Clinical Case Study

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# Perianal Disease and Granulomas: Think Out of the Box…

Ana Reis-Melo[a, b] Maria do Céu Espinheira[a] Isabel Pinto-Pais[a]
Artur Bonito Vitor[c] Jacinta Bustamante[d] Eunice Trindade[a]

[a]Pediatric Gastroenterology Unit, Centro Hospitalar Universitário do São João, Porto, Portugal; [b]Biomedicine Department, Faculdade de Medicina, Universidade do Porto, Porto, Portugal; [c]Pediatric Primary Immunodeficiencies Unit, Centro Hospitalar Universitário do São João, Porto, Portugal; [d]Centre d'Etudes des Déficits Immunitaires, Hôpital Necker Enfants-Malades, Paris, France

**Keywords**
p40$^{phox}$ deficiency · Crohn's disease · Chronic granulomatous diseases · *NCF4* gene

**Abstract**
***Background:*** Chronic granulomatous disease (CGD) is a primary immunodeficiency due to a malfunction of NADPH oxidase. It is characterized by recurrent and severe infections caused by catalase-positive microorganisms and autoinflammatory manifestations. Recently, there has been described an *NCF4* gene variant that causes a deficiency of p40$^{phox}$, a subunit of NADPH oxidase. Patients with this deficiency appear to have a less severe clinical form as compared to classic CGD. ***Case:*** A 15-year-old girl with vulvar lichen planus since she was 2 years old and suspected Crohn's disease (CD) was first seen at our hospital. At the age of 12 years, she had been submitted to sacrococcygeal cyst exeresis, without cicatrization of the surgical wound and extension of the lesion to the perianal area. The diagnosis of CD was questioned, and the patient underwent an endoscopic and radiologic assessment, which was normal. A skin biopsy from the perianal area revealed a granuloma; thus, CD with isolated perianal disease was assumed. After several different treatments including antibiotics, infliximab, and adalimumab, the perianal lesion persisted, with no associated gastrointestinal symptoms. Therefore, the hypothesis of an immunodeficiency was considered. An immunologic and genetic study revealed reduced oxidative burst in the phorbol myristate acetate test, with diminished reactive oxygen species production and a homozygous mutation in the *NCF4* gene. The adolescent started prophylactic trimethoprim-sulfamethoxazole and became asymptomatic. ***Conclusions:*** The present case highlights that alternative diagnoses to CD must be considered in the presence of isolated perianal disease with granulomatous inflammation, especially when the disease is refractory to conventional CD therapy.



**Doença perianal e granulomas: pensar fora da caixa…**

**Palavras Chave**
Deficiência da p40$^{phox}$ · Doença de Crohn · Doença granulomatosa crónica · Gene *NCF4*

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E-Mail karger@karger.com
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Ana Reis-Melo
Pediatric Department, Centro Hospital São João
Alameda Prof. Hernâni Monteiro
PT–4200-319 Porto (Portugal)
E-Mail anareismelo@gmail.com

**Resumo**

***Introdução:*** A doença granulomatosa crónica (DGC) é uma imunodeficiência primária devido a uma disfunção da NADPH oxidase. É caracterizada por infeções recorrentes e graves causadas por microrganismos catalase positivos e manifestações auto-inflamatórias. Recentemente, foi identificada uma variante do gene *NCF4* responsável por deficiência de p40$^{phox}$, uma proteína constituinte da NADPH oxidase e clinicamente esta doença manifesta-se como uma imunodeficiência menos grave quando comparada com a DGC clássica. ***Caso:*** Adolescente de 15 anos, com líquen plano vulvar desde os 2 anos. Aos 12 anos, submetida a exérese de quisto sacrococcígeo não tendo ocorrido cicatrização da ferida cirúrgica e com extensão da lesão para a região perianal. Perante a suspeita de doença Crohn (DC), realizada investigação endoscópica e radiológica que foi normal. A biópsia de pele da lesão perianal identificou granuloma, tendo sido admitido o diagnóstico de DC com apresentação perianal. Foi submetida a vários tratamentos sem resolução da lesão. Aos 15 anos, colocada a hipótese de imunodeficiência primária; o estudo imunológico mostrou diminuição da explosão oxidativa no teste de imunidade com acetato miristato de forbol, com produção reduzida de radicais livres de oxigénio (RLO). Geneticamente identificada mutação homozigótica no gene *NCF4*. Atualmente, sob antibiótico profilático e clinicamente assintomática. ***Conclusão:*** Este caso permite alertar para a investigação de diagnósticos alternativos à DC perante doença perianal isolada com inflamação granulomatosa, em particular quando é refratária à terapêutica dirigida.



## Introduction

Chronic granulomatous disease (CGD) is one of the classic and well-recognized immunodeficiencies at the pediatric age that is usually diagnosed between the age of 1 and 3 years [1]. CGD presents with severe and recurrent bacterial and fungal infections that predominantly affect the lungs and skin [2]. The clinical manifestations include pneumonia, cutaneous abscesses, lymphadenitis, and more rarely osteomyelitis. Commonly found agents include *Staphylococcus aureus*, gram-negative organisms (*Serratia marcescens* and *Burkholderia cepacia*) and *Aspergillus* [2, 3].

Some patients present with noninfectious symptoms, such as bloody diarrhea or failure to thrive, mimicking an inflammatory bowel disease. This condition correlates with a failure of phagocytosis due to modification of NADPH oxidase activity. The NADPH oxidase system comprises 6 proteins related to 6 particular genes – the membrane-bound heterodimer (p22$^{phox}$ + g91$^{phox}$), the 3 cytosolic subunits p47$^{phox}$ (*NCF1*), p67$^{phox}$ (*NCF2*) and p40$^{phox}$ (*NCF4*), and EROS (*CYBC1*) – and its function is to produce reactive oxygen species (ROS) radicals in order to eliminate microorganisms [4].

The large majority of patients have a biallelic mutation in *CYBB* encoding p91$^{phox}$, and until recently, only 1 patient had been described as having a mutation in the *NCF4* gene [5]. A new published series showed 24 patients with biallelic mutations in this gene responsible for autosomal recessive p40$^{phox}$ deficiency, which was identified to cause a reduction in NADPH oxidase activity, culminating in noninvasive infections and immunologic dysregulation less severe than with classic CGD [6].

## Case Report

A 15-year-old patient with a past history of vulvar lichen planus since the age of 2 years presented to the gastroenterology clinic at our hospital with a 4-year history of Crohn's disease (CD) unresponsive to "conventional treatment." At the age of 12 years, the patient had presented with a sacrococcygeal cyst with fistulous tracts that needed surgical exeresis, but there were posterior wound healing complications and extension of the lesion to the perianal area (Fig. 1).

After multiple antibiotics and a second repair surgery without improvement of the wound, the adolescent was investigated for CD. At that point, the patient did not present with any gastrointestinal or constitutional symptoms, and blood work was unremarkable except for high fecal calprotectin (707 μg/mL) and positive anti-*Saccharomyces cerevisiae* antibody, with IgA at >200 EU/mL and IgG at 91 EU/mL. Previous endoscopic assessments, including upper and lower digestive endoscopy and capsule endoscopy, had been considered normal, and an abdominopelvic magnetic resonance image showed only an intersphincteric fistula. Skin biopsy of the perianal lesion revealed a granuloma, and CD with perianal presentation was assumed. Tuberculosis was ruled out, as the patient had normal thoracic radiography results, negative polymerase chain reaction for *Mycobacterium tuberculosis* complex in ileal samples, and negative interferon-γ release assay results.

Several different types of drug were initiated. At the beginning, the patient was on metronidazole, ciprofloxacin, and azathioprine, with little improvement. In the next few months, the patient had several inflammatory/infectious relapses in the perianal wound, so infliximab was started. Two months later, the patient presented with several new oral and vulvar lesions, as well as perianal wound deterioration with hematic drainage. Hyperbaric oxygen therapy was also ineffective, with persistence of the perianal wound (Fig. 2), in conjunction with vulvar lesions and new nasal pustules. After innumerous antibiotic courses without improvement, biologic therapy was switched to adalimumab. At 15 years of age, the patient had de novo inverse psoriasis as a side effect of biologic therapy, which was suspended with improvement of the skin lesions.

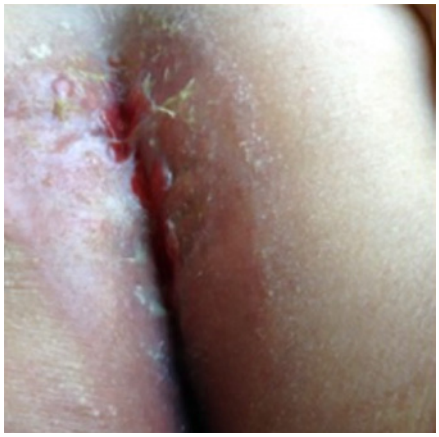

**Fig. 1.** Perianal lesion of the patient at 12 years of age.

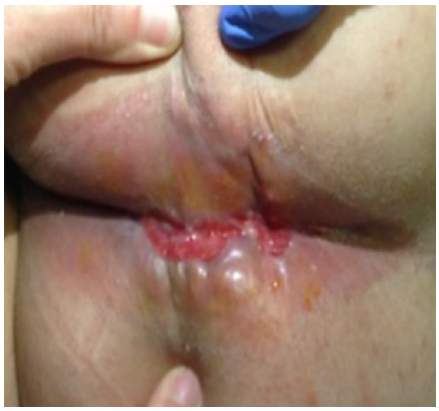

**Fig. 2.** Perianal lesions of the patient at 15 years of age.

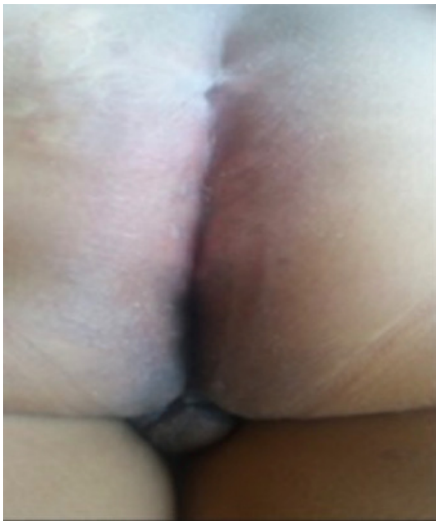

**Fig. 3.** Perianal region healed at 18 years of age.

Endoscopic examinations were repeated at our institution, and the results were again normal. The biopsies were revised and a granuloma was observed in a sample from the transverse colon. Thalidomide therapy was initiated, with mild clinical improvement for the first time. Unfortunately, the patient developed severe drug-related lymphopenia. At that time, as the patient had a long history of nonhealing wounds in the perianal and vulvar regions, as well as granulomas in skin biopsy and colonic mucosa, but no gastrointestinal symptoms, clinical suspicion for an immunodeficiency was raised. Her complete blood count and T, B, and natural killer cell subsets were normal; her serum immunoglobulin levels and response to immunizations were also normal.

Although NADPH oxidase activity was impaired in terms of superoxide and hydrogen peroxide production (after phorbol ester activation), a DHR123 (dihydrorhodamine 123) assay demonstrated that neutrophils and monocytes generated ROS upon phorbol ester (e.g., phorbol myristate acetate [PMA]), and neutrophils even produced ROS after physiological activity (e.g., fMLP [*N*-formyl-methionyl-leucyl-phenylalanine] with or without TNF-α), meaning that the patient had an impaired but not abolished NADPH activity. Monocyte-derived macrophages and monocyte-derived dendric cells showed a normal NADPH activity. Genetic panel testing was carried out, which revealed a homozygous mutation in the *NCF4* gene, conferring an AR p40$^{phox}$ deficiency. After this finding, the adolescent received intracellularly acting antibiotics – clindamycin for a short period, followed by prophylactic trimethoprim-sulfamethoxazole, with significant clinical improvement and resolution of the perianal wound (Fig. 3).

## Discussion and Conclusion

Perianal disease at diagnosis is seen in 9% of pediatric CD patients, and in these patients frequently precedes other features of the disease for months [7]. It is well known that many diseases may mimic the clinical symptoms and endoscopic and histological features of inflammatory bowel disease, particularly CD. This is relevant, which is why an extensive clinical review article on inflammatory bowel disease was published in 2018 [8], but interestingly only two entities were considered under the designation of granulomatous autoimmune diseases: (1) sarcoidosis and (2) common variable immunodeficiency. The case that we presented is an example of other diseases that may present with some features of CD and should be considered in a differential diagnosis, especially in refractory disease, regardless of the patient's age.

CGD is a rare primary immunodeficiency with an estimated incidence of 1:200,000 live births, although its true incidence or prevalence is not known [9]. The clinical course of this disease depends on the genetic variants, which can be heterogeneous and complex per se, and consequently on the NADPH oxidase subunit that is deficient. A total deficiency of the system portend an ominous clinical course with high mortality; on the other hand, diminished activity of the oxidase results in a less severe disease [3]. Generally, CGD is characterized by invasive infections caused by catalase-positive microorganisms and fungi that are directly related to the higher mortality among these patients [2]. Dysregulated inflammation is also commonly present, with cohorts showing inflammatory manifestations occurring in more than 50% of the patients and the main affected organs being the gastrointestinal tract, lungs, urogenital tract, and eyes

[10]. Diagnosis of this condition is based on evaluation of the phagocytic cells' capacity to produce superoxide or hydrogen peroxide, usually via the nitro blue tetrazolium slide assay and the DHR123 assay with PMA stimuli [3]. In our patient, the results showed an impaired production of ROS, even though it was higher when compared to classic CGD, which may explain the difference in clinical presentation. Treatment consists of antimicrobial prophylaxis and ultimately hematopoietic stem cell transplantation [10].

The first case of $p40^{phox}$ deficiency was published in 2009, a 3-year-old-boy with granulomatous colitis difficult to manage. He underwent several different treatments without improvement, including biological therapy and an ileostomy. The DHR123 assay revealed partial reduction of intracellular oxidant production, despite the fact that the production of $O_2^-$ by neutrophils in response to stimulation with PMA or fMLP was normal; immunoblot analysis of the neutrophil oxidase subunits showed reduced $p40^{phox}$ expression [5]. A recently published study showed an impact of *NCF4* mutations on NADPH oxidase activity, revealing that intracellular ROS production in $p40^{phox}$-deficient neutrophils is much higher than in classic CGD cells, and that these patients are capable of eliminating bacteria and fungi, unlike CGD patients [6]. This may explain why our patient did not have any life-threatening or invasive infections; instead, she presented with inflammatory granulomas in the gastrointestinal tract and peripheral infections in the perianal and vulvar regions. Also the use of antibiotics that act intracellularly, such as clindamycin, led to progressive improvement of the perianal wound. Maintenance treatment with prophylactic cotrimoxazole, which is preconized for these patients, allows conserving the wound healed.

Inflammatory and autoimmune manifestations appear to be characteristic of $p40^{phox}$-deficient patients rather than of classic CGD patients [6]; the cutaneous and oral/nasal lesions of our patient could be a manifestation of this. The persistently high fecal calprotectin level in our patient may infer a state of hyperinflammation, considering that this marker can be related to a colonic inflammation in CGD patients that is not evident clinically or endoscopically [11]. The possibility of occurrence of severe colitis during the course of the disease, as described in other patients with the same defect, emphasizes the need for careful and continuous monitoring [6].

Our patient was submitted to several therapies and suffered from some of their side effects. Even though anti-TNF agents are used to treat psoriasis, it is known that, paradoxically, they can cause psoriasis themselves when used to treat other diseases like CD [12]. As in other cases, it had only completely resolved after interruption of adalimumab treatment [13]. Thalidomide can be used, as a rescue therapy, in CD refractory to other treatments, and this was attempted with improvement, probably due to the immunomodulatory properties of the drug [14, 15]. In the context of its use, our patient developed leukopenia, a recognized side effect that requires thalidomide withdrawal [16].

The presence of granulomas in the skin and gastrointestinal tract in our patient implies a prolonged inflammatory response which is explained by the modified regulator protein that ultimately causes a reduced, but not null, ROS production. The present case highlights that alternative diagnoses to CD must be considered in the presence of isolated perianal disease with granulomatous inflammation, especially when the disease is refractory to conventional CD therapy. Early diagnosis of $p40^{phox}$ deficiency and initiation of appropriate prophylactic treatment allow avoiding recurrent infections and severe drug-related side effects and may improve quality of life.

## Statement of Ethics

The authors have no ethical conflicts to disclose.

## Disclosure Statement

The authors have no conflicts of interest to declare.

## Author Contributions

A.R.-M., M.d.C.E., and I.P.P.: acquisition, analysis, or interpretation of the data for the work; A.B.-V. and J.B.: revision; E.T.: drafting of the work and revision.

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