JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2009, 60, Suppl 6, 27-31
www.jpp.krakow.pl

F. INDRIO[1], G. RIEZZO[2], F. RAIMONDI[3], M. BISCEGLIA[4], L. CAVALLO[1], R. FRANCAVILLA[1]

EFFECTS OF PROBIOTIC AND PREBIOTIC ON GASTROINTESTINAL MOTILITY IN NEWBORNS

[1]Department of Pediatrics, University of Bari Policlinico Piazza G. Cesare, Bari Italy; [2]Laboratory of Experimental Pathophysiology, National Institute for Digestive Diseases I.R.C.C.S. "Saverio de Bellis", Castellana Grotte (Bari), Italy; [3]Department of Pediatrics, University Federico II, Naples, Italy; [4]Department of Pediatrics, Ospedale San Giovanni di Dio, Crotone, Italy

To fortify the biological role of milk formula has been suggested to use probiotics and prebiotics as functional components to mimic the effect of breast milk. The aim of this study was to evaluate the effects of prebiotic, probiotic added to a standard formula on gastrointestinal motility respect to placebo-formula. Cutaneous electrogastrography (EGG) and ultrasound gastric emptying (GE) were performed in 49 preterm newborns. 17 newborns were exclusively breast-fed; 32 were randomly assigned to receive prebiotic-added formula (0.8 g/dl of a mixture from scGOS and lcFOS, ratio 9:1) (10), a probiotic-added formula (*L. reuteri* at dose of $1x10^8$ colony forming units (CFU) per day) (10), a formula with placebo (12) for 30 days. No difference was seen in the nutritional parameters and no adverse events were reported. After the intervention period, the prebiotic, probiotic, and breast milk groups showed a higher percentage of EGG slow wave propagation and faster gastric half emptying time respect to placebo group (ANOVA on ranks $p<0.001$; Dunn test *vs* control: prebiotic, probiotic and breast-milk *vs* placebo formula $p<0.05$; and ANOVA on ranks $p=0.005$; Dunn test *vs* control: prebiotic, probiotic and breast-milk *vs* placebo formula $p<0.05$, respectively). Feeding preterm infants with a formula supplemented with prebiotics or probiotics may stimulate gastric emptying and improve maturation of the EGG activity mimicking the effect of breast milk.

Key words: *newborns, prebiotics, probiotics, breast milk, infant formula feed, electrogastrography, gastric emptying*

INTRODUCTION

Human breast milk is always seen as the preferred choice for infant nutrition (1). It is a wholly nutritious complete food for infants and contains many components that have important bioactive roles (2, 3). In particular, several glicoprotein and solubile oligasaccharides were found to be selectively stimulatory for bifidobacteria (4, 5). Gut flora dominated by bifidobacteria account for healthier outcome of breast-milk infants respect to formula-fed ones. Some kinds of oligosaccharides act as soluble receptors of different pathogenes at mucosal level, so demonstrating a higher immunological resistance in breast-milk infants (6).

During the last few years the role of the intestinal microflora in health and disease has become increasingly recognized and a strong indication has been arose that diet can influence the relative amount of microbial species and strains of the gastrointestinal flora (7). Much interest exists in modulating the composition of the gut towards a potentially more beneficial community. This outcome may be achieved by using targeted dietary supplementation (8). Whenever breast-feeding is not possible or not chosen, infant formulas are the alternatives. One approach to fortify the biological role of formula feeds has been to use probiotics and prebiotics as constituents (9).

Bifidobacteria and lactobacilli are the most popular micro-organism for probiotic applications and the most effective ones are of human origin (10). Probiotic supplementation in infant formulas has shown that some strains may persist in the infant gut (11, 12) and lower stool pH (13). Supplementation with LGG (14) and with *Bifidobacterium bifidum* and *Streptococcus thermophilus* have been successful in preventing viral diarrhoea in infants (15). An alternative approach for intestinal flora modulation is the use of prebiotics, nondigestible food component that selectively stimulate certain bacteria resident in the gut (16) rather than introducing exogenous species, as is the case with probiotics. Any dietary component that reaches the colon intact is a potential prebiotic but most of the interest in the development of prebiotics is aimed at non-digestible oligosaccharides. The prebiotic approach has the advantage that heat stability or exposure to O_2 is not an issue and it is concentrated towards stimulation or enhancement of the indigenous probiotic flora. Hence, for practical as well as aesthetic reasons their use in formula feeds currently seems to be more widespread than the use of probiotics. The targeted health benefits are similar. It is likely that inclusion of such dietary prebiotic components in moderate amounts may benefit formula-fed infants by establishing an intestinal flora with more bifidobacteria and less-harmful bacteria. The health aspects of this approach have not yet been determined.

A further possibility in microflora management is the use of synbiotics, the combination of probiotics and prebiotics. A synbiotic has been defined as 'a mixture of probiotics and prebiotics that beneficially affects the host by improving the

survival and implantation of live microbial dietary supplements in the GI tract, by selectively stimulating the growth and/or activating the metabolism of one or a limited number of health-promoting bacteria, and thus improving host welfare' (16). However, they have not yet entered the infant food market.

The aim of this study was to compare the effects of prebiotic, probiotic and breast-milk on gastrointestinal motility respect to a standard formula. Gastrointestinal motility was evaluated by means of non invasive techniques such as cutaneous electrogastrography and ultrasound gastric emptying recording.

PATIENTS AND METHODS

Subject and protocol

Healthy preterm, appropriate for gestational age and with normal Apgar score were enrolled in a double-blind placebo controlled study within the first week of life. Newborns with: a) respiratory distress, b) congenital malformation, c) inborn errors of metabolism, d) proven sepsis or infection were not included. Forty-nine preterm newborns completed the study. 17 newborns were exclusively breast-fed; the remaining 32 newborns randomly received prebiotic-added formula (0.8 g/dl of a mixture from scGOS and lcFOS, ratio 9:1), (10) a probiotic-added formula (*L. reuteri* at dose of 1 10^8 colony forming units (CFU) per day, delivered in an oil formulation) (10) and 12 newborns were fed with an indistinguishable placebo formulation for 30 days. All the formula-fed preterm newborns were exclusively bottle fed with the same standard formula throughout the intervention period. Written informed consent was obtained from the parents, and the study was approved by our local institutional Ethics Committee.

Anthropometric evaluation

Anthropometric measurements (body weight, length, and head circumference) were performed before the start of the study. At all subsequent visits anthropometric measurements were taken and parents were asked to complete a 24-hour dietary recall and a 24-hour tolerance recall. Adverse events were recorded throughout the study as they occurred.

Assessment of gastric electrical activity

Gastric electrical activity was recorded at baseline (time 0: within first week of life) and about 30 days after the start of the study. After overnight fasting, the EGG recordings were performed at least 30 min before and after meal using a EGG recorder (UPS 2020, Medical Management Systems, MMS, The Netherlands). Five silver-silver chloride electrodes (Clear Trace, ConMed, Utica, NY USA) were placed on the cleaned abdominal surface and the ground electrode was placed to the left costal margin as reported by Chen *et al*. (17). All recordings and analysis of the EGG parameters (dominant frequency and normal slow wave percentage) were reported in our previous paper (18). In addition to the running spectra analysis (19) available with the EGG equipment, Redtech GiPC software was used to perform further EGG data filtering and analysis and only the EGG traces with the best quality signal were used for the further analysis in the time domain. The EGG traces were visually inspected in a blind manner to compute the presence of time lag between two EGG channels on the minute-by-minute samples of the EGG traces (30 min basal or postprandial recording time). The time lag had to be at least 3 s and was calculated between the two channels placed along the antral axis (channel 2-3 and channel 3-4). Propagation was calculated as percentage of samples in which a propagation was detected respect to the whole 30 samples.

Assessment of gastric emptying

Gastric emptying was recorded at baseline (time 0: within the first week of life) and about 30 days after the start of the study. The ultrasound gastric emptying examinations were always performed by the same investigator using a real-time apparatus (Image Point HX, Hewlett Packard Company, Palo Alto, CA, USA) equipped with a 3.5 MHz linear probe. The probe was positioned at the level of the transpyloric plane for simultaneous visualization of the antrum, superior mesenteric vein and the aorta. The antral measurements were always taken from the outer profile of the wall. Since the cross section of the gastric antrum, corresponding to the sagittal plane passing through the superior mesenteric vein, is elliptical in shape, its area can be calculated by measuring the longitudinal (L) and anteroposterior (AP) diameters and applying the formula $\pi LxAP/4$. During the same EGG recording session, antral measurements were made before and immediately after the end of the test meal (time 0), and at regular 30-min intervals up to 180 min after the meal. In each patient, the gastric emptying rate was expressed as percent reduction in antral cross sectional area from time 0 to 120 min after meal ingestion (20).

Data analysis

The data were analyzed first using simple descriptive statistics of centrality and dispersion. All data were expressed as mean±SD, but non-parametric statistical tests were performed.

Table. 1. Clinical parameters at baseline recording session.

	Prebiotic group	**Probiotic group**	**Breast-milk group**	**Placebo group**
Gender	5/5	4/6	8/9	7/6
Gestational age (wks)	34±0.3	34±1.1	34±0.8	33.9±0.6
Weight (g)	2209±321	1890±432	2326±329	2041±268
Length (cm)	42.9±1.7	44.4±2.3	45.0±2.3	43.7±2.1
Head circumference (cm)	32.7±1.5	32.1±1.6	32.1±1.1	31.2±2.6

Data are expressed as mean±SD. ANOVA on ranks NS

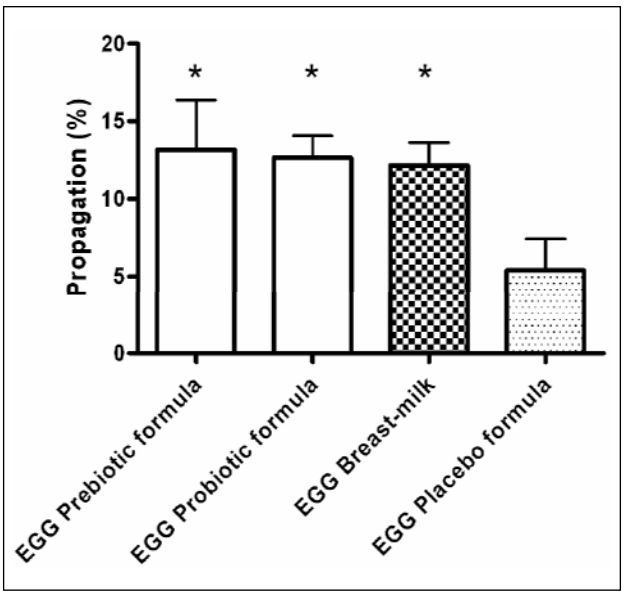

Fig. 1. The plot represents the % of time during which propagation was visually detected. It is the record after 30 days treatment - postprandial period. A clear difference is evident between the study groups respect to the placebo formula group. Data are expressed as mean±SD. ANOVA on ranks p<0.001; *post hoc* test Dunn's test *vs* control: prebiotic, probiotic formula and breast-milk *vs* placebo formula p<0.05. EGG = electrogastrography.

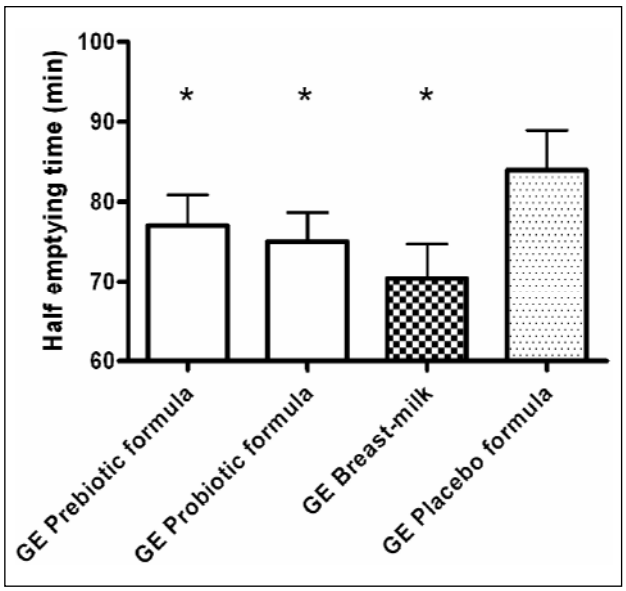

Fig. 2. The half empting times recorded after 30 days treatment is reported. A clear difference in the half emptying time was evident between the study groups and placebo formula group. Data are expressed as mean±SD. Data analysis: ANOVA on ranks p=0.005; post hoc test Dunn's test *vs* control: prebiotic, probiotic formula and breast-milk *vs* placebo formula p<0.05. GE = gastric emptying.

The difference among the groups was determined by One Way ANOVA Analysis of Variance on Ranks and Dunn's test (post hoc comparisons *vs* control (placebo formula). All the differences were considered significant at a 5% level. The software package used for the statistical analysis was STATA (STATA ver 4.0 Statistical Software, Stata Corporation).

RESULTS

The clinical parameters at the baseline are shown in *Table 1*. No difference was seen in the daily increase of body weight and no adverse events were reported. The daily increase of body weight was satisfactory being an average of 35 gr/day. No difference was seen in the time of allowed to feed at the start and at the end of the study among the groups.

At baseline, EGG and gastric emptying data were similar in all the groups. After the intervention period, EGG dominant frequency showed a normal electrical activity and no differences in newborns receiving prebiotic, probiotic formula, brast milk and placebo formula both for preprandial recording (dominant frequency: 2.8±0.4 cpm 3.0±0.4 cpm, 2.8±0.3; 2.8±0.5 cpm, respectively ANOVA on ranks: n.s.) and postprandial recording (dominant frequency: 2.8±0.3 cpm 3.0±0.3 cpm, 2.9±0.4; 2.9±0.2 cpm, respectively ANOVA: n.s.). A higher percentage of propagation was found in the newborn receiving the prebiotic and the probiotic and breast milk compared to placebo group (*Fig.1*). As regard gastric emptying, the half emptying time recorded after the intervention period was faster in the prebiotic, probiotic, and breast milk groups compared the placebo group (*Fig. 2*).

DISCUSSION

The addition of prebiotic and probiotic to a formula induces a GI motility pattern similar to that one induced by breast milk. The gastric emptying parameters showed a similar profile in all the three study groups underlying a significant difference with the placebo.

The development of gastric slow waves and effects of feeding in preterm and full term infants have been recently reported by Zhang *et al*. (21). Our study confirms a quite normal EGG activity in older preterm newborns, when frequency and percentage of normal slow waves were considered. As reported by several authors (22, 23) the presence of uncoupling, *i.e.* the lack of propagation among different EGG channels, can be associated to a normal EGG or in some case to tachygastria. The electrical uncoupling induces a collision between slow waves propagating from ectopic sites and the normal pacemaker site, disrupting peristalsis and delaying gastric emptying (24). The newborns receiving the GOS/FOS mixture had a higher percentage of propagation respect to the placebo group. The percentage of propagation is an index of efficient electrical gastrical activity lead to an efficient peristalsis and can explain the absence of EGG improvement in a previous work on probiotics in preterms (25). In our study, newborns fed with prebiotic showed a more efficient EGG activity and a faster gastric emptying time than placebo group confirming the crucial role of the electrical coupling in the modulation of mechanical activity and coordination. A faster gastric emptying time than placebo group was seen in a previous study on the effect of probiotics in preterm newborns (25).

The action of prebiotic on upper gastrointestinal motility might be explained by several physiological pathways. The most important mechanism seems to be mediated by bacterial metabolites such as SCFAs. Colonic SCFAs modify upper motility *via* polypeptide YY by inducing relaxation of the proximal stomach, lower esophageal sphincter and reducing gastric emptying (26). A faster gastric emptying in preterm infants can lead to luminal nutrients remaining in the intestine shorter and prevent the inflammation cascade and reduce the development of NEC (27).

No data are available concerning the role of the SCFA in preterm newborns, but the mechanism of probiotics on the gastric emptying may be the same as in adults. Probiotics have been used for many years in the animal fed industry, but they are now increasingly made available in many forms and can be purchased over the counter as freed-dried preparations in health food stores. Thus, possible health benefits associated with the administration of probiotic organisms are widely gaining acceptance. For example, there are several reports of disease prevention or enhancement of immune function resulting from the administration of probiotics (28). These organisms are thought to prevent the attachment of pathogens to enterocytes and invasion of these cells (29). Furthermore, probiotic bacteria have been shown to enhance the human intestinal epithelial barrier function (30) so preventing the translocation of potentially harmful organisms. In addition same strain of probiotic bacteria induces the production of the antimicrobial peptide human beta-defensin by the epithelium and immune cells, peptides that have been recognized to play a key role in the host defence. This data indicate both a direct and indirect mechanisms of controlling potentially harmful bacteria by probiotic bacteria (31).

Also prebiotic seems to play an indirect mechanism *via* the activation of a human antimicrobal protein, the chathelicidin by means of butyrate (32). Butyrate is a by-product of bacterial fiber fermentation that is produced by endogenous intestinal flora, and it is the major trofhic factor for colonocytes. A recent compelling study showed that oral butyrate treatment of Shigella-infected dysenteric rabbits led to improvement of clinical symptoms, decreased blood in the stool, and a reduction in the bacterial load in the stool (33). These data not only support the notion that cathelicidin is an essential effector molecule but also suggest that certain intestinal infections may be treatable through stimulation of epithelium-derived antibiotics.

In conclusion, feeding preterm infants with a formula supplemented with prebiotics or probiotics may stimulate gastric emptying and improve maturation of the EGG activity. The beneficial effect would provide new regimens for the prevention of the illness in preterms. Role for symbiotic formula is suggested and could represent a new challenge in the infant nutrition.

Conflict of interests: None declared.

REFERENCES

1. Cuthbertson WJF. Evolution of infant nutrition. *Br J Nutr* 1999; 81: 359-371.
2. Goldman AS, Chheda S, Garofalo R. Spectrum of immunomodulating agents in human milk. *Int J Pediatr Hematol Oncol* 1997; 4: 491-497.
3. Garofalo RP, Goldman AS. Expression of functional immunomodulatory and anti-inflammatory factors in human milk. *Clin Perinatol* 1999; 26: 361-377.
4. Gauhe A, Gyorgy P, Hoover JR, *et al*. Bifidus factor - preparation obtained from human milk. *Arch Biochem* 1954; 49: 214-224.
5. Petschow BW, Talbott RD. Response of Bifidobacterium species to growth promoters in human and cow milk. *Pediatr Res* 1991; 29: 208-213.
6. Kunz C, Rudloff S, Baier W, Klein N, Strobel S. Oligosaccharides in human milk: structural, functional, and metabolic aspects. *Ann Rev Nutr* 2000; 20: 699-722.
7. Holzapfel WH, Haberer P, Snel J, Schillinger U, Huis in't Veld JH. Overview of gut flora and probiotics. *Int J Food Microbiol* 1998; 41: 85-101.
8. Collins MD, Gibson GR. Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. *Am J Clin Nutr* 1999; 69: 1052S-1057S.
9. Gibson GR, McCartney AL. Modification of the gut flora by dietary means. *Biochem Soc Trans* 1998; 26: 222-228.
10. Dunne C, O'Mahony L, Murphy L, *et al*. In vitro selection criteria for probiotic bacteria of human origin: correlation with in vivo findings. *Am J Clin Nutr* 2001; 73: 386S-392S.
11. Bennet R, Nord CE, Zetterstrom R. Transient colonisation of the gut of newborn infants by orally administered bifidobacteria and lactobacilli. *Acta Paediatr* 1992; 81: 784-787.
12. Millar MR, Bacon C, Smith SL, Walker V, Hall MA. Enteral feeding of premature infants with Lactobacillus GG. *Arch Dis Child* 1993; 69: 483-487.
13. Langhendries JP, Detry J, Van Hees J, *et al*. Effect of a fermented infant formula containing viable bifidobacteria on the fecal flora composition and pH of healthy full-term infants. *J Pediatr Gastroenterol Nutr* 1995; 2: 177-181.
14. Isolauri E, Juntunen M, Rautanen T, Sillanaukee P, Koivula T. A human Lactobacillus strain (Lactobacillus casei strain GG) promotes recovery from acute diarrhea in children. *Pediatrics* 1991; 88: 90-97.
15. Saavedra JM, Bauman NA, Oung I, Perman JA, Yolken RH. Feeding of Bifidobacterium bifidum and Streptococcus thermophilus to infants in hospital for prevention of diarrhoea and shedding of rotavirus. *Lancet* 1994; 344: 1046-1049.
16. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microflora introducing the concept of probiotics. *J Nutr* 1995; 125: 1401-1412.
17. Chen JD, Zou X, Lin X, Ouyang S, Liang J. Detection of slow wave propagation from the cutaneous electrogastrogram. *Am J Physiol* 1999; 277: G424-G430.
18. Riezzo G, Indrio F, Montagna O, *et al*. Gastric electrical activity and gastric emptying in preterm newborns fed standard and hydrolisate formulas. *J Pediatr Gastroenterol Nutr* 2001; 33: 290-295.
19. Van Der Schee EJ, Smout AJ, Grashuis JL. Application of running spectrum analysis to electrogastrographic signals recorded from dog and man. In Motility of the Digestive Tract, Wenbeck M (ed). New York, Raven Press, 1982, pp. 1241-1250.
20. Darwiche G, Bjorgell O, Thorsson O, Almer LO. Correlation between simultaneous scintigraphy and ultrasonographic measurement of gastric emptying in patients with type 1 diabetes mellitus. *J Ultrasound Med* 2003; 22: 359-366.
21. Zhang J, Ouyang H, Zhu HB, *et al*. Development of gastric slow waves and effects of feeding in pre-term and full-term infants. *Neurogastroenterol Motil* 2006; 18: 284-291.
22. Liang J, Chen JDZ. What can be measured from surface electrogastrography. Computer simulations. *Dig Dis Sci* 1997; 42: 1331-1343.
23. Familoni B, Bowes KL, Kingma YJ, *et al*. Can transcutaneous recordings detect gastric electrical abnormalities? *Gut* 1991; 32: 141-146.
24. Biust ML, Cheng LK, Sanders KM, Pullan AJ. Multiscale modeling of the human gastric electric activity: can the electrogastrogram detect functional electrical uncoupling? *Exp Physiol* 2006; 91: 383-390.
25. Indrio F, Riezzo G, Bisceglia M, Cavallo L, Francavilla R. The effects of probiotics on feeding tolerance, bowel habits and gastrointestinal motility in preterm newborns. *J Pediatr* 2008; 152: 801-806.
26. Labayen I, Forga L, Gonzalez A, Lenoir-Wijnkoop I, Nutr R, Martinez JA. Relationship between lactose digestion, gastrointestinal transit time and symptoms in lactose

malabsorbers after dairy consumption. *Aliment Pharm Ther* 2001; 15: 543-549.
27. Clark DA, Miller MJ. Intraluminal pathogenesis of necrotizing enterocolitis. *J Pediatr* 1990; 117: 64-67.
28. Furrie E, Macfarlane S, Kennedy A, *et al*. Symbiotic therapy (Bifidobacterium longum/Sinergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomized controlled pilot trial. *Gut* 2005; 54: 242-249.
29. Lu L, Walker WA. Pathogenic and physiologic interactions of bacteria with the gastrointestinal epithelium. *Am J Clin Nutr* 2001; 73: 1124S-1130S.
30. Madsen K, Cornish A, Soper P, *et al*. Probiotic bacteria enhance murine and human intestinal epithelial barrier function. *Gastroenterology* 2001; 121: 580-591.
31. Wehkamp J, Harder J, WehkampK, *et al*. Nf-kB and AP-1-mediated induction of human beta defensin-2 in intestinal epithelial cells by *Escherichia coli* Nissle 1917: a novel effect of a probiotic bacterium. *Infect Immun* 2004; 72: 5750-5758.
32. Schauber J, Svanholm C, Termen S, *et al*. Expression of the cathelicidin LL-37 is modulated by short chain fatty acids in colonocytes:relevance of signalling pathways. *Gut* 2003; 52: 735-741.
33. Raqib R, Sarker P, Bergman P, *et al*. Improved outcome in shigellosis asociated with butyrate induction of an endogenous peptide antibiotic. *Proc Natl Acad Sci USA* 2006; 103: 9178-9183.

Received: September 9, 2009
Accepted: October 30, 2009

Author's address: Dr. Flavia Indrio, Department of Pediatrics, University of Bari, Bari, Italy, Policlinico Piazza G. Cesare, Bari, Italy; Phone and Fax +390805593624; Mobile:+393292938421; E-mail: f.indrio@neonatologia.uniba.it