

## Chronic Granulomatous Disease and Crohn's Disease Histopathological Distinctive Features: A Pediatric Study

Marta Valente Pinto, Ana Maria Palha, José Gonçalo Marques, Susana Lopes da Silva, Ana Isabel Lopes

Marta Valente Pinto, Ana Isabel Lopes, Gastroenterology Unit, Pediatric Department, Santa Maria Hospital, Centro Hospitalar Lisboa Norte, Academic Medical Center of Lisbon, Av. Professor Egas Moniz 1649-035, Lisboa, Portugal

Ana Maria Palha, Pathology Department, Santa Maria Hospital - Centro Hospitalar Lisboa Norte, Academic Medical Center of Lisbon, Av. Professor Egas Moniz 1649-035, Lisboa, Portugal

José Gonçalo Marques, Center for Primary Immunodeficiencies and Infectious Diseases Unit, Pediatric Department, Santa Maria Hospital, Centro Hospitalar Lisboa Norte, Academic Medical Center of Lisbon, Av. Professor Egas Moniz 1649-035, Lisboa, Portugal

Susana Lopes da Silva, Center for Primary Immunodeficiencies, Immuno-Allergology Department, Santa Maria Hospital, Centro Hospitalar Lisboa Norte, Academic Medical Center of Lisbon, Av. Professor Egas Moniz 1649-035, Lisboa, Portugal

Correspondence to: Ana Isabel Lopes, MD PhD, Coordinator of the Pediatric Gastroenterology Unit, Gastroenterology Unit, Pediatric Department, Santa Maria University Hospital, Centro Hospitalar Lisboa Norte, Academic Medical Center of Lisbon, Av. Professor Egas Moniz 1649-035, Lisboa, Portugal. [anaialopes@sapo.pt](mailto:anaialopes@sapo.pt)

Telephone: +351217805202 Fax: +351217805623

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### ABSTRACT

**AIM:** To identify potential distinctive histopathological features of the gastrointestinal mucosa involvement in children with Crohn's Disease (CD) and Chronic Granulomatous Disease (CGD).

**METHODS:** Comparative evaluation of mucosal gastrointestinal involvement in 4 children with CG and CD ( $n=8$ ). The evaluation included Periodic Acid Schiff (PAS) and Immunohistochemical staining for CD68 in colonic biopsies.

**RESULTS:** Endoscopy was performed at a mean age of 5.5 years and 9.1 years at CDG and CD patients, respectively. Findings were similar in both groups. Histologically, in the colon, CGD patients showed focal architectural distortion ( $n=2$ ), patchy mono/polymorphonuclear infiltrate ( $n=4$ ), focal cryptitis ( $n=3$ ) and noncaseating granulomas ( $n=3$ ); patients with CD presented focal architectural distortion ( $n=4$ ), patchy inflammatory mono/

polymorphonuclear infiltrate ( $n=4$ ), cryptitis ( $n=4$ ), crypt abscesses ( $n=3$ ), ulceration ( $n=3$ ) and noncaseating granulomas ( $n=3$ ). PAS positive pigmented histiocytes in the lamina propria were observed in all CGD patients and absent in CD. In CGD patients, CD68 positive cells with vast cytoplasm were found, mostly at a deep location in the lamina propria, and in clusters. In CD, CD68 positive cells featured a distinct pattern, with less cytoplasm and appeared predominantly isolated and scattered.

**CONCLUSION:** Although our results are preliminary, they suggest that the characterization of histiocyte infiltrate and of its topographic pattern, using additionally valuable markers as PAS and CD68 immunostaining, may further contribute to better discriminate CGD and CD.

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**Key words:** Chronic Granulomatous Disease (CGD); Crohn's Disease (CD); Colonic mucosa; Histopathology; Histiocytes; CD-68 cells

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### INTRODUCTION

Chronic Granulomatous Disease (CGD) is a primary immunodeficiency disorder of phagocytic cells caused by a dysfunction of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, with consequent decreased or absent production of reactive oxygen species (ROS)<sup>[1,2]</sup>. It is characterized by the occurrence of severe recurrent bacterial and fungal infections, as well as by a state of hyperinflammation, which leads to the formation of granulomas<sup>[1-4]</sup>. The infectious manifestations are the most common clinical features, usually evident during the first years of life, sometimes alerting to the diagnosis<sup>[3]</sup>.

Symptomatic gastrointestinal disease may occur in a significant number of patients with CGD, with a reported global prevalence reaching 25-50% of the cases<sup>[3,5-7]</sup>. It is recognized that

gastrointestinal involvement, sometimes preceding diagnosis, can occur from mouth to anus<sup>[5]</sup>. The symptoms reported by the patients depend on the affected site(s), the most common being abdominal pain, diarrhea, nausea and vomiting, constipation, failure to thrive and anemia<sup>[5,8-9]</sup>.

Regarding the age at presentation, there may be a significant overlap in clinical expression between CGD and inflammatory bowel disease (IBD), particularly Crohn's Disease (CD), representing a true challenge to their differential diagnosis. Endoscopic and histological findings are also very similar; macroscopically, both diseases are characterized by chronic active colitis with patchy friability, strictures, fissures and ulcers, with a segmental distribution in CD and a continuous distribution with variable proximal extension from rectum in ulcerative colitis (UC)<sup>[10,11]</sup>. Histologically CGD and CD are both characterized by an infiltrate of polymorphonuclear cells, cryptitis and crypt abscesses and occasional granulomas without necrosis<sup>[3,10-11]</sup>.

The recognized overlap of clinical expression between these two entities recommends the systematic exclusion of CGD in refractory and/or early onset IBD, considering the implications of a late diagnosis<sup>[5-8]</sup>.

It has been recently emphasized the importance of identifying distinctive histopathological patterns. However, knowledge is still scarce in this area and so far only a few papers have concerned pediatric age<sup>[9,12,13]</sup>. Pigmented histiocytes within the lamina propria present in colonic biopsies from patients with CGD have been suggested as the major distinctive feature that should alert for this diagnosis<sup>[12-13]</sup>. Another element that has been explored (only a case series study and a case report were so far published), is the differential expression of CD68 + cells (macrophages) in colonic mucosa<sup>[12-13]</sup>.

The aim of this study was to compare gastrointestinal involvement between children with CGD and CD, considering clinical, endoscopic and histological features, including as additional markers Period Acid Schiff (PAS) stain and CD68 immunostaining. These elements might further contribute to a better distinction between these two entities.

## METHODS

### Patients

Pediatric patients with CGD and CD followed at a Pediatric tertiary center were identified through clinical and laboratory databases. The four patients with CGD (diagnostic criteria based on functional and genetic studies) and gastrointestinal symptoms (GI) followed in our Center were included in the study. Additionally, four pediatric patients with recently diagnosed active CD (diagnosis according to Porto criteria and disease activity determined according to the Paediatric Crohn's Disease Activity Index- PCDAI) were also selected<sup>[11]</sup>. CD patients recruitment was based on age and expression of GI disease (including endoscopic features), as much similar as possible as compared to CGD patients. We conducted a retrospective study of clinical data, including review of all medical records. Demographic data (including patient age, gender and ethnicity) were recorded, as were details of the underlying disease (including subtype gene in CGD, age at onset of symptoms, age at diagnosis and clinical manifestations).

We performed a descriptive analysis of endoscopic features previously reported during the investigation of gastroenterological symptoms. The histopathological features concerning the specimens taken during the initial upper and lower gastrointestinal endoscopic evaluation (with the exception of one case of CGD, due to inaccessibility to the initial biopsies) were reviewed by the same pathologist.

Histological Assessment: Formalin-fixed (10%) paraffin-

embedded tissues from biopsies, were processed according to conventional histological technique. Serial sections (4 µm) were stained with hematoxylin-eosin (H&E) and gastric biopsies were stained with Giemsa for *Helicobacter pylori* identification. Colonic biopsies were additionally stained with: PAS staining to detect histiocyte pigment lipofuscine; immunohistochemical staining (indirect method with polymer detection system peroxidase/DAB) for CD68 (Monoclonal mouse, DAKO, Clone PG-M1). The immunostaining was performed according to standard methods, in selected representative sections. We performed a descriptive evaluation in two areas of inflammation of the colonic mucosa with magnification (×40), concerning morphological features and distribution pattern of the CD68 positive cells.

## RESULTS

Clinical Features: 4 CGD patients (3 boys, 1 girl), with a mean age of 97.5 months (39-205 months), were studied. In two cases the first manifestations of CGD were infectious diseases, in one case gastrointestinal symptoms and in the fourth case (the girl) the diagnosis was prenatal.

The diagnosis was performed by the quantitative dihydrorhodamine 123 flow cytometry assay, at a mean age of 34.8 months (0-61 months), and in 3 cases confirmed by DNA analyses. 2 boys had an X-linked disease, affecting the gp91phox subunit of NADPH oxidase, while the girl had autosomal recessive disease affecting p67phox subunits. All children were caucasian. They presented with GI symptoms at a mean age of 34 months (1-60 months), including diarrheal stools in the 4 patients (3 with blood), failure to thrive and abdominal pain (2 patients). In this setting, endoscopy was performed (upper endoscopy in 3/4 patients, total colonoscopy in the four patients with ileoscopy in 1/4 patients) at a mean age of 66 months (37-127 months), (before any specific therapy had been instituted for gastrointestinal disease).

Three of 4 children had been treated with steroids (one in association with azathioprine). The follow-up period of this group since the beginning of the treatment and the study ranged from 6 months to 12 years.

The other child was submitted to bone marrow transplantation (BMT) for the first time at the age of 9 years (with a complete graft failure 12 months later) and for the second time by the age of 12 years. After the BMT, clinical remission including gastrointestinal symptoms has been observed (1 year follow-up). Endoscopy performed 16 months after BMT revealed normal endoscopic and histological features.

This group of CGD patients was compared with a group of 4 caucasian patients with CD (one male), with a mean age of 113 months (36-186 months), presenting with GI symptoms at the mean age of 108 months (14-189 months). The GI symptoms were very similar to those observed within CGD patients and included: diarrhea (4 children) weight loss (3 children) and abdominal pain (1 child). The diagnosis was established according to Porto Criteria, between 18 and 191 months of age (mean 122 months); median PCDAI 30 (range 27,5-45).

In all children with CD an upper endoscopy and a total colonoscopy (ileoscopy in 2 cases) were performed at the mean age of 113 months (18-191 months) and 114 months (18-191 months) respectively. In both groups, infectious etiologies were excluded. All four patients were under azathioprine therapy (since recent diagnosis) and had been treated with steroids (for remission induction). At the timing of endoscopy, both groups of patients (CD and CGD) had additionally underwent a cycle of semi-elemental dietary intervention. Endoscopic and histological findings: the macroscopic findings of the

upper endoscopy and colonoscopy were similar within CGD and CD (Table 1). In these 8 patients, 108 biopsy specimens were reviewed, 26 from the upper and 82 from the lower gastrointestinal tract.

In 4/6 upper endoscopies no changes were shown. In both groups the major features were erythematous and friable mucosa (Table 1). At the colonoscopy, a friable and erythematous mucosa was observed in 7 of 8 patients, as well as a discontinuous (5 patients) and diffuse (3 patients) pattern (Table 1). Histopathologically, in gastric and duodenal biopsies, the findings in all cases characteristically included patchy chronic inflammation (2CGD/4CD) with neutrophil infiltrate (2CGD/1CD). In one child of each group, *Helicobacter pylori* was identified.

**Table 1** Macroscopic findings in the first endoscopic evaluation of CGD and CD patients.

Features	CGD <sup>1</sup> n=3				CGD <sup>1</sup> n=3			
	<sup>2</sup> E	<sup>3</sup> S	<sup>4</sup> D1	<sup>5</sup> D2	<sup>2</sup> E	<sup>3</sup> S	<sup>4</sup> D1	<sup>5</sup> D2
Upper GI tract								
Erythematous and friable mucosa	0	1	1	1	0	1	1	1
Ulcers	0	0	0	0	0	0	0	0
Nodularity	0	1	1	1	0	1	1	1
Normal	2	2	2	2	2	2	2	2
Ileum								
Friable mucosa			<sup>1</sup> n=1				<sup>1</sup> n=2	
Colon								
Friable mucosa			<sup>1</sup> n=4				<sup>1</sup> n=4	
Erythematous mucosa			4				3	
Ulcers			1 (rectum)				0	
White dots			1				1	
Micronodularity			0				2	
Inflammatory exudate			3				0	
Discontinuous pattern			3				2	

<sup>1</sup>n: Number of patients where biopsies were performed and analyzed in that specific areas; <sup>2</sup>E: esophagus; <sup>3</sup>S stomach; <sup>4</sup>D1: duodenum first portion; <sup>5</sup>D2: duodenum second portion.

The colon biopsies have shown maintenance of epithelial cell population (all) and architectural distortion, with the following findings: shortened crypts, variable crypt orientation (2CGD/4CD) associated with patchy active chronic inflammation with ulceration (1CGD/3CD), cryptitis, crypt abscess formation (3CGD/4CD), lymphoid follicles (2CGD/3CD) and occasional epithelioid granulomas without necrosis (mucosal - 3CGD/ mucosal and submucosal - 3CD). At all sites of inflammation, the cellular infiltrate contained scattered eosinophils.

Ileum was observed in 1 case of CGD, showing a conserved structure, with an inflammatory infiltrate and without granulomas.

The ileal mucosa was also observed in 2 cases of CD, showing, in both cases, histological features similar to those observed in the colonic mucosa.

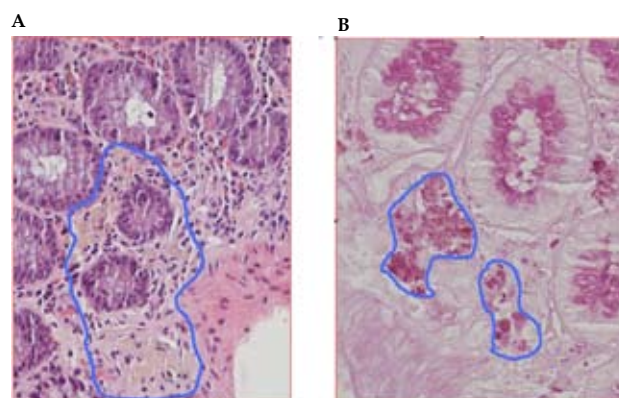
In Hematoxylin Eosin staining, pigmented histiocytes in the lamina propria, mainly distributed between the base of the crypts and muscularis mucosa, were present in all CGD patients and absent in CD patients (Figure 1). In figure 1, the blue outlined area in the first image (Figure 1A) represents the pigmented histiocytes in the lamina propria mainly between the base of the crypts and muscularis mucosa (MM), found in all CGD patients (H&E). A higher magnification showing pigmented histiocyte positive for Periodic Acid Schiff (PAS) staining - blue outlined area - is represented in the second image (Figure 1B).

Figure 2 demonstrates the absence of pigmented histiocytes (PAS staining) in CD patients

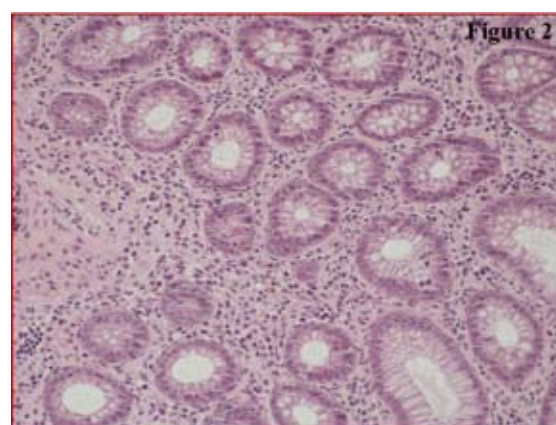
In CGD patients, CD68 positive cells with vast cytoplasm were found, mostly at a deep location in the lamina propria and in clusters (Figure 3, A and B). The image a) in figure 3 represents the CD 68 positive cells with the features described above. A higher magnification of this image is shown in image b).

In CD, the CD68 positive cells had a distinct pattern, with less cy-

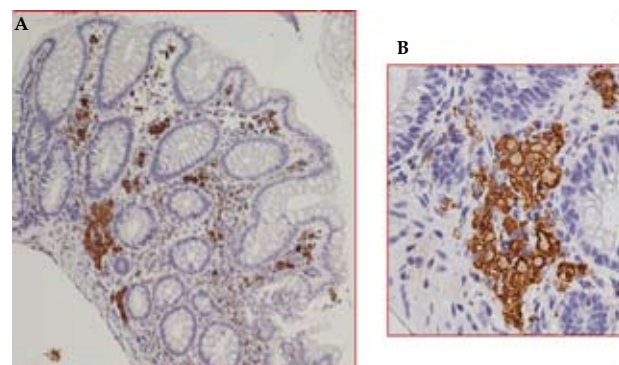
toplasm, appearing predominantly isolated and scattered (Figure 4, A) and B). The image b) is a detail of the image a).



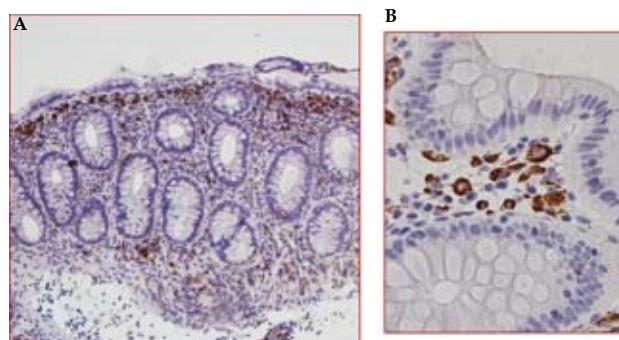
**Figure 1** Photomicrograph of colonic mucosa from a child with Chronic Granulomatous Disease.



**Figure 2** Photomicrograph of colonic mucosa from a child with Crohn's Disease.



**Figure 3** Photomicrograph of colonic mucosa from a child with Chronic Granulomatous Disease immunostaining for CD68.



**Figure 4** Photomicrograph of colonic mucosa from a child with Crohn's Disease immunostaining for CD68.

## DISCUSSION

The diagnosis of CGD may be challenging, particularly when the initial presentation is not typical, as in the absence of recurrent infections. Although the average age at onset of GI symptoms has been reported as 5 years (10 months-30 years), these may occur as soon as during the first year of life and may precede CGD diagnosis in 15% of cases<sup>[6,7]</sup>.

The GI manifestations may occur throughout the digestive tract, ranging from mild to severe<sup>[6-8,14]</sup>. Manifestations such as abdominal pain, diarrhea and weight loss are among the most frequent, as observed in our study group<sup>[5,8]</sup>.

It should be emphasized that even when the GI manifestations occur at an expected age, a misdiagnosis of IBD remains an important possibility, compromising the treatment management approach<sup>[15]</sup>. Although the optimal treatment of granulomatous colitis in this setting is not established, and the first recommended approach is similar to CD, the major difference is the possibility of cure after stem cell transplantation in CGD, proposed in cases of severe colitis refractory to treatment<sup>[1,2,7,16-17]</sup>.

The great similarities between the endoscopic features in patients with CGD and CD contribute to this misdiagnosis<sup>[6,8-9]</sup>.

The most frequently reported histological changes in CDG patients are the presence of inflammatory infiltrate by mono and polymorphonuclear cells, crypt architecture distortion and the presence of noncaseating granulomas<sup>[8-10,14,18]</sup>, these features were also found in our study, although no granulomas have been observed in upper GI tract mucosa in CGD patients.

Some recent studies have suggested that the presence of pigmented laden macrophages in the lamina propria, as observed in our study<sup>[8,9,18,19]</sup>, might be a distinctive pattern of CGD. In fact, in our 4 patients with CGD, we observed the presence of macrophages (histiocytes) in clusters, with brownish-yellow pigment (PAS +), with higher density predominately in the lower third of the mucosa. These features were not found in CD patients. Moreover, the cells were located between the base of the crypts and the muscularis mucosa.

Only one previous study had shown some of these features, such as the presence of pigmented histiocytes around the crypt bases<sup>[8]</sup>.

Considering that CD is much more frequent than CGD and that features such as granulomas, abscesses and cryptitis are more obvious, the distinction between entities such as CD and CGD becomes relevant. In fact, the presence of pigmented histiocytes might be a relevant distinguishing feature between these two entities, allowing the early establishment of the diagnosis.

Another feature addressed in our study concerns the differences in CD68 cell immunostaining in colonic mucosa of these patients. CD68 is a glycoprotein, member of the lysosomal-associated membrane protein family, that is considered a monocyte-macrophage marker and it is also found in the primary granules of neutrophils, as well as in certain epithelial cells<sup>[12]</sup>. Its function is not accurately recognized, but these cells appear to have a role in the endocytosis and lysosomal trafficking<sup>[12]</sup>.

One of the first published studies (Mitomi H, *et al*) on the expression of CD68 cells in the colonic mucosa in patients with colitis associated with CGD showed an increased expression of CD-68 cells in colonic lamina propria, when compared with CD patients<sup>[13]</sup>.

Another study from Liu S, *et al.* showed a lower expression of CD68 cells in CGD biopsies. In our study we found a different distribution pattern and although we have not performed semi-quantitative evaluation, a higher CD68 density was evident in CGD

patients.

Different patient selection criteria, degree of the disease, levels of biopsies and inter observer variance might explain these differences. All these differences can lead to the question of the relevance of CD-68 expression in colonic mucosa and its role to distinguish between these two entities.

Interestingly, other immunodeficiency spectrum diseases, such as glycogen storage disease type IB, common variable immunodeficiency (CVID), X-linked agammaglobulinemia, also characterized by the presence of colitis, share some common features with those present in CGD, such as the presence of pigmented histiocytes<sup>[9]</sup>. Despite this similarity, findings such as crypt abscesses and cryptitis have only been reported in CVID.

In conclusion, with the present study we intended to further contribute to the clinical and morphological characterization of the GI clinical spectrum of CGD and to the differential diagnosis with CD. The presence of pigmented laden histiocytes, with larger cytoplasm, with a characteristic pattern of topographic distribution, if further confirmed, could provide a contribution to the histological diagnosis of CGD.

The study also allowed us to raise the question about which other immunohistochemical assessments might be useful to the diagnosis. CD68 staining in the colonic mucosa appears to be interesting, and might possibly be used as an additional histological marker to the disease diagnosis. Studies with larger populations, ideally recruited from multicenter databases, are essential to improve our knowledge, allowing a more effective diagnosis.

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**Peer reviewers:** Giuliana Decorti, MD, Department of Life Sciences, University of Trieste, Via L. Giorgieri 7, Trieste, I-34127, Italy; Alan C Moss MD, Division of Gastroenterology, BIDMC, 330 Brookline Ave, Boston, MA 02215, USA; Takayuki Yamamoto, MD, PhD, FACC, Inflammatory Bowel Disease Center, Yokkaichi Social Insurance Hospital, 10-8 Hazuyamacho, Yokkaichi, Mie 510-0016, Japan.