ASCA IgG - Medipan Diagnostica distributed by Euribel S.A./N.V. , Brussels, Belgium). The ELISA were performed according to manufacturers instructions. Medizym ASCA IgG is only a qualitative test while Medizym ASCA IgA allows quantitative evaluation. For IgA, titers higher than 5 times the cut-off level were considered as high titers. Dosages were performed in duplicate and the result recorded was given by the mean of the 2 measures.

Statistical analysis

Comparison of clinical, epidemiological and genetic characteristics between ASCA+ and ASCA- patients were performed by Fischer's exact test. Level of significance was < 0.05. A logistic regression including all parameters tested was also performed with ASCA status as dependent variable. Further logistic regression were also performed with ASCA IgA or IgG status as dependent variables.

Results

Patients characteristics for whole population are shown in Table 1.

38.9% CD patients were negative for both IgA and IgG ASCA while 61.1% were positive (respectively IgA and IgG : 31.9% ; IgA only : 9.7% ; IgG only : 19.5%).

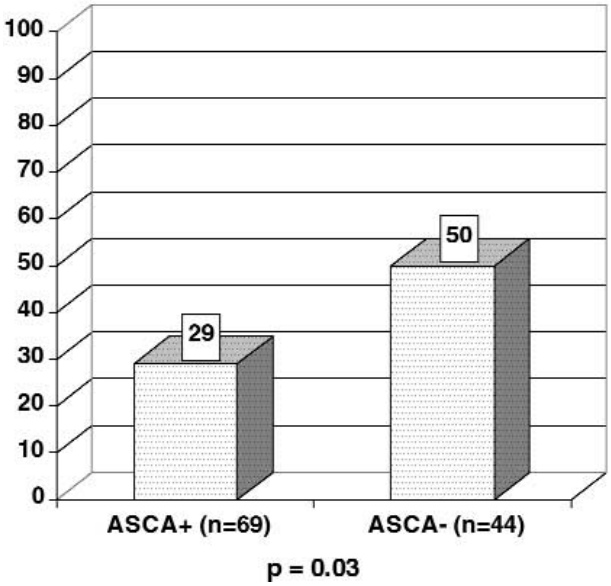

Fig. 1. — Percentage of Smokers according to ASCA Status

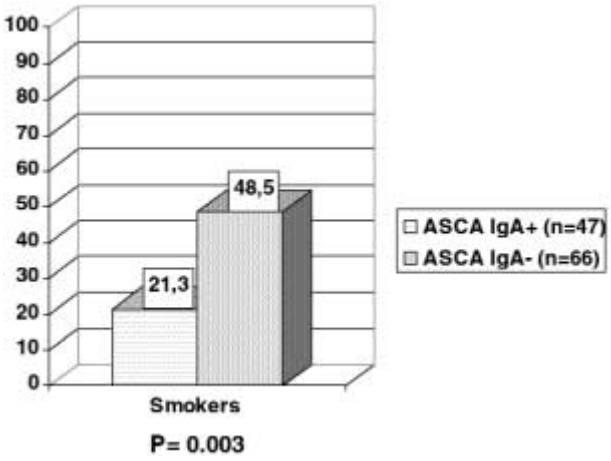

Fig. 2. — Percentage of Smokers according to ASCA IgA Status.

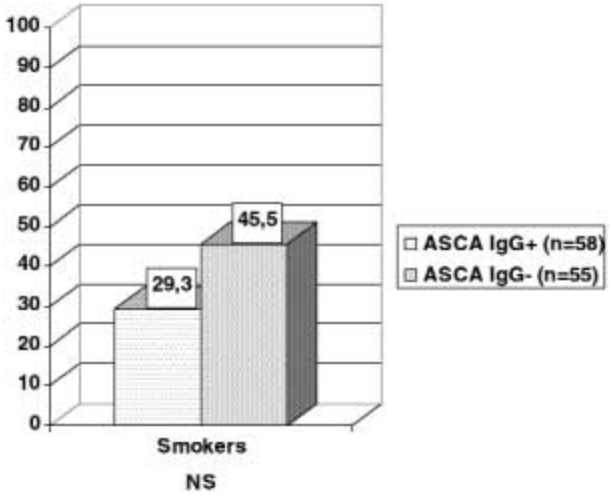

Fig. 3. — Percentage of Smokers according to ASCA IgG Status.

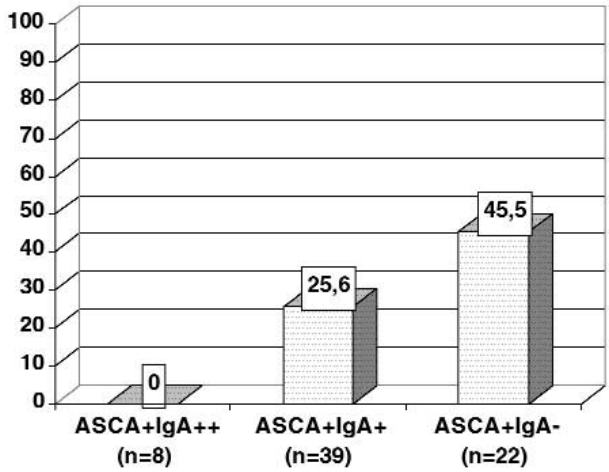

Fig. 4. — Percentage of Smokers according to Ig type in ASCA+ CD Patients.

Epidemiological, clinical and genetic characteristics in ASCA+ and ASCA- CD are shown in Table 1.

There was no significant difference between ASCA+ and ASCA- CD patients for age, gender, systemic manifestations, familial disease, parameters of the Vienna classification and genotyping for TNF. The only significant difference was for smoking habit : there were less smokers in CD ASCA+ than in CD ASCA- (29% versus 50% ; P = 0.03) (Table 1, Fig. 1).

This significant difference was maintained when ex-smoker (considered as non-smoker) were excluded from analysis (data not shown).

When studying ASCA IgA or IgG alone, this difference was only significant for ASCA IgA (21.3% smoker-ASCA IgA+ versus 48.5% smoker-ASCA IgA- ; P = 0.003) and not for ASCA IgG (29.3% smoker-ASCA IgG+ versus 45.5% smoker-ASCA IgG- ; NS) (Fig. 2-3).

This low proportion of smokers was more pronounced in IgA+ patients (IgG+ or IgG-) than in isolated IgG+ patients (respectively 25.6% smokers versus 45.5% smokers) and was the highest in patients with high titers of ASCA (0/8 smokers) (Fig. 4).

Logistic regression with ASCA status as dependent variable showed only smoking habit to be borderline for significance (P = 0.057). Other parameters were not significant. When taking only ASCA IgA positivity as dependent variable, again, smoking was borderline for significance (P = 0.066). However, when taking only ASCA IgG as dependent variable, smoking was no longer significant (P = 0.17).

Discussion

Among our population 61.1% CD patients were determined ASCA positive (respectively 31.9% IgA and IgG ; 9.7% IgA only and 19.5% IgG only) and the only significant difference between ASCA positive and negative patients was smoking habit.

This percentage of ASCA positivity confirmes previous reports (18 ;23-28). Among them a few have expressed results both for IgG and IgA ASCA. Sutton showed 53% ASCA positive patients with 35% IGA and IgG, 10% IgA only and 8% IgG only (27). Vasiliauskas (18) demonstrated 56% ASCA positive CD patients with 37.1% IgA and IgG, 12.1% IgA only and 7.1% IgG only while these percentages were respectively 63% ASCA positive, 43% IgA and IgG, 0% IgA only and 20% IgG only for Barnes (28). Thus differences exist between these studies involving much the percentage of isolated IgA or IgG than the total percentage. These differences could be explained by different assays used. Vermeire *et al.* recently studied, in IBD (CD and UC) and control population, four different assays and, among them, three commercially available assays : Prometheus Laboratories Inc. (San Diego, CA), Medipan Diagnostica (Selchow, Germany) and Quanta Lite (Inova Diagnostics, San Diego, CA) (31). A large variation in sensitivity between the assays was seen, ranging from 41% to 76%. Specificity was inversely related to sensitivity and was the highest for Medipan Diagnostica (97.5%), which is the one we used in the present study. Differences could be explained by the coating chosen (partially purified and disrupted *S. Cerevisiae* (Inova), oligopeptidomannans from the cell wall of the yeast (Prometheus) or mannoses (Medipan)), procedures followed for assays determination (dilutions of the serum used, conjugates or incubation time) or by different cutoff value chosen by the companies. Interpretation of results differs greatly and caution should thus be used when comparing results.

In all these studies however, including ours, about 40-50% of CD patients are ASCA -. Differences between ASCA + and ASCA - CD have not clearly been identified. Indeed, when considering clinical characteristics previous reports were discordant. Studies have shown either no relation between ASCA status and age (22) or more ASCA + in younger patients (18,24). Others have shown no relation with disease location (19,21,26) or a predominance of ASCA + in small bowel involvement (18,22,24). For behaviour, Ruemmele *et al.*, in pediatrics forms, didn't find relation between ASCA status and complicated forms (26) while Vasiliauskas found high ASCA titers independently associated with a tendency towards developing stricturing and penetrating small bowel complications (18). These discrepancies may relate to the classification used for disease location and behaviour. In our study, we used the most recent Vienna classification in which an effort was made to improve inter-observer reproducibility (29). However, even using this classification, location and behaviour may still change over the course of the disease (30). Therefore, to avoid an influence of disease duration on location or behaviour, we classified patients after a fixed duration of 3 years. In this conditions we found that ASCA status was not influenced by any of these clinical characteristics (age at diagnosis, location, behaviour). Other demographic characteristics, including gender, presence of systemic manifestation and a familial history of inflammatory bowel disease were neither associated with ASCA. This agrees with other studies for gender (18,22) and familial history (18).

Among genetic factors, Taylor et al found significant association between ASCA and TNF microsatellite haplotype TNFa11b4c1d3e3 in an IBD population of both CD and ulcerative colitis patients (32). We previously studied a single base pair polymorphism at position –308 in the promoter of TNF gene (16). It has been shown to have a functional significance on TNF production (33-35) and our results suggest that it may play a role in the phenotype of the disease (carriage of allele TNF2 associated with steroid-dependent disease and to a lesser extent with penetrating and colonic disease) (16). When comparing genotype and allelic frequencies for this –308 TNF gene polymorphism between ASCA positive and negative patients no difference was found in the two groups.

Smoking has been proven to be a particularly important environmental factor in CD : it is not only a risk factor for the development of CD (5,6) but it is also associated with more relapse (7,8), higher risk of operation (9-12) and complicated disease (13). Despite this influence, to our knowledge, smoking habit has rarely been taken into account when studying ASCA status in CD (36). In our study, in univariate analysis, smoking habit was the only significant character to differentiate ASCA positive and negative CD patients : there were more smokers in CD ASCA negative patients. This difference was more prominent with ASCA IgA. After logistic regression, smoking was still borderline for significance for global ASCA status as well as ASCA IgA, but not IgG. No other parameter was selected by this logistic regression. Cigarette smoking influences a variety of factors that may directly or indirectly affect the intestinal defenses. Smoking interferes with mucosal epithelial defenses (reduction of colonic mucus production, modification of intestinal permeability, influence on rectal blood flow), modifies inflammatory mediators (reduction of prostaglandin production, excessive generation of free radicals, reduction of antioxidant defenses) and certainly induces an abnormal response of the systemic and mucosal immune systems (cell mediated immunity and humoral immune markers) (37). Particularly smoking could reduce IgA ASCA production. Indeed IgA concentration in pure parotid saliva is reduced in both healthy smokers and smoking patients with epithelial head and neck tumors when compared with non-smokers (38). This could be the reason for what we found. But beyond this, it may also suggest a mechanism by which smoking may favour the development of CD. The decrease in secretory IgA may alter first line defenses against intestinal bacteria. This may be particularly relevant to the recent finding of NOD2 mutations associated with CD. Alternatively, a more specific interaction between smoking and still unidentified ASCA inducing antigen may not be excluded.

In conclusions, ASCA + patients are less often smoker than ASCA- ones. There may be an interference between smoking and development of ASCA, mainly IgA. Therefore, we suggest that smoking habit should be taken into account when considering ASCA status.

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