

CLINICAL NOTES

Fecal calprotectin (FC) in newborns: is it a predictive marker of gastrointestinal and/or allergic disease?

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Abstract

Fecal calprotectin seems to provide a safe and non-invasive means of helping differentiate between patients with organic and non-organic intestinal disease. Aim of our study was to evaluate if FC levels at birth and at first month of age can be a predictive biomarker of organic or functional gastrointestinal disease (FGIDs) and/or allergic disease diagnosed in 2 years old children. Between December 2007 and January 2008 a telephone interview has been proposed to the parents of 109 consecutive healthy children, in which FC was measured at birth two years before. For our study, a modified version of the original paediatric questionnaire on paediatric functional gastrointestinal disorders (QPGS) was used for the interview. Specific questions were added to detect allergic diseases. We did not find any statistically significant result between FC measured at birth and during first month of life in children with allergy or not. The interference of familiarity does not lead to a statistically significant change in the fecal calprotectin values during the first month of life.

Keywords: Fecal calprotectin; newborns; atopy; functional gastrointestinal

Introduction

Calprotectin is a small calcium- and zinc-binding protein complex belonging to the S-100 family (S-100A8/A9) with a molecular weight of 36.5 kDa, composed of 8 and 14 kDa subunits.⁽¹⁾ The protein is abundant predominantly in neutrophils (accounts for 5% of the total proteins and 60% of the cytosol neutrophils proteins) but also expressed on the membrane of monocytes, and is considered to have both bactericidal and fungicidal properties. Fecal calprotectin (FC) is an important clinic test in order to evaluate the inflammatory state of the whole intestinal tract. FC measurement is an easy, non-invasive and cheap test and it can be easily used in pediatric age. The increase of FC concentration is related to an increase of a turnover of leukocytes in the intestinal wall besides to an increase of neutrophils in the intestinal lumen.⁽²⁾

Food and Drug Administration, in 2006, classified FC immunological test systems into class II (special

controls) that is used in the diagnosis of inflammatory bowel diseases (IBD) and as an aid in the differentiation of IBD from irritable bowel syndrome (IBS).⁽³⁾ IBS has been included in childhood functional gastrointestinal disorders (FGIDs), a variable combination of age-dependent, chronic, or recurrent gastrointestinal symptoms not otherwise explained by structural or biochemical abnormalities. Until recently, the diagnosis of FGIDs in children was based on the exclusion of organic disease and physicians felt obliged to order a large battery of test, many invasive to confirm or rule out an organic cause.⁽⁴⁾ It is likely that mucosal inflammation may, at least in part, be a determinant of visceral hypersensitivity and sensitization, possibly enhanced by alteration of the bacterial environment or infection.⁽⁵⁾ The infant and preschool child cannot discriminate between emotional and physical distress and cannot accurately report symptoms such as nausea or pain. Therefore, clinicians depend on the reports and interpretations of parents,

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who know their child best.⁽⁶⁾ This is the reason why the determination of fecal inflammatory marker should be a simple method helping to distinguish constitutive from functional etiologies of neonate/toddler gastrointestinal symptoms.

FC, intestinal permeability and positive Rome I criteria seem to provide a safe and non-invasive means of helping differentiate between patients with organic and non-organic intestinal disease. The sensitivity and specificity of calprotectin for organic disease were 89% and 79%. Sensitivity and specificity of positive Rome criteria was 85% and 71%.⁽⁷⁾ Concentration of FC in term^(1,8) and preterm newborns^(9–11) at birth and during the first month of life are more than 5 times higher than in healthy children and adults showing a wide interindividual and age-dependent variation. Some authors hypothesized that alterations in newborns FC could be associated with specific disorders in infancy such as atopic dermatitis, cow's milk intolerance, severe infantile colic, and gastroesophageal reflux.⁽¹²⁾

Aim of study

To evaluate if FC levels at birth and at first month of age can be a predictive biomarker of organic or FGIDs and/or allergic disease diagnosed in 2-year-old children.

Patients and methods

The study was performed at the Department of the Gynaecology, Obstetrics and Neonatology of University of Bari, Italy.

An informed consent was obtained and the study was approved by our local ethical committee.

Between December 2007 and January 2008 a telephonic interview has been proposed to the parents of 109 consecutive healthy children, in which FC was measured at birth 2 years before.⁽¹⁾ In these children FC was determined in stool samples collected at day 3, 7, 15, 30 after birth. All the subjects were healthy term newborns. The samples were stored at –20°C till the dosage with a commercial kit (CALPREST, Eurospital, Trieste, Italy). We found, as previously reported,⁽¹⁾ high FC values during all the first month of life, without any interference with the type of feeding, as other authors demonstrated before.⁽⁸⁾

Questionnaires were scored for diagnosis of FGIDs in neonate/toddler using previously published criteria for the following disorders: infant regurgitation, infant rumination syndrome, cyclic vomiting syndrome, functional diarrhoea, functional constipation.⁽¹³⁾ The questionnaire contained 15 items that measure past illness, health-care use, relative's bowel problems and sociodemographic

characteristics and three sections about symptom-related items.

For our study, a modified version of the original pediatric questionnaire on pediatric functional gastrointestinal disorders (QPGS) was used for the interview.

The greatest changes were determined according to Rome III criteria published in 2006⁽⁶⁾: cyclic vomit syndrome may be diagnosed after two rather than three episodes, functional constipation may be diagnosed after 1 month of symptoms rather than 12 weeks. Specific questions were added to detect allergic diseases.

Statistical analysis

Classical descriptive statistical methods were used to describe the sample of children. A one-way analysis of variance (ANOVA) for repeated measures was performed to evaluate time variation of FC. Two-way ANOVA models for repeated measures were builded to evaluate the effect of allergy and familiarity on FC trend. SPSS (version 10.1) was used to analyze data.

Results

Of 109 families 91 (83.4%) were contacted. In 18 cases the telephone number was changed and we couldn't be able to recontact them. 89/91 (97.8%) of parents accepted to answer (86 mothers and 3 fathers). We detected in 13/89 (14.6%) children allergic symptoms. Of 13 children 11

Table 1. Characteristics of the 89 children studied.

age (month)		
mean	27	
s.d.	3.84	
sex		
Male	48	53.9%
Female	41	46.1%
Familial association		
No	52	58.8%
Yes	36	40.4%
Allergy		
No	76	85.4%
Yes	13	14.6%

Table 2. Allergic symptoms and skin prick test results.

Allergic symptoms			Skin prick test positive	
Atopic dermatitis	9	69.23%	9	81.82%
Urticaria	2	15.38%	1	9.09%
Drug allergy	1	7.69%	0	0.00
Cronic diarrheea	1	7.69%	1	9.09%
Total	13		11	

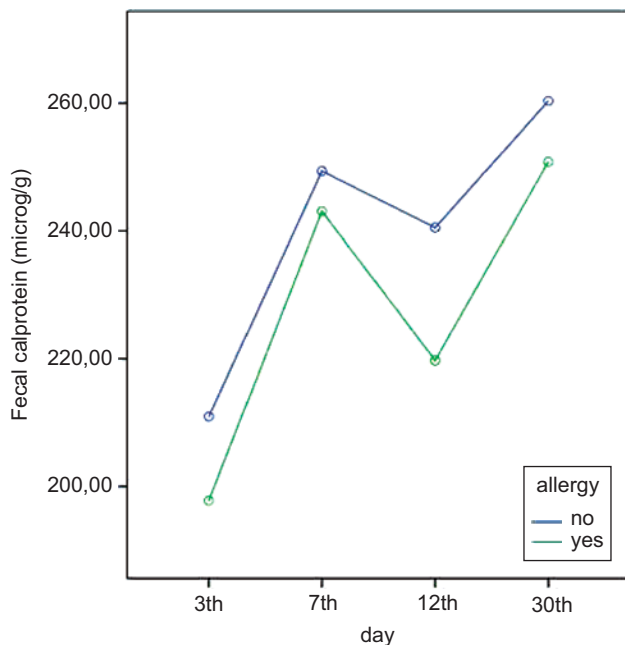


Figure 1. Comparison of the progress of calprotectin concentration in the healthy newborn child and in the newborns who develop allergy. time effect ($F=3.017$, $P=0.031$) allergy effect ($F=0.516$, $P=0.475$) interaction time $\times$ allergy effect ($F=0.058$, $P=0.981$).

children showed skin prick tests positivity to cow's milk proteins; in two cases skin prick-test were negative. Of 13 children 9 (69.2%) children presented atopic dermatitis. In 2/13 child was diagnosed urticaria (15.3%). In 1/13 (7.6%) child suffered of allergy to amoxicillin/clavulanic acid (Table 2). One child (7.6%) presented cronic diarrhea that disappeared when he began cow's milk protein free diet. We didn't find any statistically significant result between FC measured at birth and during first month of life in children with allergy or not (Figure 1).

In 1/89 (1.1%) child we made a diagnosis of regurgitation according to Roma III criteria. 45/89 (50.5%) children showed familiarity for FGIDs: 12/89 mothers (13%), 4/89 fathers (4.4%) and 29/89 (32%) of another relative suffer from FGIDs. The interference of familiarity does not lead to a statistically significant change in the FC values during the first month of life (Figure 2).

Discussion

Our study shows a higher prevalence of allergic disease (14.6%) in children younger than 3 years with respect to the prevalence reported (6%) in a recent study regarding children of the same age.⁽¹⁴⁾ Of 13 allergic children 9 children (69.2%) suffered from atopic dermatitis, with a prevalence of 10.1% in the whole study sample. Atopic dermatitis is a common and chronic inflammatory pathology of skin and, as previously reported,

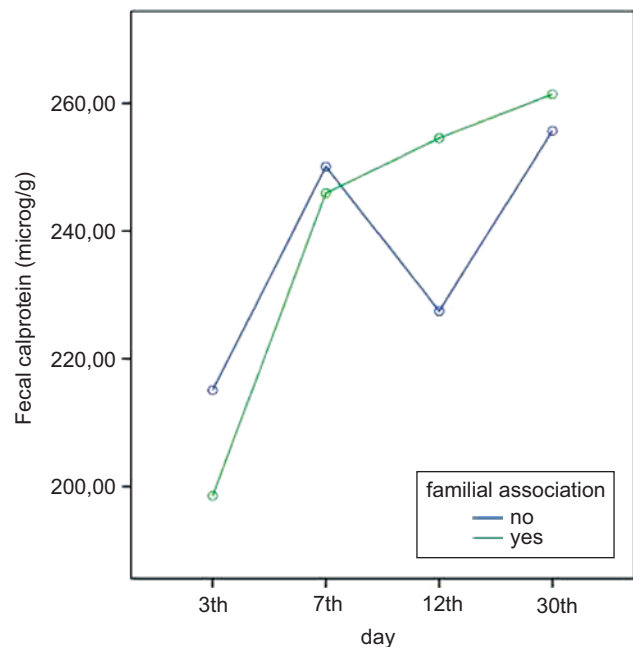


Figure 2. Effect of familiarity in the progress of FC. time effect ($F=5.509$, $P=0.001$) familial effect ($F=0.054$, $P=0.817$) interaction time $\times$ familial effect ($F=0.934$, $P=0.425$).

it affects 12–15% of children in the first years of life.^(15,16) Skin prick test for cow's milk proteins are often positive,⁽¹⁷⁾ according to our results.

Food allergy in infants could be justified not only by an immunological but also by a structural immaturity of the intestinal barrier.^(18,19) Experimental studies suggest a key role of bacterial flora in development of food allergy and food tolerance.⁽²⁰⁾

Our study aimed to test the predictive value of calprotectin measured at birth as a possible marker of allergic predisposition in the first 2 years of age. The comparison of calprotectin concentration at birth did not lead to any statistically significant result in allergic vs. non-allergic children at 2 years of age.

The levels of FC in the first month of life are not influenced by a possible individual predisposition to atopy in contrast with results reported by Savino.⁽¹²⁾

A recent study⁽⁴⁾ found a prevalence of FGIDs in Italian children aged less than 3 years old of 1.1%, which overlaps with our study results. Our study shows that the prevalence of familiarity extended to mother, father, grandparents and uncles for functional disorders is 30%. Grandparents fill the highest percentage with a quantitatively more important prevalence (46%).

The interference of familiarity does not lead to a statistically significant change in FC at birth and during the first month of life.

Our study aims to verify if the FC concentration in the first month of life may reveal the individual predisposition to intestinal flogosis, by evaluating such progress in those children who presented allergy or FGIDs after 2 years. Our

data ascertained that calprotectin has no predictive role in those pathologies. The reasons of high concentrations of FC in stools of newborns are still unknown. Evidence has recently accumulated that in addition to its cytotoxic activities, calprotectin has a cytokine-like activity. It is, therefore, possible to suppose that the protein complex or the individual subunits are not only a useful marker of inflammatory states, but also perhaps to have a positive effect to the host defense in physiological conditions, as in healthy newborns.

Starting at birth, humans and other mammals are colonized with diverse societies of bacteria that are in continuous and intimate contact with host tissues. The intestinal epithelium, in addition to present a physical barrier to microbial penetration, plays a role by producing and secreting large quantities of antimicrobial peptides, keeping commensal bacteria from penetrating host tissues and causing serious problems as inflammation and sepsis.⁽²¹⁾ Small-intestinal Paneth cells are key effectors of this type of innate defense.^(22,23) Otherwise, during the first weeks of life potent chemotactic agents produced by the intestinal flora, such as formyl-methionyl-leucyl-phenylalanine, may stimulate the transepithelial migrations of granulocytes,⁽²⁴⁾ giving a high intraluminal concentrations of calprotectin as a defense against invading or attaching pathogen agents. Calprotectin, in fact, suppresses the growth of yeast and fungi.^(25,26) Thus, it can be hypothesized that calprotectin, in concert with other factors, contributes in healthy newborns to the host defense mechanism.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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