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Reviews

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Review

Growth changes after gluteen free diet in pediatric celiac patients: a literature-review

Alessia Ghiselli[1], Barbara Bizzarri[1], Federica Gaiani[1], Francesca Semeraro[1], Silvia Iuliano[1], Francesco Di Mario[1], Antonio Nouvenne[1], Stefano Kayali[1], Gian Luigi de' Angelis[1]

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Summary. *Background:* Celiac disease is an auto-immune disorder characterized by clinical manifestations that appear in genetically predisposed subjects after gluten ingestion. In the last years, there has been a progressive change in clinical manifestations. Our aim was to evaluate the nutritional status of children with celiac disease at diagnosis and how the gluten-free diet (GFD) influences their growth. *Methods:* A search on PubMed/Medline was performed using "celiac disease", "body mass index" and "children" as key words. Medline, Scopus, PubMed publisher and Google Scholar were searched as well. We selected clinical studies describing the nutritional status of patients before and after GFD using indicators like height, weight, BMI, skeletal age. We excluded papers referred to adult population or in which other diseases were related to celiac disease. Also literature-reviews were excluded. *Results:* From 1999 to 2018, 10 studies were found. Overall, 1383 patients in pediatric age were evaluated for their nutritional status at diagnosis of celiac disease and after a variable period from 1 to 17 years of GFD. Indicators considered were height, weight, BMI and skeletal age. *Conclusions:* the nutritional status of celiac patients at diagnosis is variable including an increasing number of overweight and obese. GFD has a beneficial impact on growth changes determining a correction of BMI distribution towards a Gaussian shape. (www.actabiomedica.it)

Key words: celiac disease, BMI, growth, gluten free diet

Introduction

Celiac disease (CD), also known as gluten-sensitive enteropathy, is a life-long condition that affects the small intestine in genetically susceptible individuals (1). It is a multifactorial disease that arises from a complex interaction between genetic heritage, immunological and environmental factors. The genes involved are the major histocompatibility complex type II and, in particular, the HLA-DQ2 and HLA-DQ8 haplotypes. It's characterized by subtotal villous atrophy of the small intestine, intra-epithelial lymphocytosis and crypt hyperplasia, and is associated with a variable gluten-related presentation. (2). Young children often present with "classic" symptoms including diarrhea, abdominal distention, and growth retardation. Vomiting, abdominal pain and constipation are atypical manifestations, more common in older children and teenagers (1). Celiac disease can also present with extra-intestinal conditions such as arthritis, neurological diseases, and anemia (1).

The presentation of CD has changed over time (1). The prevalence of the classical presentation of CD has decreased in the last decades, while the prevalence of non-classical presentations has increased. Growth failure in terms of length (or height) or weight may be the earliest sign of the disease (2) but many reports show that CD can be associated with normal weight but also with overweight and obesity (3, 4). Variations in the nutritional status may be seen at diagnosis (5), which in pediatric age can be evaluated using indicators such as height, weight, BMI, skeletal age.

The quantification and the definition of BMI in children vary widely overworld, therefore it is difficult to compare papers from different countries. Anyway, the ideal definition, based on percentage of body fat, is impracticable for epidemiological use (6). Body mass index (BMI, weight/height^2) is widely used in adult populations, with a cut-off point of 30 kg/m^2 is recognized as a definition of adult obesity (6). BMI in childhood changes substantially with age and sex especially during the pubertal growth spurt (Figure 1). Therefore the BMI in pediatric age should be used not as an absolute value but as a relative value, with the support of centile curves. Clearly, a cut-off point related to age is needed to define pediatric obesity. In the United States, the 85th and 95th centiles of BMI for age and sex, have been recommended as cut off points to identify overweight and obesity (6).

It is now well established that many patients with celiac disease have a high or normal body mass index (BMI) at diagnosis. Some studies show that BMI increases on a gluten-free diet (GFD), especially in those who adhere closely to the diet while other studies show contradictory results (7). Few studies examining BMI and other growth parameters have been performed in children, with inconclusive findings (8).

The aim of this paper was to review the available literature on nutritional status of children with celiac disease at diagnosis, focusing on the influence of gluten-free diet (GFD) on growth, through the use of specific indicators.

Methods

In order to evaluate the nutritional status of children affected by CD at diagnosis, its evolution after gluten-free diet and the parameters that can be used to evaluate growth changes, we performed a literature search of PubMed database using Mesh terms "celiac disease", "body mass index" and "children". Medline, Scopus, PubMed publisher and Google Scholar were searched as well. The entire databases were considered, without restrictions of time. We included only full text papers selected with two filters "humans" and "language". Only papers in English were included.

Exclusion criteria were:

- papers referred to children affected by CD with other comorbidities such as arthritis, diabetes and thyroiditis.
- papers referred to adult population
- studies in which nutritional status of CD at diagnosis was compared with health children and not with the same group after GFD
- literature-review articles

Each article was tabulated in chronological order from the oldest to the most recent as follows: author and year of the study, number of patients, demographic data, period of follow up, growth indicators at diagnosis (BMI, skeletal age), growth indicators after gluten-free diet (BMI, skeletal age) (Table 1). Further parameters to evaluate nutritional status such as biochemical tests were not considered.

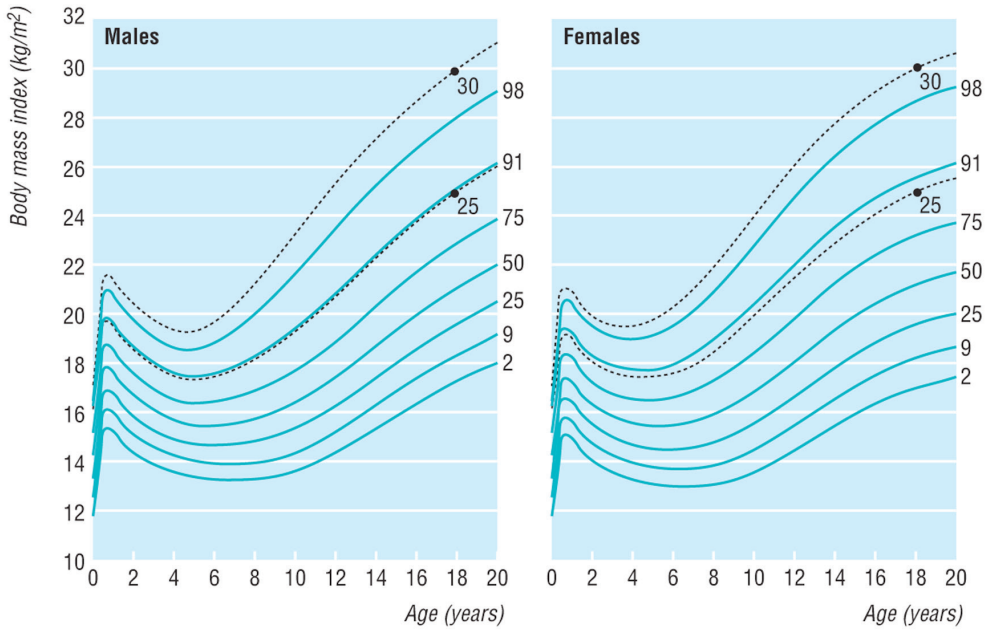
The publications were manually screened and reviewed to identify reports, and data were extracted from the papers according to the predetermined criteria. Two investigators independently reviewed and extracted data from the papers according to the predetermined criteria.

Results

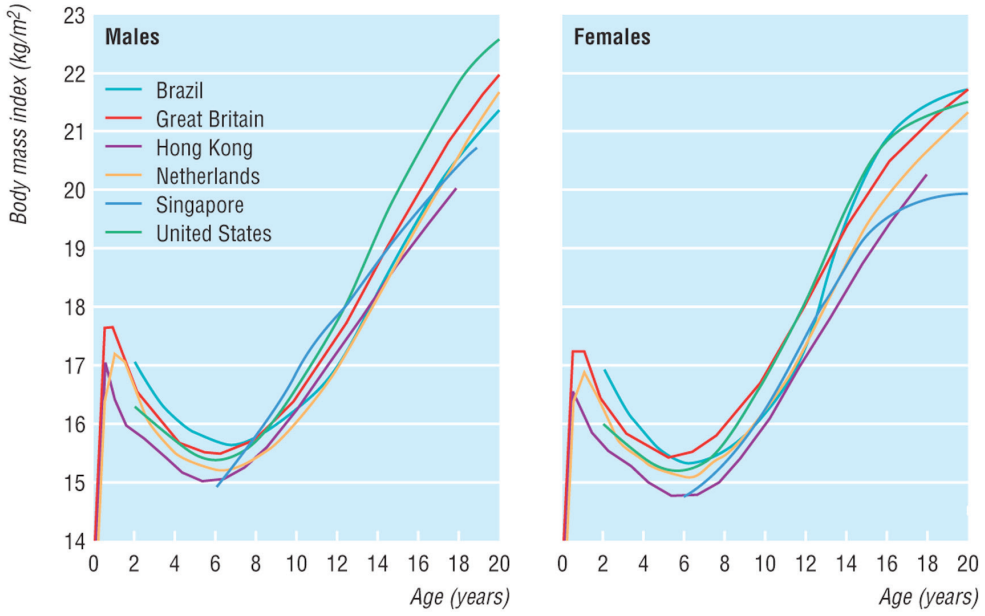
After a first research, 147 abstracts were found. Among them, 127 full text papers were analyzed. Finally, applying the filters "humans" and "language" and including only English papers, 105 papers were obtained. After manual screening according to established criteria, we selected 10 retrospective articles, published from 1999 to 2018.

The numerosity of the cohorts among papers varied widely, from a case reports study with 1 patient (9) up to the paper including the highest number of patients, with 445 subjects (10). Overall 1383 patient in pediatric age (from 1 to 17 years old) were evaluated for their nutritional status at diagnosis of celiac disease and after a variable period from 1 to 17 years of GFD.

Only in one study (11) skeletal age was used as growth indicator together with other parameters such as height and weight. In five papers (2, 9, 12-14), centile curves of BMI were considered as principal indicator for evaluation of growth changes after GFD. In the other four studies (8, 10, 15, 16) only weight was



Centiles for body mass index for British males and females. Centile curves are spaced two thirds of z score apart. Also shown are body mass index values of 25 and 30 kg/m² at age 18, with extra centile curves drawn through them



Median body mass index by age and sex in six nationally representative datasets

Figure 1.

Table 1. Papers included in the literature review

Author, year	N° patients	Demographic data	Period of follow up	Growth indicators at diagnosis (BMI, skeletal age)	Growth indicators after gluten-free diet (BMI, skeletal age)
Gemme G. 1999	26 (11 M, 15 F)	<3 years	15,3 years	Tendency to short stature, underweight and retarded skeletal age	Skeletal retardation, slightly below average height
Patwari AK, 2005	50	2-10 years	1-4 years	Short stature, underweight.	Normalisation of body mass, incomplete recovery in height
Oso O, 2006	1	14 years	3 years	BMI 37,2 Kg/m2	BMI 42,7 Kg/m2
Telega G, 2008	143 (93 F, 50 M)	1-17 years	17 years	11,2% overweight (BMI >90%)	-
Van Dommelen, 2008	134	<2,5 years	-	BMI decrement in patients with clinical manifestations	-
Valletta E., 2010	149	Children (median age 6,2 years)	1 year	5% malnutrition 23% underweight 11% overweight 3% obese	- - 21% overweight 4 % obese
Venkatasubramani N. 2010	143	childhood	1 year	7 pz BMI >95%	4 pz decreased 2 pz increased 1 pz not available
Reilly NR 2011	142	13 months-19 years	3 years	75% normal 13% overweight 6% obese	13% of normal became overweihgt 75% of overwheigt decreased BMI
Brambilla P, 2013	150	2-16 years	4 years	16% underweight 12% overweight or obese	8% underweight minimal increase of overweight
Capriati T., 2016	445	children	-	7,8 % overweight or obese	9,8 % overweight or obese

considered with the consequent identification of five categories: malnutrition, underweight, normal-weight, overweight and obese.

Discussion

Celiac disease is one of the most common chronic diseases in childhood (7). Children may present with the classical manifestations of disease characterized by chronic diarrhea, failure to thrive and abdominal distention or with non-classical features including gastrointestinal symptoms as well as extra-intestinal manifestations (3). Because of the damage to the small intestinal mucosa, it can be expected to result in malabsorption of nutrients leading to poor weight gain, weight loss and undernutrition. However, a few recent reports have identified an increasing number of adults, adolescents and children who are overweight, or even obese, at the time of initial diagnosis of CD (3). This concept has been well described by Dickey et al in 2006 (17) who reviewed a database of 371 celiac

patients showing how only few patients were underweight while a large part was overweight/obese.

In adult population affected by celiac disease it is easier to examine the relationship between the disease and nutritional status because BMI is a standardized and recognized parameter for the evaluation of corporeal mass. Cheng et al in 2010 (18) and Ukkola et al in 2012 (19) investigated BMI before and after GFD in celiac patients. Both concluded that GFD has a beneficial impact on BMI, underweight patients gain weight and overweight/obese patients lose weight.

In pediatric age, the relationship between nutritional status in CD before and after GFD is more difficult to evaluate because of the relative value of BMI and the necessity to use centile curves. Gemme et al in 1999 (11) was the only author to use skeletal age in addition to height, weight and BMI, and he concluded that after a period of GFD patients didn't catch up completely in height and skeletal age. Patwari et al in 2005 (15) used as anthropometric parameters only weight and height expressed as Z scores relative to National Center for Health Statistics standards. In this study 50 patients were analyzed and showed short stature and underweight before diet; after GFD they achieved a normalization of body mass but an incomplete recovery in height. The only case report study of Oso et al in 2006 (9) reported the case of a 14-y-old boy with celiac disease with a BMI at presentation of 37.2 kg/m^2 (>99.9th centile) that increased to 42.7 kg/m^2 despite dietary support confirming that obesity in childhood doesn't exclude the diagnosis of CD. Telega et al in 2008 (12) confirmed the presence of overweight in celiac patients at diagnosis. The same results can be observed in a study by van Dommelen et al in 2008 (2) in which she concluded that BMI is a better predictor for nutritional status than weight and much better than length or height. Valletta et al in 2010 (16) observed, in a population of 149 children after 1 year of gluten-free diet, a doubling of overweight while obese remain unchanged. Similar results were obtained from Brambilla et al in 2013 (8) where after 4 years of follow up he observed a halving of underweight and a minimal increase of overweight, and from Venkatasubramani et al in 2010 where, in a case study with 5% of obese (BMI > 95%), two-thirds decreased after GFD while one-third increased. Capriati et al in 2016 (10) presented an increase of both overweight and obese patients after GFD. BMI was used for this evaluation as a measure of nutritional status according to Italian growth charts of Cacciari. Instead Reilly et al in 2011 (14) after 3 years of observation, showed substantially a decrease of BMI in overweight. Overall considering all growth indicators, with limits due to demographic data, it can be concluded that GFD improves nutritional status of CD with a reduction of underweight and obese but also with a minimal increase of overweight. This determines a modification of BMI distribution towards a bell shape which better represents the distribution of biological variables.

Conclusions

Celiac disease is now considered a common chronic disease in childhood and no more a rare condition. Children more frequently present with atypical symptoms than with classical features and variations in nutrition may be seen at diagnosis. Many indicators can be used to evaluate the influence of GFD on growth even if BMI seems to be the best predictor. Overall GFD has a beneficial impact on growth changes determining a correction of BMI distribution towards a Gaussian shape. A careful follow-up of nutritional status after the diagnosis of CD is necessary, as new morbidities could also emerge in children strictly compliant with GFD, especially in overweight and obese.

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Review

Thyroid and celiac disease in pediatric age: a literature review

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Summary. Chronic autoimmune thyroid disease or Hashimoto thyroiditis (HT) and Graves-Basedow disease (GD) are the main autoimmune thyroid diseases in pediatric age. Both are characterized by the production of anti-thyroid antibodies, by an infiltration of autoreactive B and T lymphocytes into the thyroid parenchyma and by alterations in thyroid function (hyperthyroidism in GD, normal function or subclinical hypothyroidism in HT with possible evolution towards manifest hypothyroidism). Celiac disease (CD) is a systemic autoimmune disease caused by gluten ingestion in genetically predisposed subjects, its prevalence is around 1% in Western Countries. It presents with a pathognomonic enteropathy, a variety of clinical manifestations, positivity for specific antibodies, positivity for typical haplotypes HLA DQ2/DQ8. The clinical manifestations may vary among four types: typical, atypical, silent and latent. Diagnosis can be made in presence of specific histopathologic findings in duodenal biopsies and antibodies positivity. Celiac disease is associated to various endocrine autoimmunities such as thyropathies, diabetes mellitus type 1, Addison disease, multiendocrine syndromes. The most frequent associated thyropaties are HT and GD. The present review aims to explore the associations between thyropathies and celiac disease in pediatric age. (www.actabiomedica.it)

Key words: autoimmunity, autoimmune thyropathies, celiac disease

Background

The global prevalence of autoimmune diseases in pediatric age is about 5%. Among them, the most frequent autoimmunities are represented by autoimmune thyroid diseases (AITD). Hashimoto Thyroiditis (HT) can be considered the prototype of organ-specific autoimmunity and represents the most common cause of acquired hypothyroidism in geographic areas with lack of iodine. Its prevalence is about 3% in pediatric age. Graves Disease (GD) represents the most common cause of hyperthyroidism in pediatric age, with a prevalence of about 1:5000 children; the incidence increases progressively with age, reaching a peak in adolescence up to 3:100.000 adolescents/year. All the scientific advances in the last 10 years have allowed at least partially the comprehension of the marked preponderance of AITD in females (Females: Males ratio 5:1) with the phenomena of fetal microchimerism (1) and X chromosome inactivation. Autoimmune thyropathies can be associated with other autoimmunities such as celiac disease (CD), diabetes mellitus type 1 (T1DM), Addison disease, multiendocrine syndromes, alopecia, idiopathic juvenile arthritis. While AITD in adults are strongly associated with rheumatoid arthritis, psoriatic arthritis and connective tissue diseases, they are more frequently associated to T1DM and CD in pediatric age (3, 4).

Autoimmune thyropathies

In AITD the morpho-functional damage represents the direct consequence of the interaction be-

tween environmental and genetic factors. Linkage studies have allowed the identification of two groups of susceptibility genes: haplotypes of Human Leucocyte Antigen (HLA) DQA1, DQ2and DRB1-1401. The T cells regulating gene (CTLA-4) is a transmembrane protein belonging to the superfamily of immunoglobulins and acts by reducing T lymphocytes activation; CD40, one of the receptors of Tumor Necrosis Factor (TNF) plays a critical role in adaptative immunity and is also located on antigen presenting cells (APC) and on epithelial thyroid cells; the gene encoding for thyrosin-phosphatase protein 22 (PTPN22) is one of the inhibitors of the signal pathway of T cells receptor; finally, FCRL3, IL2RA and FOXP3 are also crucial genes in the pathogenesis of autoimmune thyropathies. Thyroid-specific genes are thyroglobulin gene (TG) and thyrotropin (TSH). The recently discovered thyrotropin receptor (TSHR) Single Nucleotide Polymorphisms (SNPs) seem to be associated specifically to GD but not to HT, although the functional consequence of these intronic polymorphisms is still not clear. Anyway it is believed that they could generate RNA variants with an increase of TSHR α subunits, which could represent potential autoantigens (5). Another hypothesis suggests that SNPs could lead to a lower amount of TSHR thymic transcripts, with a consequent decrease in central immune tolerance for TSHR (6). Considering that these polymorphisms are located on intronic regions, therefore not coding, it is reasonable to assume that genetic mechanisms are much more complex (7). Studies conducted on twins demonstrate that genetic factors contribution in the determination of AITD accounts for about 70%. Moreover, homozygous twins present more pathologic similarities than heterozygous twins. Nevertheless, the rate of pathologic concordance is of about 50%, even between homozygous twins, showing once again how other environmental factors are determining in the onset of autoimmunity. Among the considered environmental factors of AITD, particular attention should be paid to obesity, according to the "accelerator hypothesis". Starting from the assumption that obese children are hyperleptinemic, it is well known that leptin promotes cells-mediated immune responses among its function, being therefore capable of promoting the onset of autoimmune response in AITD. Besides obesity, other potential "accelerators" are represented by high alimentary iodine intake as it increases antigenic sites in TG, smoke, stress, drugs including amiodarone, lithium, α-interferon, interleukin 2, antiretrovirals, vitamin D gene polymorphisms and low serum doses of selenium. With regards to selenium, literature shows controversial results: after supplementation with selenium, the reduction of anti-thyroperoxydase (TPO) was not uniform; this phenomenon was only observed in geographic areas lacking selenium (8). Finally, some authors have demonstrated the seasonal and the geographic influences in the onset of AITD, especially for GD, and the role of infectious agents (*Yersinia enterocolitica*, Coxsackie B virus, Retrovirus, Helicobacter pylori). Recently, a high prevalence of AITD has been demonstrated among patients affected by Hepatitis C Virus (HCV). Actually, HCV can infect thyrocytes, causing an increase in the production of pro-inflammatory cytokines, which could be responsible for the autoimmune aggression of the thyroid (9). In pediatric age, HT most frequently presents during puberty, while it is rare in children younger than 3 years. Graves Disease is responsible for about 95% of cases of hyperthyroidism in pediatrics, with an annual incidence of 8 patients per 1.000.000 children younger than 4 years (10).

Celiac disease

Celiac disease is a chronic autoimmune disorder which manifests in genetically susceptible subjects triggered by the ingestion of gluten. At present, the only available treatment is a strict gluten-free diet. Epidemiologic studies demonstrate that CD is a common condition allover the world, with a predilection of Caucasian populations and a prevalence varying between 1:266 and 1:80 individuals. The disease is more common among females and may present at any age, although the onset seems to be more frequent in early childhood and around the fifth decade. Celiac disease presents a familiar predisposition, as it presents more frequently among first-degree relatives, reaching the highest concordance between homozygous twins up to 70% (12). This genetic predisposition is strongly dependent from the HLA setting. About 90-95% of

patients affected by CD presents the HLA haplotype DR3-DQ2, encoding for DQA1*0301/DQB1*0302 (12).

Considering auto-antibodies development, various antibodies have been tested for the serological diagnosis of CD, for the identification of subjects at risk who are candidates for undergoing esophagogastroduodenoscopy with duodenal biopsy, and for the monitoring of the gluten-free diet. Anti-reticulin antibodies and anti-gliadin antibodies were the first tests available, but nowadays anti-endomysium antibodies (EMA) and anti-transglutaminase antibodies IgA (t-TGAbs) are used for the higher sensitivity and specificity (12, 13). According to international guidelines, the diagnosis of CD is based on specific histopathologic findings in duodenal biopsies, on positive values of the specific antibodies, regardless of patients' symptoms (14, 15).

Gliadin, a type of prolamin and a component of gluten, is a class of proteins present in wheat and several other cereals within the grass genus Triticum. Gliadin is an excellent substrate for tissue transglutaminase, the enzyme identified as the principal auto-antigen in CD. This enzyme plays a crucial role in the maintenance of cellular homeostasis, by regulating the duplication cell cycle, differentiation and apoptosis. Studies have demonstrated the presence of HLA DQ2/DQ8 specific T-cells in mucosal lesions of celiac patients. Antigen presenting cells expose and present pre-digested gluten to T-lymphocytes CD4 positive, thanks to their molecules HLA DQ2. Transglutaminase then modifies gliadin peptides by deaminating glutamine residues, therefore facilitating the binding of gliadin peptides to HLA. This provokes an increase in the binding-affinity, which exacerbates T cells reactivity into the intestinal mucosa with a consequent local immune response that continues until gliadin is ingested. Stimulated T helper 1 (Th1) cells secrete cytokines such as TNFα and interferon-γ, which can further damage the intestinal mucosa. At the same time, a T helper 2 (Th2) response manifests, with the consequent production of t-TGAbs. These autoantibodies are able to inhibit *in vitro* the differentiation of cryptic epithelial cells, anyway the same effect remains to be established in vivo (13). Besides gluten, various environmental factors influence mode and type of clinical presentation of CD: viral antigens (e.g. *Rotavirus*) increasing intestinal mucosa permeability, the length of breastfeeding period, type and mode of weaning including the age of introduction of gluten in the infant diet seem to play a role.

With regards to clinical presentation, CD may manifest in four different forms: typical, with gastrointestinal symptoms, manifest malabsorption, weight loss and/or growth delay; atypical, with extraintestinal manifestations such ad herpetiform dermatitis or amelogenesis imperfecta; silent, with positive serum markers and positive histopathology but lack of symptoms or signs of malabsorption; latent, with positive serum markers but normal duodenal biopsies.

Prevalence of autoimmune thyropathies in CD patients

As well as for adults, an association between CD and AITD has been demonstrated also in pediatric age, in variable percentages between 2% and 7.8%, three times higher than in general population (16). A study conducted on CD children living in Sardinia, demonstrated a prevalence of AITD of 10.5%, therefore 4 times higher than in general population (17). Further studies evaluating the prevalence of AITD in CD patients have proven that a percentage variable between 2.4% and 40.4% of the patients in the cohort was affected (17-23). Details are shown in table 1. Two hypotheses have been suggested to explain this association: firstly, CD and AITD share one or more genes; secondly, a continued introduction of glu-

Tabella 1. Prevalence of AITD (clinical, subclinical, potential) in patients with CD

Country	Authors	N. patients	Pevalence AITD
Italia	Meloni (17)	34	10.5
Italia	Ventura (18)	90	14.4
Polonia	Kowalska (19)	34	41
Italia	Oderda (20)	41	2.4
Brasile	Da Silva (21)	52	40.4
Turchia	Kalyoncu (22)	67	4.5
Italia	Diamanti (23)	558	12

Tabella 2. Prevalence of CD in patients affected by AITD

Country	Authors	Type of AITD	CD prevalence %	N. patients with CD	N. patients with AITD
Italia	Larizza (31)	Hypo	8.8	6	68
Italia	Larizza (31)	Hyper	4.6	1	22
Polonia	Kaczorowska (32)	Hypo/Hyper	4.3	2	47
Turchia	Sari (33)	Hypo	5.0	5	101
Italia	De Martino (34)	Hypo/Hyper	9.9	9	91
Polonia	Grzenda-Adamek (35)	Hypo/Hyper	0.6	7	115

ten in celiac patients not on a gluten-free diet (GFD) may lead to a loss of integrity of the intestinal barrier, with a consequent alteration in the systemic immune response which may favor the onset of other autoimmune diseases (17, 24). Despite, other studies have demonstrated that the duration of the exposition to gluten in CD does not correlate with the risk of developing further autoimmune diseases, and in parallel that the cessation of gluten ingestion is not protective against autoimmunities (17, 25-27). These results are anyway controversial, as other authors suggest that a strict adhesion to the GFD is associated with a reduction of the risk of developing AITD and anti-thyroid antibodies disappear on a gluten-free diet (18, 28). Finally, GFD seems to have a favorable effect on other autoimmune comorbidities, although it is not able to stop the progression of an autoimmune process which has already started (29).

Prevalence of CD in patients with AITD

A recent meta-analysis has observed the prevalence of CD in patients affected by AITD (30). The prevalence has been demonstrated to be higher in children (6.2%) compared to adults (2.7%). Celiac disease was more prevalent in patients with hyperthyroidism (2.6%) compared to patients with hypothyroidism (1.4%) (31-35). Details are shown in table 2. Literature clearly shows a strong correlation between CD and AITD and the importance of investigating AITD in celiac children, either clinic, subclinic or potential, performing an accurate familial and personal history, a careful clinical examination, a dosage of TSH, Free T4 (FT4) and anti-thyroid antibodies, as well as a thyroid ultrasound.

Similarly, current international guidelines recommend performing the screening for CD in children affected by AITD (36). It is recommended to perform an accurate familiar and personal history of children affected by AITD, as well as an attentive research of typical and atypical signs and symptoms od CD, moreover it is recommended to dose EMA and t-TG IgA antibodies at diagnosis and every 2-3 years if negative (37).

Conclusions

A clear and strong association between CD and AITD has been demonstrated, therefore it is of paramount importance to carefully investigate both CD patients and AITD patients at diagnosis and during follow up, to precociously diagnose the simultaneous presence of these two autoimmunities. This awareness is even more essential if we think at the frequency of subclinic presentations both of CD and AITD, which could delay diagnosis. Overall, a multidisciplinary approach with the cooperation between gastroenterologists and endocrinologists should always be encouraged to optimize the management of patients affected by CD and AITD.

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Review

Genetic susceptibilty and celiac disease: what role do HLA haplotypes play?

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Summary. Celiac disease is a chronic immune-mediated enteropathy triggered by exposure to dietary gluten in genetically predisposed individuals. Many genes involved in the pathogenesis have been identified and a crucial role is known to be played by the Human Leukocyte Antigen (HLA) system. The main determinants for genetic susceptibility are HLA-DQA1 and HLA-DQB1 genes encoding for HLA-DQ2 and HLA-DQ8 molecules, carried by almost all patients affected. However, since HLA-DQ2 and HLA-DQ8 heterodimers explain almost 40% of the disease heritability, HLA typing should not be applied in diagnosis, but exclusively to clarify uncertain diagnoses, considering its negative predictive value. (www.actabiomedica.it)

Key words: celiac disease, HLA typing, diagnostics, genetic predisposition

Introduction

Celiac disease is a chronic immune-mediated enteropathy triggered by exposure to dietary gluten in genetically predisposed individuals (1). In celiac patients, the ingestion of gluten leads to the activation of both the innate and adaptive response of the immune system, with a subsequent chronic inflammation that determines changes in the mucosal structure including villous atrophy, crypt hyperplasia and lymphocyte infiltration. These changes in structure cause subsequent loss of function by the intestinal mucosa and the onset of symptoms brought by nutrient malabsorption.

The range of clinical manifestations in celiac disease varies widely, with a high prevalence of asymptomatic individuals. For these reasons, the disease itself has been represented as an iceberg: the tip is associated with classical symptoms of nutrient malabsorption; the largest part of the iceberg corresponds to atypical manifestations, as well as silent and latent phenotypes (2).

Diagnosis is based on serological tests, whose aim is to search for auto-antibodies produced by the activation of B lymphocytes following gluten ingestion, and small bowel biopsy.

According to the European Society of Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN), the diagnosis of celiac disease can be confirmed by an elevation in the specific antibody titer associated to an alteration of the duodenal mucosa seen at the histologic analysis, or if the antibody titer exceeds 10 times the upper limits in association to typical symptoms of malabsorption (1).

The main auto-antibodies used in the diagnostic process are anti-tissue transglutaminase (anti-TG2) and anti-endomysium (EMA) antibodies. Deamidated Gliadin Peptide (AGA-DPG) antibodies are used along with anti-TG2 in diagnosing celiac disease in the pediatric population younger than 2 years of age.

Small intestine biopsy is performed during esophagogastroduodenoscopy (EGDS) and it is followed by

histological analysis. The disease activity grade according to the histological findings on the biopsy is classified based on Marsh-Oberhüber criteria.

So far, the only available and effective treatment for celiac disease is the adoption of a life-long gluten-free diet, that could lead to the normalization of serological parameters and the regression of mucosal damage.

Celiac disease and genetics

Celiac disease has a multifactorial etiology, linked to the contribution of both the genetic predisposition and various environmental factors, including gluten and the timing of its introduction in infant diet. Other possible environmental factors are under study as possible concurring element in etiology, such as an alteration in the intestinal microbiota, an alteration in the intestinal mucosa permeability, and infections.

CD development depends on the presence of key genes that orchestrate the immunological response to dietary gluten. Genetic risk genes are searched with the help of two complementary methods: genetic linkage and genetic association studies. Genetic linkage studies identify common chromosomal regions shared by affected siblings using Single Nucleotide Polymorphisms (SNPs) as genetic markers. After linkage has been identified, association studies are used to identify the disease-specific gene from the candidate gene locus. This type of study compares frequencies of genetic variants in patients with those in controls (3).

Many genes involved in the pathogenesis have been identified and a crucial role is known to be played by the Human Leukocyte Antigen (HLA) system. The HLA super-locus is a genomic region placed on the short arm of chromosome 6 (6p21), where it encompasses approximately 4000 kb. This super-locus can be separated into five HLA regions: the extended class I, class I, class III, class II and extended class II regions. It contains hundreds of genes with immunological functions and it is characterized by a high gene density and variability and an extensive linkage disequilibrium. This phenomenon, where certain combinations of alleles are passed on to the offspring more often than it is usually expected, makes it very difficult to determine whether a specific gene is involved directly in the disease susceptibility as a causal genetic variant or whether it marks the effect of linked genes. The term "haplotype" is used to refer to the set of alleles on a single chromosome that are inherited together (4, 6).

In the case of Celiac Disease specific alleles of the HLA system are involved in the pathogenesis of the disease.

HLA class I and II regions comprise genes encoding for proteins that have important roles in the regulation of the immune system, such as glycoproteins for antigen presentation to the immune cells, as well as some other fundamental molecular and cellular processes (4, 5).

Glycoproteins for antigen presentation encoded by HLA class II are heterodimers constituted by an alpha-heavy chain and a small beta2-microglobulin chain whose genes map on the HLA-D region, comprising HLA-DP, HLA-DQ and HLA-DR genes (6). These molecules present exogenous antigens to CD4+ lymphocytes, which activate the humoral response.

Glycoproteins encoded by HLA class I (A, B and C) are instead involved in the presentation of endogenous antigens to CD8+ lymphocytes that trigger an immune cytotoxic response.

In particular, HLA-DQA1 and HLA-DQB1 genes are the main determinants for genetic susceptibility, referred to as CELIAC1 by the HUGO Gene Nomenclature Committee (http://www.genenames.org/) and encoding for HLA-DQ2 and HLA-DQ8 molecules, carried by almost all patients presenting the disease (7).

Almost 95% of patients with CD express HLA-DQ2 and the rest of them usually carry the HLA-DQ8 heterodimer, encoded by DQA1*0301-DQB1*0201 alleles.

HLA-DQ2 heterodimers are encoded by DQA1*05 and DQB1*02 alleles, which are involved in the formation respectively of the α and β chains of the heterodimer. They could be inherited in one out of two different configurations: DQ2.5*cis,* on the same chromosome, or *DQ2.5trans*, where each allele is encoded on one of the two homologous chromosomes, one chromosome from each parent (6). The DQ2.5*cis* very frequently appears in linkage disequilibrium with DRB1*03:01 allele, which was first associated with CD risk (8).

However, the presence of these alleles is necessary but not sufficient for disease development: in fact, although HLA-DQ2 allele is common in the white population (30% of people are carriers), almost 3% of them will develop CD (9, 10). Risk of developing CD for people carrying the risk alleles is estimated being between 36-53% (11). Furthermore, risk of developing CD depends on gene dose, so far only demonstrated for HLA-DQ2: homozygous individuals have a risk at least 5 times higher than heterozygous individuals (12). A familiar aggregation has been found in 5-15% of patients as well as a higher concordance rate of celiac disease in monozygotic than in dizygotic twins (83-86% vs. 11%) (3).

Genetic linkage studies have identified other three chromosomal regions officially recognized as genetic predisposing factors for celiac disease so far: 5q31-q33 (CELIAC2), 2q33 (CELIAC3) and 19p13.1 (CELIAC4).

HLA influence on CD susceptibility shows a dose effect. CD risk can be classified according to the number of DQA1*05 and DQB1*02 alleles carried by the individual. Homozygosity for DQ2.5*cis* and heterozygosity for DQ2.5*cis* with a chromosome possessing a second DQB1*02 allele (DQ2.2) confer the highest risk to develop CD. Heterozygosity for DQ2.5*cis* in individuals with a single copy of DQB1*02 (non-DQ2.2) or presence of DQ2.5*trans* confer intermediate risk. DQ2 negativity suggests an extremely low chance of developing CD (13).

Since HLA-DQ2 and HLA-DQ8 heterodimers explain almost 40% of the disease heritability, the remaining 60% is estimated to be shared between an unknown number of non-HLA genes. Recently, genome-wide association studies identified many non-HLA genes that may be involved in the risk of developing CD. They are involved in controlling the immune response and among them we can find genes encoding for IL2, IL21, CTLA4, CCR3, IL 12A, AH2B3 and TAGAP (14).

Although their contribution to the onset of the disease is weak (almost 15%) it has been shown that they could aid to identify those individuals who are at higher risk for CD (14, 15).

Interestingly, it seems that the presence of the haplotype AH 8.1 could be an additional factor risk to CD. Together with AH 18.2, they are called "ancestral haplotypes" and are made up of DQA1*05, DQB1*02 and DRB1*03:01 alleles. These alleles can also be found within non-specific allelic combinations and constitute other less frequent haplotypes. DRB1*03:01 haplotypes have been associated to numerous immune-mediated disorders, as type 1 diabetes, multiple sclerosis or selective IgA deficiency. In some cases, different DRB1*03:01 haplotypes showed a different contribution to the risk of developing the disease (13).

A study conducted in the Saharawi population demonstrated how the genes located in the previously mentioned haplotype (here mentioned as B8/DR3/DQ2) could also be related to specific clinical manifestations of the disease. The study showed how its presence was more related to atypical forms than typical ones and was not significantly implicated in the susceptibility of CD (16). This could be considered a probable future implication of HLA-DQ2 in the clinical practice: not only used as a risk-predictor, but also as a parameter to foresee the possible clinical manifestations of the disease.

HLA typing in clinical practice

Up to now, genetic testing in the clinical practice of celiac disease has been proved to be useful only for HLA-DQA1 and HLA-DQB1 genes, which are strongly associated with the risk of disease onset.

However, HLA typing should not be applied in diagnosis, but exclusively used to clarify uncertain diagnoses, considering its negative predictive value: its positivity does not necessarily predict the certain onset of the disease, but indicates a genetic predisposition to develop the disease. On the other hand, when predisposing genes are not present, it is very unlikely for the patient to develop celiac disease in the future (6, 17).

According to ESPGHAN 2012 guidelines, HLA typing should be performed in patients with uncertain diagnosis of CD: this category includes patients with negative serology and mild infiltrative changes in small intestinal biopsy specimens (1).

In pediatric age, HLA typing should be performed to add strength to diagnosis in presence of clinical symptoms referred to CD and positive serol-

ogy (anti-TG2 antibodies higher than 10 times the upper limit threshold and positive EMA). In this case small bowel biopsy may be omitted.

Furthermore, in asymptomatic patients at risk for developing CD, HLA typing could be used to select those who will need a strict follow-up based on periodical (usually annual) antibody testing. This group of patients includes those people who are more likely to develop CD than the general population, such as patients affected by another autoimmune-mediated disease such as Diabetes Mellitus Type I, autoimmune thyroid diseases, autoimmune liver diseases or by chromosomal disease such as Down syndrome, Turner syndrome, Williams syndrome, (1). Diabetes Mellitus Type I (T1D) and CD are both multifactorial disease sand co-occur in families and even in single patients, more often than expected in the general population. Approximately 4-9% of patients with T1D also have CD while patients with CD are at increased risk of developing T1D.

Genetic testing is also an important tool for the screening of celiac patients' first degree-relatives who present uncertain serology or symptoms suggestive of malabsorption, in order to identify those who will need to undergo serological follow-up. In fact, it has been demonstrated that 20% of siblings and 6% of parents who had a positive genetic test, resulted to be affected with celiac disease. The risk is very high for DQ2 and/or DQ8 alleles carriers, but interestingly a high risk seems to be associated even to the presence of just DQB1*02 allele, carried in double dose, with the absence of DQA1*05 allele (18, 19).

Targeted screening for CD is recommended both for a benefit in management of other autoimmune diseases and for the risk, in untreated patients, of iron deficiency anemia, growth retardation, osteoporosis, fertility problems, neurologic disease and gastrointestinal malignancies such as intestinal lymphoma.

Besides, HLA typing is not recommended as an initial only screening test in people at average risk for celiac disease due to its poor positive predictive value.

Discussion

Over the last years, great importance has been given to HLA genotyping in the prediction and prognosis of many autoimmune diseases including celiac disease. Almost 95% of patients with CD express HLA-DQ2 and the rest of them usually carry the HLA-DQ8 heterodimer, encoded by DQA1*0301-DQB1*0201 alleles. However about 30%-40% of the general population carry HLA-DQ2 and/or DQ8 without developing CD. HLA typing is considered an excellent tool to be used in association to more specific tests like endoscopy, when serology is ambiguous in subjects being investigated for CD (4-8). Additionally, in individuals considered at high risk for CD as first-degree relatives, patients with immunoglobulin A deficiency, other autoimmune diseases or with Down, Turner or Williams syndromes, the HLA typing is used to identify those people in which these alleles are absent and exclude them from further investigations. The absence of predisposing alleles spares unnecessary serial serological testing while confirmation of their presence reflects an increased likelihood of developing CD. Thereby, in individuals disclosing HLA predisposing alleles, periodic screening for auto-antibodies against IgA tTG and IgA EMA should be considered to avoid a misdiagnosis of subclinical or silent forms of the disease.

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Review

Peripheral neuropathy and gastroenterologic disorders: an overview on an underrecognized association

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Summary. *Background and aim of the work:* Although peripheral neuropathies in children are often of genetic origin, acquired causes should be carefully looked for and ruled out also in the pediatric age. Gastroenterological disorders can be complicated by peripheral neuropathy as a result of micronutrients deficiency, drug toxicity or because of shared pathophysiological mechanisms. *Methods:* In this descriptive review we sought to give an overview on the most relevant clinical conditions in which peripheral neuropathies are associated with gastrointestinal disorders or symptoms. *Results:* We describe the clinical, demographic, and electrophysiological features of peripheral neuropathy in three main clinical scenarios: in the context of common gastroenterological disorders (inflammatory bowel and celiac disease), in the context of micronutrients deficiencies arising from malabsorption irrespective of etiology, and in a rare degenerative mitochondrial disorder, mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) disorder. *Conclusions:* The association between gastrointestinal and peripheral nervous system symptoms is probably still underrecognized but has to be actively sought, in order to provide prompt diagnosis resulting in optimal care and long-term management with the aim to improve quality of life and, at least in some conditions, try to impact on prognosis. (www.actabiomedica.it)

Key words: peripheral neuropathy, inflammatory bowel disease, ulcerative colitis, Crohn disease, celiac disease, malabsorption, vitamin deficiency, mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) disease, Guillain-Barré syndromes

Background and aim of the work

Unlike in adult age, pediatric-onset peripheral neuropathies are often of genetic origin. Charcot-Marie-Tooth (CMT), typically presenting with distal weakness and wasting, reduced deep tendon reflexes, contractures and skeletal deformities, is considered the most common neuromuscular disorder (1), and can challenge the clinician with wide range of age of onset (including congenital cases (2)) phenotypic (associated peripheral and central involvement (3), marked sensory or upper limbs involvement, visual/hearing impairment, pyramidal signs (4), intellectual disability) and genetic heterogeneity. Additional genetically-determined peripheral neuropathies, which can be encountered in clinical practice, include hereditary neuropathy with liability to pressure palsy (HNPP) (5), most frequently presenting with acute-onset, non-painful focal sensory and motor mononeuropathy, but also with atypical phenotypes, including chronic cramps and exercise-induced myalgia ((6)) and variable electrophysiological features (5, 7).

Although the presence of an underlying genetic etiology, acquired causes represent a fairly common clinical scenario in children, developing as a consequence of trauma or a complication of a chronic disorder. Toxic effects of medications or a long-term consequence of nutritional deficits also have to be ruled out in the diagnostic work-up.

Gastrointestinal diseases are occasionally associated with neurologic manifestations, including peripheral neuropathies. In most cases, signs and symptoms of peripheral nervous system involvement occur in the setting of a known gastrointestinal disease, but on rarer occasions, neurologic symptoms predate gastrointestinal ones, therefore both the gastroenterologist and the neurologist need to be aware of this potential association. In both cases, a high index of suspicion is crucial for a prompt diagnosis.

We performed a descriptive review with the aim to give an overview of gastroenterological conditions with the higher risk of development of an associated peripheral neuropathy in their natural history.

Methods

We decided to focus our review on two highly prevalent gastroenterological diseases (inflammatory bowel disease and celiac disease) due to their known association with peripheral neuropathy and their frequency in the general population. We also collected data on nutritional deficiencies which could result in peripheral nervous system complications in any gastroenterological condition determining malabsorption. Due to the very high frequency of gastro-enteritis in the pediatric age, we will briefly discuss Guillain-Barré syndrome as a potential complication of gastro-intestinal infections in children. Finally, we reported on a rare, severe mitochondrial disorder, mitochondrial neurogastrointestinal encephalopathy (MNGIE) disorder, as an example of a complex clinical condition for which patients will most probably seek gastroenterological advice, but will have associated neurologic manifestations to be actively sought, especially in the early stages of the disease. We reviewed papers we used the search terms “inflammatory bowel disease” and “peripheral neuropathy”, “celiac disease” and “peripheral neuropathy”, mitochondrial neurogastrointestinal encephalopathy (MNGIE) disorder. We excluded case reports and articles not written in English.

Results

1.1 Inflammatory bowel disease

Inflammatory bowel disease (IBD) includes ulcerative colitis (UC) and Crohn’s disease (CD). Neurologic involvement (including peripheral and central nervous system) is rare and has been reported both before and after the onset of intestinal symptoms. The most frequent neurologic complications include inflammatory and axonal neuropathies, cerebrovascular and demyelinating disease (8).

Although peripheral neuropathy (PN) is known to be related to IBD, its real prevalence remains largely unknown. Neuromuscular signs and symptoms are reportedly 3-7 times more prevalent in patients with IBD than in controls, while large-fiber peripheral neuropathy is 5-7 times more prevalent than in controls (9). In a population-based retrospective (1940-2004) cohort of adult patients with newly diagnosed IBD (Figueroa et al.), the neuropathy incidence rate was 72 cases per 100,000 IBD person-years with a cumulative incidence rate of 2.4% after 30 years (10).

Different types of neuropathy have been reported including sensory, motor, autonomic and mixed (axonal and demyelinating), acute and chronic. In a recent retrospective review, more than two-thirds of patients with IBD had axonal neuropathy, with sensory predominance, and only one third developed demyelinating neuropathy (11). Carpal tunnel syndrome seems to be more common in Ulcerative colitis (UC) than in patients with Crohn disease (CD). In UC the most frequent diagnosis is acute inflammatory demyelinating polyradiculoneuropathy, but also mononeuritis multiplex and brachial plexopathy are reported (12). In contrast, patients with CD most commonly demonstrate axonal motor and sensory neuropathy (13). CD patients also present autonomic neuropathy early in their disease course (14).

Interestingly, peripheral neuropathies are not related to disease activity (their onset can occur in peri-

ods of quiescence) and do not respond to treatment of the underlying IBD (15). Demyelinating neuropathies respond better to immunomodulatory therapy than axonal neuropathy (16).

The pathogenesis of peripheral nervous system damage in inflammatory bowel disease is still unclear, although most likely related to immune mechanisms. In addition, it can be iatrogenic or result from micronutrient deficiencies (17).

While T cells are clearly involved in the pathogenesis of demyelinating neuropathies, the relationship between axonal damage and immune system derangements remains unclear, although empirically supported by the observed clinical improvement with immunomodulatory therapies (11).

1.2 Non-drug-induced peripheral neuropathy in IBD

When causes of secondary neuropathy are excluded, the reported frequency of peripheral neuropathy in IBD will vary greatly (0-39%) due to selection bias, use of different definitions, or different population characteristics (18).

1.3 Secondary peripheral neuropathies

In addition to primary causes, patients with IBD may experience severe nutritional and iatrogenic neuropathies which can be more disabling than the bowel disorder (18).

1.3.1 Drug-induced neurologic manifestations of IBD

Biological agents

TNF inhibitors

The proinflammatory cytokine TNF-α plays a main role in the inflammatory cascade in IBD; consequently, anti-TNF-α drugs mitigate this inflammatory process. Many TNF-α inhibitors have been used in IBD therapy, predominantly in severe and moderate cases.

Infliximab was the first TNF inhibitor successfully used in IBD. Additional drugs with demonstrated efficacy include adalimumab and golimumab (humanized monoclonal antibodies), and certolizumab pegol (humanized anti-TNF-α antibody Fab' fragment conjugated with a polyethylene glycol molecule) (18).

Cases of neurologic toxicity of infliximab and adalimumab include peripheral neuropathies in 42% of cases. Demyelinating neuropathies, either acute or chronic, compatible with Guillain-Barré syndrome (GBS), Miller Fisher syndrome, Lewis–Sumner syndrome (a rare acquired demyelinating polyneuropathy characterized by asymmetrical distal weakness of the upper or lower extremities and motor dysfunction with adult onset) or chronic inflammatory demyelinating polyneuropathy (CIDP) have been described in IBD patients receiving anti-TNF-α agents (19). GBS cases account for the majority, developing between 6 days and 2 years after initiating anti-TNF-α drugs. In CIDP cases, elevated serum anti-ganglioside antibodies have been described, suggesting an abnormal immune reaction against myelin (19). Moreover, there are case reports of MMN following infliximab treatment; in these cases, patients developed asymmetric progressive weakness and conduction block (20). Finally, axonal sensory neuropathy, mononeuritis multiplex or sensorimotor polyneuropathy was also reported in patients treated with infliximab (21).

The proposed pathogenesis includes a T-cell and humoral immune attack against peripheral nerve myelin, vasculitis-induced nerve ischemia, and inhibition of signaling support for axons (91). Prognosis is usually favorable if treatment is discontinued, which is the first step for the management of these conditions. In patients not achieving clinical recovery, immunomodulatory therapy with steroids or intravenous immunoglobulins should be started (21). Use of TNF inhibitors should be avoided in patients with peripheral neuropathy or multiple sclerosis. Patients with IBD should be examined for neuropathy before initiating anti-TNF-α treatment.

Non-biological agents

Peripheral neuropathy is also a frequent neurologic complication in IBD patients treated with metronidazole, thalidomide, or cyclosporine.

Metronidazole

Metronidazole has been used in IBD for decades but the incidence of PN remains controversial. In one

study, the incidence of PN reached 50% of the IBD patients receiving metronidazole, but diagnosis was clinically based, without neurophysiological confirmation (22). Conversely, in a CD population, no significant differences in PN occurrence were found between patients taking metronidazole (daily dose ≤800 mg) and patients never been treated with metronidazole (23). Metronidazole-induced PN occurs more frequently in patients receiving more than 1.5 g daily of metronidazole for more than 30 days (24).

The genesis of the toxic nerve damage has not been clarified but an increase in free radicals has been hypothesized (25). ENG studies usually demonstrate a pure sensory deficit or autonomic neuropathy, while motor disturbances develop in severe cases. Neuropathy usually resolves completely once metronidazole is discontinued (26), but recovery might be protracted (23).

Thalidomide

Thalidomide is a small molecule with anti-TNF-α, immunomodulatory and antiangiogenic properties. It is used in clinical practice as third line therapy to maintain remission in CD (27). Although used infrequently in the management of pediatric Crohn's disease, it has an important role in treating patients losing response to standard treatment options. The mechanism of action is unclear, but the drug inhibits both angiogenesis and tumor necrosis factor release by leukocytes. A randomized, double-blind controlled trial of thalidomide in pediatric Crohn's disease demonstrated a remission rate of 63% (versus 12% in the placebo group) (28).

Symptomatic PN is frequent, from 20% (29) to approximately 50-55% in pediatric cohorts (30) and even 72.5% (31). Sixteen patients aged 6-24 years received thalidomide for Crohn's disease from 2002 to 2012. Nine subjects had electrophysiologic evidence of sensorimotor axonal polyneuropathy, the vast majority (8/9) with sensory and/or motor symptoms (32).

The underlying mechanisms of nerve damage are still obscure, but most likely involve capillary damage, secondary hypoxemia in nerve fibers and acceleration of neuronal cell death secondary to downregulation of TNF-α.

Given the potential neurotoxicity, patients treated with thalidomide should undergo regular clinical examination and ENG to detect presymptomatic or progressive peripheral neuropathy (33).

Some categories of patients seem to be at increased risk of developing a peripheral neuropathy. There seem to be a dose-dependent effect, as doses > 60 g (32) or >50 g (34) seem to be associated with an increased risk. The risk also increases depending on the mean daily dose. Therapy duration is also a factor, as 4 out of the 5 subjects receiving thalidomide for > 20 months developed polyneuropathy (32). In one study, the median period of treatment before neuropathy developed was 16.5 months; the percentage of neuropathy-free patients was 70% and 35.6% at 12 and 24 months of treatment, respectively. Interestingly, in patients with neuropathy receiving therapy for > 24 months and having ≥3 electromyography studies, the neuropathy severity plateaued (31). Aside from the total administered dose, additional factors might contribute to the risk profile, including pharmacogenetic susceptibility, involving C-hydroxylation and acetylation reactions (35, 36). Single nucleotide polymorphisms in ICAM1 (rs1799969) and SERPINB2 (rs6103) genes were found to be protective against thalidomide-induced PN and favored its resolution, supporting the hypothesis that genetic susceptibility may have a role in the natural history of thalidomide-induced polyneuropathy. ICAM1 gene encodes for an intercellular adhesion molecule playing a role in inflammatory processes (37), involved in nerve repair after traumatic injury (38). SERPINB2 is a serine protease inhibitor with cytoprotective effects whose levels increase during cellular stress (39, 40). Conversely, no risk factor related to IBD characteristics emerged (31). The association between thalidomide and metronidazole does not seem to increase the risk of TiPN (31).

The pathology of thalidomide neuropathy is characterized by loss of large sensory fibers with apparent preservation of small sensory fibers and no apparent demyelination (41); however, 3 among the 7 patients reporting mild sensory symptoms had normal electrophysiological studies, raising the possibility of a small fiber neuropathy, not detectable with routine nerve conduction study.

Children, adolescents, and young adults receiving thalidomide should undergo regular neurophysiological studies to monitor for neuropathy (32). Thalido-

mide-induced PN is generally reversible with dose reduction or drug discontinuation, although irreversible cases have been reported, therefore discontinuation or dose reduction are mandatory. In one study, clinical symptoms resolved in approximately 90% of cases, but nerve conduction studies abnormalities persisted in more than half of the patients for months after drug withdrawal (31).

Cyclosporine

Cyclosporine is a potent immunomodulatory drug, effective in the treatment of IBD, which can also induce peripheral neuropathy, in most cases with mild and reversible symptoms, not necessarily requiring dose reduction (42).

1.4 Clinical approach to peripheral neuropathy in IBD patients

The presence of suspected neuropathic symptoms (weakness, paresthesias) in patients with gastroenterological symptoms should prompt a thorough neurologic evaluation and the execution of neurophysiologic testing. A diagnosis of IBD-associated peripheral neuropathy may be established after exclusion of other causes, including complications of therapy or secondary micronutrients deficiencies. A tentative treatment with immunotherapy should be indicated irrespective of the underlying mechanism of nerve damage. If a secondary peripheral neuropathy is suspected, levels of vitamins and micronutrients (vitamins B1, B12 and E, folate, and copper) should be obtained and a supplementation started in case of documented deficiency. Any drug used for IBD with potential deleterious effects on peripheral nerve should be withdrawn (17).

Nutritional deficiency-induced peripheral neuropathies

Vitamin deficiencies are well-recognized causes of PN in IBD and celiac disease, most commonly vitamin B group deficiency.

Vitamin B12 deficiency

Vitamin B12 and folate deficiencies are common in patients with IBD, particularly in CD (43). The primary function of cobalamin is to provide enzymatic activity for the synthesis of methionine and succinyl coenzyme A, essential for axon formation. The most typical neurologic manifestation of vitamin B12 deficiency is subacute combined degeneration, involving both the posterior columns of the spinal cord and the peripheral nerves, resulting in sensory ataxia and loss of cutaneous sensation (12, 15). ENG studies demonstrate axonal neuropathy, reversible after supplementation (44, 45). Central nervous systems symptoms can also develop.

Other vitamin B group deficiencies

Vitamin B1 deficiency is mainly associated with Wernicke encephalopathy. Peripheral neuropathy, usually axonal, either sensory, motor, or both, has been reported. It may run a rapidly progressive or chronic course (12). It may recover after vitamin supplementation (46, 47).

Folate deficiency can cause axonal neuropathy with slowly progressive course (48, 49).

Vitamin E deficiency

Severe vitamin E deficiency may be genetic (50) or due to malabsorption (gastric and intestinal surgery, biliary and pancreatic diseases, celiac disease, IBD, but also common variable immunodeficiency and cystic fibrosis) (51). Ataxia (from posterior column involvement) is the most common manifestation, along with progressive sensory axonal peripheral neuropathy, especially involving large fibers. Peripheral axonal loss has been demonstrated by both electrodiagnostic and pathologic examinations. Pathologic changes also include degeneration of large myelinated fibers in the posterior columns, sensory roots, and peripheral nerves (52).

Copper deficiency

Bowel surgery in patients with CD has been associated with copper deficiency. Its symptoms are indistinguishable from those of vitamin B12 deficiency (53).

Gluten-related neurologic disorders

Gluten is a product of wheat, rye, and barley. Its breakdown products are responsible for a group of immune-mediated disorders including celiac disease (15).

Celiac disease is a chronic, immune-mediated, inflammatory small bowel enteropathy triggered by the ingestion of gluten by genetically susceptible individuals expressing the HLA class II molecules DQ2 or DQ8. Clinical presentation can vary widely, from typical gastrointestinal manifestations to minimal, unusual or even absent intestinal complaints with extraintestinal manifestations (54). The prevalence of pediatric-onset coeliac disease varies between 0.4% and 1.3% (55). A wide spectrum of associated neurological and psychiatric conditions has been reported (myelopathy, myopathy, brainstem encephalitis, epilepsy, headache), but typically cardinal features are ataxia and peripheral neuropathy. Antibodies associated with the disease occur in 16-57% of individuals with neurological dysfunction (56).

The pathogenesis of neurological manifestations is multifactorial. Some may be secondary to vitamin B12 deficiency (e.g. myelopathy and neuropathy), vitamin D malabsorption (e.g. myopathy), or vitamin E deficiency (e.g. cerebellar ataxia and myopathy), as a consequence of malabsorption (56). However, as neurologic complications are also frequent without malabsorption, other factors (namely humoral mechanisms) likely contribute to the pathogenesis of neurologic deficits. However, antigliadin (AGA), anti-transglutaminase–2 (TG2) and endomysial (EMA) IgA and IgG antibodies can be negative in patients without intestinal manifestations. IgG antibody reactivity to peripheral nerve antigens has been recorded in individuals with celiac disease and peripheral neuropathy (57), while patients with ataxia may have positive antiTG6 antibodies. In individuals with celiac ataxia, antibodies against Purkinje cells and a cross-reactivity between anti-gliadin antibodies and epitopes on Purkinje cells have been demonstrated by some research groups (58). Whether these antibodies are pathogenic or rather represent a non-specific marker is still unclear. Diffuse infiltration of cerebellum and peripheral nervous system by T lymphocytes and perivascular cuffing with inflammatory cells have also been reported (59).

Estimates of the frequency of neurologic manifestations in patients with established celiac disease range from 10% to 22% (60). In one series, 7% of patients with CD presented first with neurologic symptoms (61).

Neuropathy is present in up to 23% of patients with celiac disease. Very few studies have addressed the prevalence of peripheral neuropathy in childhood, and its potential association with celiac disease (54). In one study, 7.4% of children with celiac disease on a gluten-free diet had peripheral polyneuropathy with mixed patterns of axonal motor and sensory polyneuropathy and pure sensory polyneuropathy (including children non-compliant with gluten-free diet) (62).

Most patients present with primarily sensory symptoms and a distal, symmetric neuropathy (12). Peripheral neuropathy may precede, coincide with, or follow gastro-intestinal manifestations (63). Most commonly, it is a slowly progressive, usually slightly asymmetric, distal, painful sensory focal neuropathy with adult onset around the sixth decade, sometimes accompanied by clinical or subclinical autonomic dysfunction (60). Distal large-fiber axonal (and less often, demyelinating) sensory and motor peripheral neuropathy is reported less frequently, followed by multifocal neuropathy, pure motor neuropathy, and sensory neuronopathy (64).

Compared to the general population, celiac disease is associated with a 2.5-fold increased risk of later neuropathy, a 3-fold increased risk of chronic inflammatory demyelinating neuropathy, a 4-fold increased risk of autonomic neuropathy, and 8-fold increased risk of mononeuritis multiplex, but no association with acute inflammatory demyelinating polyneuropathy (65).

Changes consistent with a primarily axonal sensory neuropathy are usually demonstrated by ENG and biopsies. In at least one case, mononeuritis multiplex occurred in the context of vasculitis and responded to corticosteroid therapy (66). According to some authors, clinical improvement is obtained with a gluten-free diet after 1 year (67). The only systematic controlled trial of gluten-free diet in gluten sensitivity-related peripheral neuropathy (in patients with and without enteropathy) outlined a greater benefit if enteropathy was absent and if the duration of symptoms

was shorter (68). Studies on the effect of a gluten-free diet on peripheral neuropathy are conflicting, with some authors reporting clinical improvement while others concluding for a lack of relevant response (69, 70).

The lack of benefit from a gluten-free diet might be explained by inadequate dietary compliance resulting in rekindling of inflammatory response (68), or irreversible damage to peripheral nerves or dorsal root ganglia. Intravenous immunoglobulins have successfully been administered in a few cases of CD and GS-related ataxia and peripheral neuropathy (71). In one study, one female (out of 835 children with coeliac disease, 0.1% (72)) affected by an acute, predominantly motor, demyelinating peripheral neuropathy, experienced relapses upon accidentally reintroducing gluten and remitted on institution of a GFD regimen.

Patients with known celiac disease should be followed-up with an awareness that neurologic manifestations might occur, although uncommonly. Undiagnosed neurologic manifestations compatible with gluten-associated disorders should include gluten sensitivity in the differential diagnosis. This is particularly relevant in patients with appropriate HLA genotype, mild gastrointestinal symptoms, or other known autoimmune diseases, such as type-1 diabetes mellitus, autoimmune thyroid disease, primary biliary cirrhosis, Sjögren's syndrome, or rheumatoid arthritis. In addition, as celiac patients (even in asymptomatic cases) are at risk of refractory iron deficiency, folate deficiency, and osteopenia, the detection of any of these conditions should prompt investigations for celiac disease in neurologic patients.

Campylobacter-associated Guillain-Barré syndrome

Guillain-Barré syndrome (GBS) is an acute inflammatory polyneuropathy with acute onset of progressive ascending symmetric weakness and areflexia, of which two main neurophysiologic subgroups exist: inflammatory demyelinating and acute motor axonal neuropathy. As the pathophysiology is based on an abnormal post-infectious immune response, the onset of symptoms is often preceded by infections, either involving the upper respiratory tract or the gastro-intestinal tract (involved in up to 75% of pediatric cases), with many diverse etiologies being identified (73). *Campylobacter jejuni* is an important epidemiological cause of infectious diarrhea. Typical incubation period is 24-72 hours, but occasionally 1 week or longer. Nonspecific prodromal symptoms include headache, myalgias, chills, and fever, usually lasting approximately 24 hours. The peak of symptoms usually occurs at 24-48 hours before resolving within 1 week. The subtype O:19 has a higher tendency to result in GBS (74). *C. jejuni* infections associated with antibodies against GM1 and GD1b gangliosides tend to be associated with a severe, pure motor form of GBs (75). This group of patients seems to respond better to early treatment with high dose immunoglobulin therapy than to plasma exchange (76).

Mitochondrial neurogastrointestinal encephalopathy ("MNGIE") disease

Mitochondrial neurogastrointestinal encephalopathy (MNGIE) disease is characterized by progressive gastrointestinal symptoms, cachexia, ptosis, ophthalmoplegia/ophtalmoparesis, central and peripheral nervous system involvement (77).

A long history of ill-defined symptoms, such as fatigability, mild gastrointestinal complaints, or thin body habitus can precede the onset of more overt symptoms. To further complicate diagnosis, the order in which symptoms appear is unpredictable, although in a review of 102 patients the first symptoms were gastrointestinal (~57%), ptosis/ophthalmoplegia (~19%), peripheral neuropathy (~14%), and myopathy (~5%) (78). Onset is before 20 years of age in 60% of cases, while the earliest reported onset was at five months (78).

Prevalence is unknown. Parental consanguinity is common, as the disease is transmitted as an autosomal recessive condition.

Progressive gastrointestinal dysmotility, caused by enteric myopathy, occurs in virtually all affected individuals (79) and is carachterized by gastric and small bowel hypomotility resulting in early satiety, nausea, dysphagia, gastroesophageal reflux, postprandial emesis, episodic abdominal pain and/or distention, and

diarrhea. Despite severe GI dysfunction, serum concentrations of micronutrients and vitamins are usually normal.

Rectal biopsy can show eosinophilic cytoplasmic inclusions in the submucosal ganglion cells, corresponding to abnormal mitochondria (80). Duodenal pathology can demonstrate focal muscle atrophy with increased nerve numbers, serosal granulomas, and focal loss of Auerbach's plexus with fibrosis (81). Mitochondrial DNA depletion, mitochondrial proliferation, and smooth cell atrophy are observed in the external layer of the muscularis propria in the stomach and small intestine (79, 82). Loss of the pacemaker cells stimulating gut contraction (interstitial cells of Cajal) is also documented in the small bowel (83).

Neurologic presentation includes ptosis, ophthalmoplegia or ophthalmoparesis, leukoencephalopathy (usually asymptomatic and detected by brain MRI) and demyelinating PN.

All individuals with MNGIE disease develop peripheral neuropathy (84), which is demyelinating in all, with associated axonal neuropathy in half of the cases. In some, the first symptoms are paresthesias (with stocking-glove distribution) and weakness (usually symmetric and distal). The severity of neuropathic symptoms is often fluctuating during the early stages of the disease.

Segmental demyelination is hypothesized to be caused by uneven distribution of mtDNA abnormalities (depletion, single-nucleotide variants, deletions, duplications) along the nerve.

Electrodiagnostic features include decreased motor and sensory nerve conduction velocities, prolonged F-wave latency, and partial conduction block. Myopathic changes are common.

Histologically, demyelination and remyelination are observed, along with loss of large myelinated fibers.

The clinical diagnosis of MNGIE disease is based on the presence of cardinal symptoms, and a family history consistent with autosomal recessive inheritance. The diagnosis can be established by detection of one of the following: (1) biallelic pathogenic variants in *TYMP* (thymidine phosphorylase); (2) markedly reduced levels of thymidine phosphorylase enzyme activity (thymidine phosphorylase enzyme activity in buffy coat <8% of the control mean; less severely reduced (<18% of the control mean) in late-onset cases (85,86)); or (3) elevated plasma concentrations of thymidine and deoxyuridine (thymidine concentration >3 μmol/L or deoxyuridine concentration >5 μmol/L (86)).

Increased CSF protein (typically ≥60 -100 mg/dL), lactic acidemia and hyperalaninemia are common. Lactic acidosis is unusual (86).

Allogenic stem cells transplantation has been performed in MNGIE patients. It is hampered by a high mortality rate (62.5%) (87). In survivors, an increase in thymidine phosphorylase activity from undetectable to normal levels and an improvement of body mass index, gastrointestinal manifestations, and peripheral neuropathy were reported in patients engrafted and living more than 2 years after transplantation (87). Liver transplantation has been proposed as an alternative treatment (88).

Conclusions

The occurrence of PN in gastro-intestinal diseases is probably under-estimated and still not thoroughly characterized, especially in children. It is sustained by a multifactorial pathogenesis, challenging the clinician in all phases of management of the disease (diagnosis, therapy and follow-up). Promoting a high index of suspicion on frequent associations can result in a potential to significantly impact on patients' quality of life, by initiating the correct treatments or discontinuing neuro-toxic agents. Awareness about the existence of exceedingly rare but severe, degenerative conditions such as MNGIE can result in a reduction of the diagnostic delay and the possibly to address patients to highly specialized centers with the aim to modify their grim outcome or provide the best supportive care.

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Review

Endoscopic ultrasound in pediatric population: a comprehensive review of the literature

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Summary. *Background and aim:* Endoscopic ultrasonography (EUS) with or without fine needle aspiration/biopsy (FNA/B) is a well-established diagnostic tool in adults for the evaluation and management of gastrointestinal (GI) tract disorders. Its use in children is still limited as well as literature in pediatric age is limited, although the application of EUS is now increasing. The present article aims to review the current literature about EUS indication, accuracy and safety in pediatric age. *Methods:* Electronic literature searches were conducted using Pubmed, Medline, Embase, and the Cochrane Central Register of Controlled Trials using the word pediatric endoscopic ultrasound, pediatric pancreaticobiliary AND/OR EUS, pediatric EUS technique. Main patients and procedures characteristics were analyzed. The primary endpoint was the indication of EUS. Secondary endpoints were the accuracy of the technique and the incidence of complications. *Results:* Data were extracted from 19 articles. A total of 571 patients were investigated, with a median age of 12,7 years. A total of 634 EUS procedures were performed. The majority of EUS procedures investigated the pancreaticobiliary tract (77,7%). Most studies showed a high positive impact on management with a median value of 81,7%. No major complications were reported. Five studies reported minor complications with a median value of 2%. *Conclusions:* EUS is safe and has a significant role in the diagnosis of pancreaticobiliary and GI diseases even in children, with a high therapeutic success. An increasing EUS utilization by pediatric gastroenterologists is expected and offering dedicated EUS training to some selected pediatric gastroenterologists might be indicated. (www.actabiomedica.it)

Key words: indication, accuracy, safety of EUS in children

Background and aim

Available since the 1980s, endoscopic ultrasound (EUS) allows detailed anatomical visualization of structures around the gastrointestinal (GI) tract, including the individual layers of the GI tract (1). However, EUS has not had a relevant role in Gastroenterology, until the advent of the fine needle aspiration (FNA) in 1991 (2). After the introduction of FNA, EUS has increasingly been applied in the field of Gastroenterology and nowadays EUS use in adults is well established and widespread.

Although the application of endoscopic ultrasound in children is increasing, EUS and EUS-FNA in children are supported by limited number of studies. This is mainly because of the lower incidence of pancreaticobiliary and GI neoplasms, presumptive size limitations of EUS equipment for pediatric patients, the need for sedation or anesthesia, the lack of skilled pediatric endosonographers and limited awareness among pediatricians of EUS diagnostic and therapeutic capabilities (3).

Benefits include absence of ionized radiation exposure, excellent axial resolution providing detailed

real-time imaging of structures and wall layers, and the capacity to sample tissue and fluid collections via FNA and fine needle biopsy (FNB) (4).

The aim of this paper is to review the current literature for EUS indication, accuracy and safety in pediatric age.

Equipment

EUS is composed of different instruments: the echoendoscopes in which US transducer is placed on the tip and a water-filled balloon surrounding the transducer, the miniprobes that can be inserted inside the biopsy channel of a standard endoscope.

Although the application of traditional linear echo-endoscopes may be feasible in patients weighing at least 15-25 kg, data evaluating EUS in patients of this size are limited (4, 5). This limit can be overcome by using smaller (6.3-6.9 mm outer diameter) endobronchial ultrasound equipment in the GI tract. With this equipment, EUS can successfully be performed in children as young as 2 months of age (6). In alternative, EUS can be performed using miniprobes through the working channel of a standard pediatric endoscope.

Radial EUS scopes provide a 360° sonographic view, which is perpendicular to the tip of the endoscope. Radial scope is generally used to study the upper digestive system because it allows complete and simple anatomical orientation and faster exploration of large areas of the gastro-intestinal tract and adjacent organs (7). Linear EUS scopes provide a 150° sector view, which is parallel to the long axis of the endoscope. It shows the gastrointestinal wall worse than the radial one, but it is in general used for the organs outside the gastrointestinal tract (e.g. pancreas), and it is indispensable for FNA (8). Both endoscopes operate a frequency between 5 and 10 MegaHz.,

Methods

Data sources

Electronic literature searches were conducted using Pubmed, Medline, Embase and the Cochrane, Scopus from 1 January 1993 to 1 September 2018.

Search Mesh terms included: "pediatric endoscopic ultrasound", "pediatric pancreaticobiliary AND/OR EUS", "pediatric EUS technique". Additional articles were selected reviewing the references of the papers identified using these key words. No attempt was made to locate unpublished material.

Study selection (inclusion/exclusion criteria)

Published studies were included if they met the following criteria:

(1) the indications of EUS in children were investigated, (2) studies involving only patients in pediatric age, (3) no gender restrictions, (4) final diagnosis was indicated (5) complication were reported, and (6) publication in English. We excluded (1) systematic reviews, abstracts, and guidelines; (2) studies involving adult patients; (3) studies not in English; (4) animal and ex vivo studies.

Data extraction

Data extraction was conducted independently by 2 investigators (B.B. and G.L.de'A.), with the discrepancies resolved by the consensus of these 2 investigators. The publications were manually screened and reviewed to identify reports for pediatric EUS.

This study did not require ethical approval as all the used data have been published previously.

The following details were recorded: Patients (the total number of patients enrolled in each study indication of EUS, mean age); Intervention (with or without FNA, Therapeutic procedures); Outcomes (diagnosis, treatment, clinical impact, complications).

Results

After removal of duplicates and screening for inclusion and exclusion criteria a total of 19 studies (3, 9-26) from 1998 and 2018 were included. Eleven studies were performed in the USA (11, 12, 15-20, 23, 25-26), 4 in Europe (3, 9, 14, 22) and 4 in Asia (10, 13, 21, 24).

Patients and procedures characteristics

The main findings are reported in Table 1. A total of 571 patients were investigated with EUS. The patients age ranged between 0,5 and 21 years old, with a median age among the studies of 12,7 years old. A total of 634 EUS procedures were performed. In 21,2% of cases a EUS-FNA or a Tru-Cut Biopsy (TCB) was performed. A 16,4% of operative EUS including drainage of pseudocyst, celiac plexus block, cyst gastrostomy with stents placement, transluminal biliary drainage following failed Endoscopic Retrograde Cholangio-Pancreatography (ERCP) cannulation were reported.

Indications and impact

Details of the indications and impacts are presented in Table 2. The majority of EUS investigated the pancreaticobiliary tract (77,7%), followed by the upper GI tract, including the evaluation of the esophagus, stomach and duodenum (15,4%), rectum (4%), and other indication (such as evaluation of lymph-nodes, mediastinal/abdominal mass) (2,9%).

Most studies about EUS in children showed a high positive impact on management ranging from 35,5 % to 100 % (media=81,7%).

Complications

There were no reported main complications in the included studies (Table 3). Five studies reported minor complications from 2 to 22% with a mean value of 2%. The reported minor complications included self-limited bleeding without need for intervention or hospitalization (19, 21) during FNA/TCB, late bleeding (19th day) after pseudocyst drainage (3), mild pancreatitis (20), transient desaturation after conscious sedation (21), postprocedural fever after cystogastrostomy (21) and after pseudocyst drainage (26), intra-procedural anesthesia-related complications (laryngospasm and hypoxemia (26).

Table 1. Patients and procedures characteristics

Studies	No patients	No procedures	Age yrs	FNA/TCB%	Therapetic procedures %
Roseau et al 1998	18	23	4-16(12)	0	0
Usui et al 2002	2	2	0,5-4 (2,25)	0	0
Nadler et al 2002	1	1	13 (13)	100	0
Varadarajulu et al. 2005	14	15	5-17 (13)	FNA (20)	0
Cohen et al 2008	32	32	1,5-18 (12)	FNA (21,9)	0
Bjerring et al. 2008	18	18	0,5-15 (12)	0	0
Attila et al. 2009	38	40	3-17 (13,5)	FNA (30)	5,2
Rosen et al 2010	25	42	NA (14)	0	0
Al-Rashdan et al. 2010	56	58	4-18 (16)	FNA (25,9)	8,9
Jazrawi et al 2011	10	10	4-17 (11,8)	FNA (20)	80
Larissa et al 2012	9	9	9-18 (13,6)	FNA/TCB 100	0
Scheers et al. 2015	48	52	2-17 (12)	FNA (25)	8,3
Gordon et al. 2015	43	51	4-18 (14,5)	FNA (25,5)	0
Mahajan et al. 2016	121	125	3-18 (15,2)	FNA (5,6)	0
Fugazza et al 2017	40	47	3-18 (15,1)	FNA (6,4)	2,5
Law et al 2018	1	1	8 (8)	0	100
Singh et al 2018	32	32	8-18 (14)	0	0
Jia et al 2018	5	6	6-17 (13)	0	100
Raina et al 2018	58	70	6-21 (18)	FNA (22,4)	6,9
Total	571	634			
Mean			12,7	21,2	16,4

Table 2. Main indications and impact of EUS procedures

Studies	Indication %				Impact%
	PB	Upper GI	Rectum	Other	
Roseau et al 1998	34,8	34,7	26,1	4,3	NA
Usui et al 2002	0	100	0	0	100
Nadler et al 2002	100	0	0	0	100
Varadarajulu et al. 2005	100	0	0	0	93
Cohen et al 2008	59,4	34,4	6,3	0	44
Bjerring et al. 2008	61,1	16,7	0	22,2	78
Attila et al. 2009	62,5	17,5	2,5	17,5	NA
Rosen et al 2010	100	0	0	0	NA
Al-Rashdan et al. 2010	72,4	3,4	6,9	0	86
Jazrawi et al 2011	100	0	0	0	86
Larissa et al 2012	100	0	0	0	86
Scheers et al. 2015	100	0	0	0	98
Gordon et al. 2015	66,7	11,8	1,9	0	80
Mahajan et al. 2016	94,4	1,6	0	0	35,5
Fugazza et al 2017	59,6	47,3	31,9	0	87,2
Law et al 2018	100	0	0	0	NA
Singh et al 2018	100	0	0	0	NA
Jia et al 2018	100	0	0	0	Na
Raina et al 2018	65,6	24,1	0	10,3	88
Mean	77,7	15,4	4	2,9	81,7

Table 3. Complications

Studies	Minor complications %	Major complications %
Roseau et al 1998	nil	nil
Usui et al 2002	nil	nil
Nadler et al 2002	nil	nil
Varadarajulu et al. 2005	nil	nil
Cohen et al 2008	nil	nil
Bjerring et al. 2008	nil	nil
Attila et al. 2009	nil	nil
Rosen et al 2010	NA	NA
Al-Rashdan et al. 2010	nil	nil
Jazrawi et al 2011	nil	nil
Larissa et al 2012	22	nil
Scheers et al. 2015	2	Nil
Gordon et al. 2015	2,3	Nil
Mahajan et al. 2016	2,4	Nil
Fugazza et al 2017	nil	Nil
Law et al 2018	nil	Nil
Singh et al 2018	nil	Nil
Jia et al 2018	nil	Nil
Raina et al 2018	8,6	Nil
Total	2	0

Discussion

Compared to the firmly established role of EUS/EUS-FNA in adults, data in pediatric patients are still scarce. Moreover, most studies focus on diagnostic indication for EUS and only few provide information on its therapeutic role in this population (12, 17). A limit of EUS in pediatrics is the presumptive size of EUS equipment, especially when therapeutic EUS procedures are required. Although literature showed that the application of linear echo-endoscopes may be feasible in patients weighing at least 15-25 kg, data regarding this modality are still limited (27). More limits are due to the lack of experience of operators as well as the rarity of diseases that require EUS evaluation in children. Even though studies in pediatrics described that only 21,2% of patients underwent FNA/TCB, currently available literature suggest that EUS-guided pancreatic tissue sampling can be performed with technical and clinical results corresponding to the procedures in adults for similar indications (3, 9, 12, 14, 17). Literature suggests that EUS-FNA is technically

successful in more than 95% of cases if carried out by an experienced endosonographer with a sensitivity of 87% and a specificity close to 100% (28). The most recurrent scenario in which EUS-FNA is reported in children include pancreatic tissue sampling in the setting of pancreatic mass or suspected Acute Idiopathic Pancreatitis (AIP) (4).

Regarding the indications, pancreaticobiliary disease is the most common reason for EUS referrals in the pediatric population. Various pancreaticobiliary diseases may require EUS evaluation also in pediatric age such as inflammatory conditions (suspected choledocholithiasis/microlithiasis, recurrent/chronic/autoimmune pancreatitis), congenital conditions (choledochal cyst, anomalous pancreaticobiliary junction, pancreas divisum, duodenal duplication, ectopic pancreas), cystic lesions (pancreatic pseudocyst, mucinous/serous cystic neoplasms), neoplastic conditions (neuroendocrine tumors, solid pseudopapillary tumor, lymphoma) (5).

EUS is known to be a sensitive procedure to evaluate both biliopancreatic diseases and gastrointestinal diseases, due to the peculiar ability to visualize early pathological changes in the pancreatic gland and to differentiate the 5 GI layers (29).

In acute pancreatitis, EUS is not indicated in the evaluation of the extension of the inflammatory process, where CT remains the gold standard, nevertheless, EUS is indicated to study the etiology of pancreatitis, being choledocholithiasis the most common cause of acute pancreatitis (78.9%) (16).

In lithiasis EUS has been shown to be 95% to 100% accurate for diagnosing such diseases as suspected choledocholithiasis and microlithiasis (13). EUS has a higher resolution and is more sensitive especially for microlithiasis (less than 3 mm in size) when compared to magnetic resonance cholangiopancreatography, or CT (30, 31). Moreover, children with EUS showing no evidence of microlithiasis can potentially avoid unnecessary cholecystectomy or ERCP (5). Therefore, EUS can identify patients with biliary pancreatitis in which the ERCP will be useful and replace diagnostic ERCP which is more invasive and associated with more risks (12). Actually, one study demonstrated that ERCP was avoided in 13 out of 17 children due to findings noted on EUS (32).

Pancreas divisum is the most common pancreatic congenital anomaly and it may play a role in "idiopathic" acute and chronic pancreatitis (33).

EUS can be performed to exclude pancreas divisum with a sensitivity of 100% and specificity of 96%, avoiding the risks associated with ERCP (5).

Pseudocyst is the most common pancreatic cystic lesion in childhood, about 75% of all cases (18). The common causes of pancreatic fluid collections (PFCs) in children worldwide are trauma (leading cause up to 50% of cases), gallstone, idiopathic, hereditary, viral, or toxin-mediated pancreatitis (34).

The rates of technical success were significantly better for the EUS-guided approach with even minor complications compared to gastrostomy (18), a success rate of 94% and with long-term pseudocyst resolution in 85% of cases (18). One recent study demonstrated successful pediatric therapeutic EUS procedures in a 6 years old child weighting 18,5 kg (25). But data in pediatric population are still limited, as reported in this review with a 16,4% of therapeutic procedures, compared to adult population (65,6% of pseudocyst requires percutaneous/endoscopic or EUS drainage) (35). Pancreatic neoplasms are rare in children. Pancreatic necrosis, solid pseudopapillary tumor, neuroendocrine tumor, insulinoma and gastrinoma, and lymphoma are the most frequent in pediatric patients (4,5). EUS has proven to be sensitive and specific in diagnosing pancreatic masses in pediatric population. Contrast-enhanced harmonic EUS and EUS elastography can be helpful to improve the accuracy of EUS (36, 37).

This review reveals that EUS played a significant role in establishing a definitive diagnosis and managing pediatric disorders with an important clinical impact ranging from 35,5-100%, with a median value of 81,7%. The authors explain that the possible reason for the low impact factor (35,5) are the lack of follow up in recurrent acute pancreatitis, the largest subgroup (59%) (21), the avoidance of ERCP by EUS in previous studies was taken as a positive impact in management and the less stringent criteria to define a positive impact in some studies (12-14, 17).

The main complications reported in children are related to therapeutic procedures (19-21, 26). Complications rates regarding perforation are similar compared with standard endoscopy. The risk of iatro-

genic pancreatitis as a result of EUS-FNA arises in patients undergoing FNA of pancreatic masses, cysts or the pancreatic duct, involving direct passage of needle through pancreatic tissue (38). Despite the use of Doppler, bleeding is reported with an incidence of 1-4,4% as intraluminal hemorrhage, and 1,3% as extraluminal hemorrhage (39). Other complications are anesthesia-related ones, since in pediatric age these procedures need sedation or general anesthesia.

Conclusions

EUS is an emerging modality even in pediatric age that provides detailed evaluation of the pancreatic parenchyma and GI system due to its high sensitivity and accuracy (5). Until now, EUS has been performed in a relatively small number of pediatric patients and the majority of endosonographers are adult gastroenterologist who usually work with instruments for adults.

Moreover, EUS enables to obtain FNA/FNB sampling or larger core tissue biopsies that may be beneficial in the diagnosis of certain pathologies such as neoplasms or AIP. Compared to ERCP, it is a safe modality with minimal risk (40-42) and diagnostic ERCP can be therefore avoided. EUS is a safe and useful outpatient procedure with minimal morbidity, although requires sedation preferably with anesthesiologic support, especially for pediatric patients. Therefore, the complex management required for the pediatric patients may limit the use of EUS in children to highly trained experts and to tertiary care centers.

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Review

Applications of wireless capsule endoscopy in pediatric age: an update

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Summary. *Background:* The small bowel has often been considered the mysterious "black hole" of the gastrointestinal tract. With regards to this, the development of the wireless capsule endoscopy (WCE) has represented a turning point. It is a non-invasive technique, enabling an excellent visualization of the small bowel (SB) mucosa without the use of radiation. The WCE was approved by the Food and Drug Administration (FDA) in 2001 for adults and in 2004 for children. The aim of the present review is to provide an update on indications, diagnostic yield, safety and limitations of WCE in children. Even though literature regarding the use of WCE in pediatric age is more limited than in adults, WCE is a useful and safe diagnostic tool for the exploration of the small bowel also in children. The indications for WCE are similar at any age, however the main indication in children is Crohn's disease (CD), while in the adults is the research of SB bleeding. The main limitation in pediatric age is the possibility for younger children to swallow the capsule. WCE in pediatric is a rapidly advancing technology and has the potential to further transform the evaluation and management of SB disease. (www.actabiomedica.it)

Key words: wireless capsule endoscopy, pediatrics, small bowel diseases, indications, safety

Introduction

The small bowel has often been considered the mysterious "black hole" of the gastrointestinal tract. With regards to this, the development of the wireless capsule endoscopy (WCE) has represented a turning point. It is a non-invasive technique, enabling an excellent visualization of the small bowel (SB) mucosa without the use of radiation. The WCE was first used in humans in 1999, in 2001 it was approved by the Food and Drug Administration (FDA) as an adjunct tool for the evaluation of SB diseases in adults and later 2003 as a first-choice diagnostic method for investigating SB diseases. In 2004 WCE was approved as a diagnostic tool also for children of 10 years or older.

Supported by additional experience in younger children, in 2009 the FDA expanded the diagnostic role of WCE and of the patency capsule (PC) for their use in children of two years or older (1). Patency capsule is a capsule with identical size of a standard capsule, containing a mixture of barium, that gradually dissolves if passage does not occur within 30 hours from the administration.

Anyway, case reports have demonstrated a safe use also in younger children, down to 8 months or 7,9 kg (2, 3). Overall, the use of WCE in children has some limitations mainly due to eventual difficulties for children in swallowing the capsule, and due to a scarcity of literature in pediatrics. The present review will provide data on the indications, diagnostic yield, safety and limitations of WCE in children.

Wireless capsule endoscopy

Nowadays, four types of small bowel capsules (PillCamSB, EndoCapsule, Miro capsule, and Capso Cam) and one esophageal capsule (PillCam ESO) are available. A capsule for the exploration of the colon is also available in Europe, in the United States and in Japan (Pill Cam Colon). The PillCam is now a third-generation capsule, with improved resolution and a variable frame rate. The frame rate increases to six frames per second when moving quickly and slows to two frames per second when moving slowly or when it is stationary. The EndoCapsule is similar to Pillcam but has a charge-coupled device chip instead of a complementary metal oxide semiconductor chip. The EndoCapsule has been replaced by the endoCapsule10 with increased resolution and three-dimensional location software.

The Miro Cam capsule uses a novel mode of transmission called electric field propagation, which uses the human body as a conductive medium to transmit images (4).

The CapsoCam is equipped with four cameras giving a 360° field of view and a variable frame rate. Wireless capsule evolution sees not only a continuous improvement in the optical lenses and image resolution, but also the software present constant advances. The resolution of modern capsule images is at a maximum 512x512 pixel (5). With the help of software algorithms picture, quality is enhanced to provide enough details for all relevant findings.

Unfortunately, there are no commercially available capsules capable of taking biopsies yet.

At present, all the capsule devices available have a battery life of 12 hours or grater. This extended battery life reduces the chance of incomplete transit in the non-obstructed small bowel.

Indications and diagnostic yield

Certainly, WCE has represented a great advance in the study of SB disease in adults. Similarly, in pediatric age, as it is a non-invasive technique which avoids the use general anesthesia and ionizing radiation, WCE represents a real advantage in diagnostic, therefore its use has experienced a significant expansion. The are several important differences in the use of WCE between adults and children (6). In pediatrics, one of the most important considerations to be made before undertaking WCE is the evaluation of the patient's ability to swallow the capsule. Patients may be helped learning to swallow the capsule, by practicing swallowing candies (7). According to reported evidence, the most physiologic way to proceed is swallowing the capsule with some water, which is safe feasible even for children 4 to 5 years old (8). In a review by Cohen et al, including 824 children among whom the youngest was 4 years old, 88,4% of the patients were able to swallow the capsule (6). Anyway, the ability to swallow the capsule is not exclusively dependent on age. Up to 1,1-1,5% of adults and older children are unable to ingest the capsule that is bigger than 1x2 cm in size. In case of impossibility for the patient to swallow the capsule due to any reasons (including swallowing disorders, dysphagia, etc.), the capsule can be safely delivered into the duodenum with various techniques during a standard endoscopy (9, 10). There are different devices for endoscopic capsule delivery, such as polypectomy snares, foreign body baskets or specific delivery devices (11) (Figure 1).

The advanCE device allows endoscopic delivery of the capsule. The system is a disposable catheter with a sheath diameter of 2,5 mm that can be preloaded through the appropriate operative channel of a standard endoscope. The placement of the capsule into the duodenum is relevant to ensure the visualization of the entire SB, avoiding the risk of delay in the passage from the stomach to the duodenum which is frequent when the capsule is swallowed. Sometimes, especially in younger children, only the tip of the endoscope may

Figure 1.

be driven into the duodenum, consequently the capsule may return into the stomach. Moreover, is case of endoscopic capsule delivery, the patient should be sedated and eventually intubated (12).

In 2015 a panel of experts in the field of WCE belonging to the Spanish Society for Pediatric Gastroenterology, Hepatology and Nutrition (SEGHNP) and to the Spanish Society for Digestive Disease (SEPD) established guidelines for the use of WCE in children (12).

The indications for the use of WCE are similar in children and adults, (table 1) however according to the available scientific evidence, the most frequent indication for WCE in children is inflammatory bowel disease (IBD) both for diagnosis and disease extension assessment, while it is the diagnostic of obscure gastrointestinal bleeding (OGIB) in adults (6). On the other hand, obscure gastrointestinal bleeding, malabsorption and protein-losing enteropathies, small bowel polyps, tumors and in general all the clinical situations where small bowel pathology is suspected are other reported indications for WCE in pediatric population, as in adults (13, 14).

A recent study regarding the use of WCE in children compared indications for WCE among 1013 procedures in pediatric patients and 22840 procedures in adults and concluded that in pediatric patients, 63% of WCE had been performed for Crohn Disease (CD), 15% for OGIB, 10% for abdominal pain/diarrhea and 8% for polyposis (15). In contrast, in adults 66% of WCE had been performed for OGIB and 10% for CD (16). However, it is of note that OGIB is more frequent than CD in pediatric population younger than 8 years of age (6).

Table 1. Indications to perform small bowel Wireless Capsule Endoscopy

Indications for small bowel WCE in children
Small bowel Crohn's disease
Diagnosis and extent evaluation
Occult/obscure intestinal bleeding
Small bowel polyps
Familial and non-familial polyposis
Malabsorption and protein losing enteropathies
Celiac disease
Eosinophilic and food allergic enteropathies
Intestinal lymphangiectasia
Small bowel tumor
Lymphoma
Leiomyoma
Carcinoid and other

Wireless capsule endoscopy and Inflammatory Bowel Disease (IBD)

In the investigation of IBD, WCE may be used at different times during the course of the disease: at diagnosis, in the differentiation of ulcerative colitis (UC) or indeterminate colitis (IBDU) from CD, in the investigation of CD extension, activity, response to treatment, or later in the disease course to differentiate active disease from contemporary functional complaints (17).

In 2009 the world Organization of Digestive Endoscopy (OMED) and the European Crohn's and Colitis Organization (ECCO) recommended to perform WCE in children for the diagnosis of CD when conventional upper and lower endoscopy and radiographic imaging are not conclusive (18). The recommendations of ESPGHAN have established that WCE is a useful alternative to identify small bowel mucosal lesions in children with suspected Crohn disease in whom conventional endoscopy and imaging tools have been non-diagnostic or in whom Magnetic Resonance Enterography (MRE) cannot be performed due to young age or in settings where MRI is not available or not feasible. A normal WCE study has a high negative predictive value for active small bowel CD (19). The main advantages of WCE are the ability to visualize the entire small bowel with minimal disconfort and to detect mucosal lesions with a higher sensitivity than MRE. The risk of capsule retention, and the inability to control capsule movement are the main limitation (20). It is therefore recommended that MRE or Patency capsule should precede WCE in order to identify strictures that may cause capsule retention (12). Moreover, WCE has a high rate of incidental findings, and therefore a low specificity. False positive features are found in 10-21% of healthy people, particularly in case

of non-steroidal anti-inflammatory drugs use which can cause erosions and ulcers. In a meta-analysis in pediatric onset IBD the diagnostic yield for WCE ranged from 58 to 72% whereas it was 0 to 61% for ileocolonoscopy (16). In a prospective pediatric controlled study conducted on 20 children with suspected IBD with either normal or non-specific findings on conventional imaging, WCE confirmed the diagnosis of CD in 12 (60%) (21). In different reports both sensitivity and specificity of WCE range from 77,8% to 94,6% while MRE shows a sensitivity of 75-85,7% and a specificity of 70% (22). Anyway, both MRE and CE should be considered complementary and accurate methods in patient with suspected CD (23).

Intestinal polyposis

A few well-designed large studies have been reported that evaluate the use of WCE for the diagnosis and surveillance of small intestinal polyposis. Peutz-Jeghers syndrome (PJS) is the most frequent polyposis syndrome during childhood. PJS is associated with chronic bleeding, anemia and bowel obstruction and intussusception requiring surgery. Polyp related complications could develop in childhood since the age of 10 years. Guidelines recommend screening patients with PJS every 2 to 3 years beginning around the age 8 years for small bowel polyps, and earlier if symptomatic (24). Mostly retrospective case series have shown WCE to be an accurate diagnostic tool compared with small bowel through imaging of the intestine (13). Tomasa et al have raised concern about the use of WCE for polyp screening because of reports of proximal jejunal, duodenal polyps and tumors that were missed by WCE and properly identified by double balloon enteroscopy (DBE) (25). Ohmiya et al found no difference in the detection rates of either all type of polyps or large polyps (>10 mm) (26). Postgate et al compared the yield of WCE with the one of barium enterography in children with PJS and concluded that WCE is a feasible, safe and accurate tool for small bowel polyp surveillance in children with PJS (27). Moreover, the assessment of polyposis syndrome has the highest diagnostic yield by indication of WCE in children. Around 80,2% of the procedures lead to significant findings, a greater percentage than in adults. Hence, WCE should be considered as a first line diagnostic method in small bowel polyposis syndrome. Studies in children and adults with PJS and other small bowel polyposis syndromes will need to be performed to clarify the relative roles of DBE, CE and MRE in these clinical conditions.

Obscure gastrointestinal bleeding and iron-deficiency anemia

OGIB, whether occult or apparent, is the most frequent indication for CE in children younger than 8 years (16). Positive findings have been reported in 42% of pediatric patients (16) compared to 60% in adults with OGIB or persistent iron deficiency anemia (28). It is important to take into account that active bleeding lesions are more likely detected when WCE is used within the first 3 days (95%) or 2 weeks (93,1%) after the bleeding event, compared to more delayed procedures (57,1% after 2 weeks) (29). WCE may be useful in several gastrointestinal disorders in childhood, such as celiac disease, protein losing enteropathy, intestinal lymphangiectasia, graft versus host disease, chronic abdominal pain and failure to thrive. However, regarding these conditions, only data on isolated case reports have been published and scientific evidence is low.

Preparation

The inability to establish the exact location of the capsule in the small bowel and the inability to flush or aspirate make adequate bowel cleaning particularly important for SB WCE (30). Since cleaning the small bowel prior to examination may improve the diagnostic yield, CE preparation regimens have been proposed, mainly by using the same products adopted for colonoscopy preparation (31). The optimal preparation regimen is yet to be established. A meta-analysis study in adults demonstrated that a protocol including a combination of simethicone and polyethylene glycol appears to be the best approach. Oliva et al, in their randomized single blind study in children, demonstrated that low volume PEG (25 ml/kg) assumed the

evening before the study and 376 mg simethicone in 20 ml water 30 minutes before the procedure achieved better visualization scores (32). A 10-12 hour fasting before the testing is generally recommended.

Adverse events

WCE is, in general, a well-tolerated and safe procedure. Two complications have been noted in WCE, namely capsule retention and capsule inhalation. Capsule inhalation is an extremely rare event and has not been reported in children (33); this complication is observed particularly in individuals with neurological or swallowing disorders (34-35). The main complication of WCE in children is capsule retention in the small bowel, defined as missed expulsion of the capsule within 2 weeks from the administration or the need for directed intervention before that time. Its incidence in most studies ranges from 1,5 to 3,5%. However, there are numerous series in which this complication was not observed (36, 37) and series that reported retention in about 20% of procedures (38). Several risk factors have been associated with capsule retention. Firstly, it might be thought that patient size may play a role. However, this is not clearly observed in published pediatric series. Younger children may have more difficulties in swallowing the capsule, but they do not retain it more often than adults. Retention usually relates to an intestinal stricture, which may be inflammatory (e.g. CD, nonsteroidal anti-inflammatory drug enteropathy, and actinic enteritis), post anastomotic or due to the presence of small bowel intestinal tumors (28). Retention associated with the presence of polyps has also been reported in pediatrics (6, 33). Studies conducted both in adult and in pediatric age have demonstrated the usefulness of patency capsule in predicting the uneventful passage of the capsule (10). Retained capsules may resolve in time and therefore, if bowel obstruction does not occur, removal may be delayed. Capsules retained due to small bowel strictures, causing bowel obstruction, may need to be removed endoscopically or surgically; however, if an inflammatory stricture is suspected and the scenario does not show a clinical emergency, medical treatment of the underlying condition (e.g. steroids for inflammatory strictures) may be attempted before surgical intervention (39).

Patency capsule

Patency capsule (PC) is a capsule of identical size of a standard capsule and consists of a small identification tag (RFID), detectable by radiofrequency, which is surrounded by an absorbable material with a small amount of barium, all this covered by an external cover. The first version had a single timer plug that degraded at 40 h. The currently available version has dual timer plugs that gradually implodes if passage does not occur within 30 hours from the administration. Both retrospectives (40) and a prospective (41) studies have been performed in pediatric IBD using PC prior to WCE. In the retrospective analysis, a PC before WCE in 23 patients allowed 22 WCE to proceed with only 1 retained capsule. In the prospective trial conducted on 18 patients (age 10-16 years) who ingested the PC, 15 excreted an intact PC without any PC or WCE retentions or adverse events. The PC can serve as a useful tool before performing WCE, as it may lower the likelihood of WCE retention, particularly in known or suspected CD where the risk of retention is the greatest.

Conclusions

WCE is a useful and safe diagnostic tool for small bowel that has particular benefits in children, because it does not usually require ionizing radiation, deep sedation or general anesthesia.

The indications for performing WCE in children are similar to those in adults, however the main one in children is CD to establish both a diagnosis and disease extension, while it is obscure gastrointestinal bleeding (OGIB) in adults and in children younger than 8 years of age.

Moreover, only few limitations in the use of WCE are known in children. The main one is the difficulty for younger children to swallow the capsule, which turns WCE into an invasive method because of the

need to deliver the WCE into the duodenum using an endoscope under deep sedation or general anesthesia.

The risk of retention appears to be dependent on indication rather than the age of the patient, confirming that WCE is a safe procedure with a significant diagnostic yield.

WCE is a rapidly advancing technology and has the potential to further transform the evaluation and management of SB disease, even in pediatric age. Although it has evolved significantly since 2000, many areas for further research are open.

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Review

Digestive disorders and Intestinal microbiota

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Summary. In the last decade, a barge body of scientific literature has suggested that specific alterations of the gut microbiota may be associated with ther development and clinical course of several gastrointestinal diseases, including irritable bowel syndrome, inflammatory bowel disease, celiac disease, gastrointestinal cancer and *Clostridium difficile* infection. These alterations are often referred to as "dysbiosis", a generic term designing reduction of gut microbiota biodiversity and alterations in its composition. Here, we provide a synthetic overview of the key concepts on the relationship between intestinal microbiota and gastrointestinal diseases, focusing on the translation of these concepts into clinical practice. (www.actabiomedica.it)

Key words: dysbiosis, microbiome, IBD, celiac disease, *Clostridium difficile*

Dysbiosis

Alongside the definition of the "normal" human intestinal microbiota and the factors that may condition its composition, in recent years biomedical research has made an enormous effort to try to identify any "abnormality" of the microbiota composition associated with various acute and chronic diseases, and to establish possible cause-effect links (1). In some cases, a clinically significant contribution of the microbiota to the pathogenesis and clinical progress of a disease has been demonstrated. While this is fairly intuitive for diseases that primarily involve the bowel, such as intestinal inflammatory diseases (IBD) and *Clostridium difficile* enterocolitis, on the other hand, it is certainly less for diseases that they involve organs anatomically very far from the intestine. However, this has allowed to hypothesize and, in some cases, to demonstrate the presence of metabolic, endocrine and systemic mechanisms through which the microbiota can, through the GI tract, influence the pathophysiology of the whole body (1-2).

The pathological changes of the human intestinal microbiota can be generalized, when they concern the balance of the microbial population as a whole, or limited to a single or a small group of minor players, which, thanks to particular metabolic activities or a high pathogenic potential, may alone to influence the onset of a disease, either in a negative (absence of a bacterium with protective activity) or positive (presence of a pathogenic bacterium or with harmful activity) way (1-2).

When the alteration of the intestinal microbiota is generalized, we generally speak of dysbiosis, meaning a generic variation in the global composition of the microbial population, with an increase in the relative abundance of some taxa and reduction of the relative abundance of others. In many cases, dysbiosis is associated with increased representation of pathobionts, that is, taxa with potential pathogenic activity such as *Escherichia* and *Klebsiella* spp, at the expense of a reduced representation of taxa with possible beneficial metabolic activity, including lactobacilli and bifidobacteria. Dysbiosis is also associated with reduced biodi-

versity, that is, number of microbial species present in the microbiome and lower complexity of the microbial community (3-4). However, this concept is not uniformly shared by the entire scientific community, and its clinical implications are still unclear. From a medical point of view, the dysbiosis is neither a disease nor a symptom, but a condition that is associated with certain diseases or that can increase the risk. Moreover, the boundaries between dysbiosis and normal individual variability in the composition of the microbiota are still undefined, and therefore it is sometimes particularly difficult to determine whether a given microbiota profile is affected by dysbiosis or not (3-4).

The intestinal microbiota can therefore influence the human pathology at different levels and its alterations can be both cause and consequence of a state of illness. In recent years, many studies have tried to identify the main abnormalities of the intestinal microbiota associated with a long series of acute and chronic human diseases, and to clarify how these anomalies can be linked to the pathogenesis of the diseases themselves. The following is an overview, far from being exhaustive, on the main results of these studies focused on gastrointestinal diseases (1).

Inflammatory Bowel Disease

In IBD there is generally a reduction of the *Firmicutes*, in particular of the species with anti-inflammatory activity *Faecalibacterium prausnitzii*, and an increase in the relative abundance of the *Bacteroidetes*, in particular of the species *Bacteroides fragilis* (5-6). There is also an increase in *Proteobacteria*, and namely a blooming of *Enterobacteriaceae*, including the opportunistic pathogens *Escherichia coli* and *Klebsiella pneumoniae*, which help to support inflammation of the mucosa and increase the risk of infections (7). Overall, this framework leads to strong dysbiosis, with a reduction in microbial diversity, the number of bacteria and their metabolic activity (8). Microbiota manipulation techniques, such as the administration of probiotics and the fecal microbiota transplantation can determine beneficial clinical consequences on the progress of the disease (9-10).

Irritable Bowel Syndrome

Although the pathogenesis of IBS is not fully understood, the role of the microbiota appears to be relevant. In fact, a significant percentage of patients shows intestinal bacterial overgrowth (SIBO). Many studies have shown a reduction of *Bifidobacteria* and *Lactobacilli* and an increase in *Enterobacter* especially in patients with IBS and diarrhea (IBS-D). Other studies have also documented an increase in *Veillonella* in patients with IBS and constipation (IBS-C). Other authors associate IBS and *Campylobacter, Yersinia, Salmonella, Shigella* and *E. Coli*. The great heterogeneity of results is also due to the multiple methods used to determine the microbiota and to the different patient inclusion criteria. Finally, there is also evidence that viruses, parasites and fungi may also play a primary role in the pathophysiology of IBS (11).

Celiac disease

The faecal microbiota of subjects with active celiac disease is associated with a greater microbial diversity compared to the healthy subject, with the expansion of *taxa Bacteroides*, *Prevotella*, *Clostridium* and *Staphylococcus* and a significant decline of bacteria with anti-inflammatory and mucosa protection activities such as *Bifidobacterium* and *Lactobacillus*. It has been proposed that this dysbiosis may play a role in sensitization to gluten and subsequent inflammation (12).

Clostridium difficile infection

The susceptibility to *Clostridium difficile* infection generally depends on a very pronounced dysbiosis, with a reduction in the number of microbiota species and microbial complexity and profound changes in the overall composition of the same (13-15). There is generally a depletion of both the bacteria that are part of the *core microbiota* and of *minor players* like bacteria producing short-chain fatty acids, non-pathogenic clostridia, *Alistipes* and *Bilophila*, which can play a central role in preventing the colonization of intestine by *C. difficile* (13-15). Moreover, *Clostridium difficile*

colitis is associated with a reduced representation of bacteria able to metabolize biliary acids, resulting in a misregulation of the entero-hepatic circle of these substances (13-15). The resulting alterations of gastrointestinal lumen milieu favour the expansion of toxinogenic *Clostridium difficile* populations. These changes are generally caused by prolonged antibiotic therapies and are more pronounced in the elderly subject with multimorbidity (13,16). An additional role could be played by non-antibiotic drugs, such as proton pump inhibitors (PPI), that have a recognized effect of modification of gut microbiota composition and are linked to increased risk of *Clostridium difficile* colitis in some studies (17). The fecal microbiota transplatation, counteracting the extreme intestinal dysbiosis associated with infection and contributing to restore a favorable biochemical milieu in the enteric lumen, is able to clinically prevent recurrences and to significantly modify the natural history of the infection (18).

Colon cancer

Alterations of the intestinal microbiota could play a role in promoting tumorigenesis at the level of the colon (19). However, these alterations probably involve only minor modifications, confined to some species with pathogenic properties and ability to locally invade the intestinal mucosa, without causing acute infectious diseases. Among these modifications, the expansion of *Enterococcus faecalis*, *Bacteroides fragilis* and *Streptococcus gallolyticus* could have the greatest relevance (20). Some metabolic products of the *genus Salmonella* are also able to activate the intracellular signaling of β-catenin which promotes epithelial proliferation (21). Furthermore, the depletion bacteria able to produce butyric acid (22) and the interaction between dietary variations and *Prevotella* enterotype (23) could promote the accumulation of metabolites with oncogenic potential in the intestinal lumen. However, at the current literature state-of-art, the role of the microbiome in gastrointestinal tumorigenesis is far from understood, and the microbiota probably represents only a cofactor in the complex pathogenic pathway, rather than a direct oncogenic player.

Esophageal cancer

There are few studies on the effects of microbiota in the development of esophageal cancer. However, it is plausible that alterations of gastric microbiota near the gastro-esophageal junction may contribute to an increased risk of cancer of the esophagus. Moreover, some studies have shown that the esophageal microbiota of patients with Barrett's esophagus is significantly different from healthy subjects (11). However, more research is needed in this field before definitive conclusions can be made.

Gastric cancer

The inflammatory cascade (Correa's cascade), that causes the progression from chronic gastritis to metaplasia to atrophy to gastric cancer, is known to be triggered by *Helicobacter Pylori* infection. However, it is noteworthy that only a minority of patients infected with *Helicobacter Pylori* develop cancer and the bacterium is often absent or present in minimal concentrations in neoplastic lesions. Some studies have shown significant differences in gastric microbiota with *H. pylori* or without *H. Pylori*. Some authors propose a modification of the current model of gastric carcinogenesis focusing the attention on the interactions between microorganisms and gastric mucosa with possible modifications of the gastric microbiota, whose alterations could determine *per se* gastric atrophy. It is possible that the "new" microbiota developing in the stomach in response to low acidity takes part to gastric carcinogenesis with a complementary (or alternative) role to the more virulent strains of *H. Pylori* (e.g. Cag A) (11).

Liver disease

The liver function is strongly influenced by the metabolism of the intestinal microbiota and of the phenomena of endotoxinemia, namely the absorption by the intestinal mucosa of the lipopolysaccharide produced by the microbiota (24-25). In non-alcoholic fatty liver disease a certain degree of gut microbiota

dysbiosis has been demonstrated, with reduced representation of butyrate-producing bacteria, such as *Faecalibacterium prausnitzii*, *Anaerotruncus colihominis* and *Butyrivibrio crossotus*, and increased representation of bacteria such as *Ruminococcus*, *Campylobacter* and *Shigella*, some of which produce the metabolite acetate, which is a lipogenic substrate (26). In alcoholic liver disease, however, a deeper dysbiosis is observed, with depletion of *Bacteroidetes* and increase of *Enterobacteriaceae*. This is linked to the effect of alcohol on the intestinal mucosa, with increased permeability and perturbation of immune function resulting in an altered equilibrium between gut bacteria and immune system (24). Similar alterations of the intestinal microbiota also occur in non-alcoholic cirrhosis of the liver, where an intestinal colonization by bacteria normally present in the oral cavity microbiota is also observed (27). Moreover, in the phases of decompensation of cirrhosis, most complications (spontaneous bacterial peritonitis, hepatic encephalopathy) are related to the intestinal microbiota and to the alteration of intestinal mucosal permeability (24).

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Review

The impact of intestinal microbiota on bio-medical research: definitions, techniques and physiology of a "new frontier"

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Summary. In recent years the metagenomics techniques have allowed to study composition and function of the intestinal microbiota. The microbiota is a new frontier of biomedical research to be explored and there is growing evidence of its fundamental health-promoting activity. The present review gives a synthetic overview on the characteristics and the role of the microbiota in the adult with particular reference to physiology, pathophysiology and relationships with the host and the environment. (www.actabiomedica.it)

Key words: microbiome, dysbiosis, metagenomics, aging, nutrition, exercise

The intestinal microbiota and the metagenomic approach

The human intestinal microbiome is the ensemble of microorganisms (mainly bacteria, but also viruses, fungi, protozoa and Archaea) that physiologically live in symbiosis with the host at the level of the digestive tract (1). The term microbiome is often used interchangeably with microbiota, which, instead, on a purely semantic level, identifies the set of proteins synthesized by such microorganisms (1).

Although there is a growing interest in the study of fungi and intestinal symbiotic viruses (so-called "mycoma" and "viroma"), actually most of the studies have analyzed the bacterial component of the intestinal microbiome. Therefore, in the common scientific language, when we speak of "microbiota" or "microbiome", we substantially refer to all intestinal bacteria, which in the past were designated with the improper terms of "microbial flora" or "resident bacterial flora" (2).

It is an extremely complex microbial community, with ecological characteristics not yet fully understood, including a large number of bacterial species (at least 1100, but some studies have hypothesized that this number exceeds 2000) (3-4). On overall, the intestinal microbiota of a healthy man can contain up to 10^{14} bacteria, with a genome that, in quantitative terms, is about 150 times larger than that of the host organism (3-4). The genome of bacteria hosted in the gastrointestinal tract is usually referred to as the "metagenome" of the host, and its study with sequencing techniques is called "metagenomics". It has been estimated that the entire human intestinal microbiota contributes to the body weight for a quota ranging from 175 g to 1.5 kg (5).

The concentration and the type of bacteria living in the intestinal lumen change according to the anatomical segment considered. In general, bacterial density increases from the proximal sections (duodenum, small intestine) to the distal ones (caecum, colon, sigma). The most represented bacteria are the obligate or optional anaerobes, especially at the colon level. The faecal microbiota is generally considered a reliable estimate of the microbiota present at the level of the lumen of the digestive tract. However, analy-

ses of stool samples are of course inaccurate to detect segment-specific alterations of the gut microbiome. Some techniques have been recently developed for the microbiota determination on intestinal biopsy samples (*mucosa- associated microbiota*) (6). These techniques have the great advantage of examining the microbiota present in a specific segment of the intestine and, therefore, of checking its possible interactions with the mucosa, but obviously they require invasive procedures (gastroscopy, operative colonoscopy) for sample collection. Thus, most of the studies on human intestinal microbiota have been conducted on faecal samples.

Most of the intestinal bacteria cannot be cultivated, even when using the most innovative and sensitive laboratory methods. It is estimated that around 60-80% of the bacterial species physiologically present in the gut microbiota share this characteristic. Thus, the complexity and diversity of the species contained in the human intestinal microbiota have been understood in only very recent years, thanks to the advent of laboratory methods of detection and identification of bacteria that are independent from culture media (*culture-independent*) and non-species-specific (7).

The "classical" microbiological techniques, currently applied until now in all clinical microbiological laboratories of the world, have in fact the great limitation of being partially or totally species-specific. So, in a biological sample with high microbial concentration such as a stool sample, they can only identify a single species or a limited range of species, for which a clinical question is posed, or a group of bacterial *taxa* that share certain biochemical and metabolic characteristics (7)

The culture-independent microbiological techniques, developed during the last decade, are based on the *high-throughput* sequencing of the bacterial DNA, and so fall within the definition of metagenomics. They allow to virtually identify all the bacterial species present in a complex ecosystem, basing on the genetic polymorphisms of some genes common to all prokaryotes and the subsequent comparison with genomic databases for taxonomic identification (8-9).

Namely, the most used technique in current microbiological research is based on the identification of the polymorphisms of the bacterial gene encoding the 16S rRNA (*16S rRNA microbial profiling).* Each 16S rRNA gene sequence detected in a fecal sample is thus assigned to a specific operational taxonomic unit (OTU) basing on the degree of homology with other detected sequences. Then, each detected 16S rRNA gene sequence, corresponding to an OTU, is assigned to a given taxon (i.e. genus and species) or, in case of mismatch, a higher taxonomic level (phylum, class, order, family, genus) by means of bioinformatics analyses, by comparison with known sequences from taxonomic databases (8-9).

These techniques assure considerable advantages in the study of the human microbiota (9):

1. They allow the simultaneous identification of a large number of taxa that permit to understand the complexity and the diversity of the human intestinal microbiota better than any other currently known technique;
2. They allow to identify also bacterial species usually not cultivable or hardly cultivable, thus overcoming many limits of the classical microbiological techniques;
3. They allow the detection of previously unknown bacterial *taxa* in the human microbiota and assign them taxonomically to an order or family with a high degree of precision;
4. They allow us to estimate the relative abundance of the individual *taxa*, providing very important quantitative information to understand the structure of the microbiota.

Therefore, the metagenomics study of the human fecal microbiota have made it possible to clarify a long series of aspects, previously unknown, on its physiology, its interactions with the mucosa of the GI tract and with other organs, its alterations during acute or chronic diseases, its possible role in the pathogenesis of a long series of diseases, not only gastrointestinal (10-11). Furthermore, the scientific community has begun to develop "new generation" techniques for the manipulation of the human intestinal microbiota (12-13), with the aim of verifying the effects on the development and the progress of some diseases, reaching in some cases, as in *Clostridium difficile* enterocolitis, extremely significant results both from the biological and clinical point of view (14).

The study of the human intestinal microbiota represents therefore a "frontier" of translational biomedical

research and a topic of great relevance in medicine. At the date of 14th November 2018, there were 12897 scientific articles on "human gut microbiota" listed in the international database PubMed (https://www.ncbi.nlm.nih.gov/pubmed), including 5072 reviews, as a proof of the extreme relevance of the topic. Of these papers, only 266 (2.06%) were published before the year 2008 while 3199 (24.8%) went back to the year 2017 only.

Despite the huge amount of literature, there are still many areas of uncertainty and the understanding of the role of intestinal microbiota in human physiopathology is still far from being sufficient to significantly affect clinical practice, with probably the only exception of *Clostridium difficile* enterocolitis (13). In particular, there are still several uncertainties regarding the definition of the "normal" microbiota and the consequent clinical interpretation of a certain intestinal microbial profile. Moreover, for many chronic diseases, from IBD to kidney stones, the involvement of the microbiota in the pathophysiology and in the etiopathogenesis is not yet completely clear, although it is fully hypothesized in the light of existing evidence (15).

The physiology of the intestinal microbiota in adult

The first in-depth knowledge on the characteristics and composition of the normal human intestinal microbiota was acquired thanks to the Human Microbiome Project, a population study in which the composition of the faecal microbiota of 242 healthy adults between the ages of 18 and 40 was determined (14). This study has made it possible to clarify that in the human intestinal microbiota 10 bacterial *phyla* are generally represented, even though the large majority of the identified bacteria belong to two of them: *Bacteroidetes* and *Firmicutes*. There is generally an inverse relationship between the relative abundance of the bacteria belonging to one or the other *phylum*, so the subjects that have a microbiota rich in *Firmicutes* have a reduced representation of *Bacteroidetes* and vice versa (14). This project also allowed to clarify other very important concepts in the physiology of adult microbiota (14):

- even if, in the complexity of the microbial population, there is a high inter-individual variability, the most represented *taxa* in faecal samples of the healthy population are a relatively small number and constitute the so-called "*core microbiota*";
- there is a very high number of *taxa*, whose presence is inconstant across different individuals, that could play important metabolic and pathophysiological roles despite the low quantitative representation in absolute terms ("*minor players*");
- the microbiota composition of each individual remains stable over time during the adult life.

Analyzing the composition of the faecal microbiota of 39 healthy adults, Arumugam and colleagues later confirmed the presence of a high inter-individual variability, identifying however the presence of some "enterotypes", that is, groups of individuals characterized by the presence of a very similar *core microbiota*. In particular, enterotype 1 is rich in *Bacteroides spp*. and *Parabacteroides spp.*, enterotype 2 is characterized by a high relative abundance of *Prevotella spp*. and *Desulfovibrio spp*., and enterotype 3 is rich in bacteria with mucin degrading capacity, such as *Ruminococcus spp*. and *Akkermansia spp*. (16). The factors that affect the presence of one or the other enterotype remain largely unknown, as dietary patterns do not seem to have a predominant role except for enterotype 2, where the abundance of *Prevotella spp*. can be positively related to fiber consumption and negatively to the consumption of animal proteins (16).

However, the diet and the place of residence remain two important factors in determining the composition of the intestinal microbiota (17-18). Yatsunenko and colleagues (17) showed significant differences in the composition of the *core microbiota* between two groups of subjects, one resident in Malawi and the other in the United States of America, hypothesizing the presence of environmental factors (diet, food preservation methods, exposure to animals, domestic hygiene) as the reason of these differences.

A diet rich in animal protein can increase the relative abundance of bacteria tolerating the exposure to high concentrations of bile acids (*Bacteroides, Alistipes, Bilophila*), and decrease the relative abundance of bacteria of the *phylum Firmicutes* metabolizing vegetal polysaccharides (*Roseburia, Eubacterium, Ruminococcus*) (18). A mainly vegetarian or vegan diet is instead associated with a greater abundance of the genus *Prevotella*, which is significantly correlated with the dietary fiber

intake (18). Furthermore, a diet with a high content of animal proteins is generally associated with a reduced complexity of the intestinal microbiota, and therefore with reduced microbial diversity (19). Finally, Wu and colleagues have shown that enterotypes, or at least the presence of a *Bacteroides* enterotype compared to a *Prevotella* enterotype, are significantly related to long-term eating habits and not to nutrient intake in the days or weeks preceding the analysis of the microbiota (20). These results partly discard the initial conclusion of Arumugam and colleagues that diet only marginally influences the *core microbiota* and enterotypes (16).

Other studies have analyzed the relationship between diet and human intestinal microbiota focusing only on specific nutrients, without reaching conclusive evidence (21). For example, a diet rich in non-digestible waxes is associated with an increase in the relative abundance of bacteria capable of degrading such compounds, such as *Eubacterium rectale* and *Oscillobacter spp*. (22). A high dietary intake of inulin, a fiber present in some vegetables, and fruit-oligosaccharides is associated with the increase of *Bifidobacterium* bacteria (23-24), while a diet rich in polyunsaturated fatty acids is associated with an increase in relative abundance of *Eubacterium rectale* and *Clostridium coccoides* (25). These changes are generally of little importance in absolute quantitative terms, mainly regarding "*minor players*" in the microbiota. However, they could assume great importance from a metabolic and functional point of view (21).

In fact, an intestinal microbiota characterized by a high diversity ("species richness") is generally considered a marker of good health and it is associated with a lower body adiposity, a greater tendency to maintain body weight over time and a better metabolic profile with reduced insulin resistance (26). Compared to the normal-weight subjects, a reduced microbial diversity is often found in overweight individuals, probably due to different eating habits and lifestyle (26).

Among other factors related to lifestyle, physical exercise also seems to be a determinant of the composition of the intestinal microbiota in healthy adults. Clarke and colleagues have shown that agonistic sport practice is associated with a greater intestinal microbial diversity compared to a sedentary lifestyle, independently of dietary caloric intake and body mass index (19). Furthermore, the peak of oxygen consumption under stress, i.e., cardiorespiratory fitness index, is correlated with the microbial diversity of the intestinal microbiota according to a study performed in a group of Canadian young adults (27). Similar results have also been obtained in studies conducted on animal models (28-30), allowing to hypothesize that at least part of the health benefits of physical exercise are mediated by exercise-related improvement of microbial diversity in the GI tract (31).

Even the events of the infantile age, and in particular the type of childbirth, breastfeeding and the age of weaning, may have significant repercussions on the composition of the adult microbiota. In fact, at the moment of birth, the intestinal microbiota is substantially absent or characterized by extreme simplicity with low bacterial load. The intestine is therefore contaminated with the microbial flora present in the birth canal (for those born by eutocic delivery) or with that present on the maternal skin (for those born by cesarean delivery). Therefore, in the newborn the intestinal microbiota is dominated by *Lactobacillus spp*. if the birth was eutocic or from *Staphylococcus spp*. if the birth was cesarean (32). With breastfeeding, part of the microbiota present on the skin of the mother's breast and part of milk microbiota are transmitted to the infant, contributing to increase the intestinal microbial complexity (33). After weaning, there is a noticeable increase in microbial diversity, with progressive reduction of *taxa* such as *Lactobacillus* and *Staphylococcus* and increase of those *taxa* representing the adult *core microbiota*, such as *Bacteroides* and *Prevotella* (34). At the age of 3 years, the microbiota then reaches a composition that, from a quantitative and qualitative point of view, is very similar to that of an adult (17).

However, special events that occur in childhood, such as diseases and/or exposure to drugs including antibiotics, can induce significant changes in the intestinal microbiota under development, which are maintained over time even in adolescence and adult age (35). Likewise, prematurity can also lead to alterations in the development of the microbiota that are maintained in later adult life (35).

The type of living environment in childhood also plays an important role in shaping the intestinal microbiota. The presence of siblings (36) and domestic

animals (37) is in fact capable of influencing the microbial populations present in the children's microbiota. It has also been shown that people living in the same domestic environment, regardless of age, and even their animals, share some common characteristics in their microbiota (38).

It has also been postulated that genetic factors of the host may influence the type of intestinal microbiota during the development phases. The study by Yatsunenko and colleagues (17) seems to disprove this hypothesis, since it found significant differences in the composition of the intestinal microbiota of mono- and dizygotic twins of different geographic origin. Bonder and colleagues (39) have instead recently demonstrated through genome-wide analysis, conducted on 1514 healthy adults, that some host *loci* are related to the relative abundance of some intestinal *taxa* such as *Bifidobacterium*. These *loci* are related to the function of the immune system and to some receptors or adhesion molecules expressed by the intestinal epithelium.

Two fundamental characteristics of the intestinal microbiota of healthy adults are stability over time and resilience. It is in fact known that, if no perturbative factors are involved, the composition of the microbiota can be estimated as constant from adolescent age up to the age of 60-65 years (40). Indeed, very complex balances are established in the relative abundance of the individual components of the microbiota, which depend on the availability of the metabolic substrates, dietary habits, function of the intestinal mucosa and the activity of the local immune system (11). In these balances, some *taxa* grow to form the *core microbiota*, while others remain confined to some ecological niches, for which they present a relative lower abundance (minor players). These balances are to a certain extent predictable through complex mathematical models that refer to the law of the equilibrium of Nash (41).

When a perturbative event, such as an acute illness, infection, antibiotic therapy, or a sudden change in dietary habits, occurs, the equilibrium changes due to the new factor (41-42). For example, in a study conducted on 10 healthy volunteers, the rapid change of diet (from high protein to vegetarian and vice-versa) caused significant changes in the relative abundance of some *taxa*, which however rapidly disappeared with resoration of steady state when the restrictive diet was suddenly suspended (18). This phenomenon, whereby the global composition of the microbiota tends to return spontaneously to the pre-existing equilibrium, is called *resilience* and is a fundamental characteristic of the intestinal microbiota of healthy adults (41-43).

Because of this resilience, age is generally not a factor influencing the composition of the intestinal microbiota in the range between 10/15 and 60/65 years old (40). In the elderly, however, some physiological changes occur that may have biological and clinical relevance.

Much of the current knowledge on the intestinal microbiota of the elderly comes from the study by Claesson and colleagues, published in Nature in 2012 (44), which analyzed the intestinal microbiota of a group of 178 Irish older subjects, either institutionalized or community-dwelling, followed-up for one year. Briefly, these authors have shown that, over 65 years of age, inter-individual variability increases, while microbial diversity, i.e., the number of species detectable with metagenomics techniques, is reduced. These changes, which may in part derive from changes in dietary habits, are more pronounced in those who exhibit a lower degree of functional autonomy, in those who live in nursing homes (44,45) and in patients with polypharmacy (46). The most interesting finding of these studies is the circumstance that the reduction of microbial diversity and therefore the alterations in the overall composition of the microbiota do not depend so much on the "chronological" age, but rather on the "biological" age or on the functional performance (47). Thus, frailty, the age-related reduction of homeostasis and functional reserve preceding disability, may be significantly associated with the microbiota composition (48).

Further studies have shown that, in healthy older individuals, the *core microbiota* tends to be maintained both qualitatively and quantitatively. Conversely, in frail or institutionalized elderly subjects, a quantitative reduction of the *core microbiota* can be detected, with a simultaneous increase of taxa such as *Anerotruncus*, *Desulfovibrio* and *Coprobacillus*, that can be considered as biomarkers of reduced health status (46).

These changes, which occur slowly over time, are accompanied by a labile equilibrium. Thus, the intestinal microbiota becomes more sensitive to possible per-

turbators and, ultimately, shows a lower resilience (50). In a study performed on a group of 728 elderly women, the Frailty Index, a global measure of fitness, was positively correlated with the relative abundance of species such as *Eggerthella lenta* and *Eubacterium dolicum* and inversely related to *Faecalibacterium prausnitzii* in the intestinal microbiota (50). Moreover, specific alterations in the intestinal microbiota of older individuals seem to be associated with reduced cognitive performances, and even be involved in the pathophysiology of Alzheimer's disease (51).

Aging then results in a reduced relative abundance of a series of bacteria, including bifidobacteria (52-53), whose metabolic activities have been defined as health-promoting (52-53). These alterations can be reflected in a reduced cross-talk between the microbiota and the intestinal mucosa, with greater activation of the local and systemic inflammatory response and less functionality of the cells of the innate immune system, with negative effects not only on the GI tract but also on the whole body (54).

Some studies on the intestinal microbiota of centenarians and supercentenarians have shown that extreme longevity, albeit accompanied by a reduction in intestinal microbial diversity, is associated with the expansion of the representation of bacterial *taxa* with health-promoting activity, such as *Eggerthella, Anaerotruncus, Bilophila* and *Akkermansia*, and *taxa* with still unclear

Table 1. Overview of physiological and pathological factors influencing the composition of the intestinal microbiota in adult subjects

Involved factors	Comment
Physiological factors	
Dietary habits	- Influence on the enterotype – Influence on microbial diversity – Influence on the relative abundance of some taxa by particular metabolic substrates (eg waxes, fibers) or sensitive to different concentrations of bile acids
Geographic origin	- Influence mediated by dietary habits, methods of food storage, exposure to animals, domestic hygiene
Physical activity	- Increase in microbial diversity and in the concentration of health-promoting bacteria
Type of childbirth, breastfeeding/lactation, age of weaning	- They can influence the overall composition of the microbiota in childhood, leaving a fingerprint even in adulthood
Presence of cohabitants and pets	- Over the time the microbiota of people and pets that live in close contact tends to resemble each other in the global composition
Genetic factors	- The presence of some taxa depends on the types of receptors expressed by epithelial cells of the mucosa
Living environment (home vs. institution)	- Reduction of microbial complexity with high inter-individual variability in institutionalized subjects
Age	- The microbiota is stable in adulthood up to 65-70 years – Then there is an increase in inter-individual variability with a reduced number of species and a tendency to dysbiosis
Pathological factors	
Direct exposure (therapy) or indirect (environmental contamination) to antibiotics	- It causes dysbiosis with profound changes in the composition of the microbiota that are not necessarily associated with a decrease in the number of bacteria – Dysbiosis depends on the type of antibiotic taken, the dose and duration of therapy
Chronic pharmacological therapies	- The main evidence is for antiblastic chemotherapy. On overall, polypharmacy is related to dysbiosis
Immunological alterations	- Immunosuppression promotes the growth of pathogenic strains

activity, such as *Oscillospira*, *Odoribacter* and *Butyricimonas*, at the expense of other bacteria with beneficial metabolic activities such as *Faecalibacterium prausnitzii* (55-57). These results allow at least to hypothesize an active role of the intestinal microbiota in the phenomena of aging and in the promotion of longevity, also through the modulation of inflammation (55).

A summary of the main factors involved in modulating the composition of the intestinal microbiota in adult is shown in Table 1.

Conclusions

The study of the intestinal microbiota with metagenomics techniques offers a new point of view for the understanding of human physiology and physiopathology. Growing evidence suggests a significant role of the microbiota in the maintenance of the homeostasis of the body and in helping to determine the state of health or illness. Biomedical research in the near future will have to focus on clarify microbiota-host relationships and on planning microbiota manipulation to prevent and possibly modify the natural history of many diseases.

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Review

The role of diet in the prevention and treatment of Inflammatory Bowel Diseases

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Summary. Inflammatory bowel diseases (IBD) – Crohn's disease (CD) and ulcerative colitis (UC) – are chronic conditions characterised by relapsing inflammation of the gastrointestinal tract. They represent an increasing public health concern and an aetiological enigma due to unknown causal factors. The current knowledge on the pathogenesis of IBD is that genetically susceptible individuals develop intolerance to a dysregulated gut microflora (dysbiosis) and chronic inflammation develops as a result of environmental triggers. Among the environmental factors associated with IBD, diet plays an important role in modulating the gut microbiome, and, consequently, it could have a therapeutic impact on the disease course. An overabundance of calories and some macronutrients typical of the Western dietetic pattern increase gut inflammation, whereas several micronutrients characteristic of the Mediterranean Diet have the potential to modulate gut inflammation, according to recent evidence. Immunonutrition has emerged as a new concept putting forward the role of vitamins such as vitamins A, C, E, and D, folic acid, beta carotene and trace elements such as zinc, selenium, manganese and iron. However, when assessed in clinical trials, specific micronutrients showed a limited benefit. Further research is required to evaluate the role of individual food compounds and complex nutritional interventions with the potential to decrease inflammation as a means of prevention and management of IBD. The current dietary recommendations for disease prevention and management are scarce and non evidence-based. This review summarizes the current knowledge on the complex interaction between diet, microbiome and immune-modulation in IBD, with particular focus to the role of the Mediterranean Diet as a tool for prevention and treatment of the disease. (www.actabiomedica.it)

Key words: mediterranean diet, IBD, Crohn Disease, ulcerative colitis, inflammation, nutrition, gut inflammation, micronutrients

Introduction

Inflammatory bowel disease (IBD) has been a global healthcare problem with a sustained increasing incidence over the last decades (1). IBD includes two major forms: Crohn's disease (CD) and ulcerative colitis (UC), which are distinct chronic bowel-relapsing inflammatory disorders. CD causes transmural inflammation and can affect any part of the gastrointestinal tract (most commonly the terminal ileum and the colon) in a non-continuous pattern. CD is commonly associated with complications such as abscesses, fistulas, strictures and perianal involvment. In contrast, UC is characterized by mucosal inflammation and it is organ-specific, affecting only the colon (2). Although the etiology of IBD remains largely unknown, recent

evidence indicates that individual's genetic susceptibility, environment, intestinal microbial flora and immune responses are all factors involved and functionally integrated in the pathogenesis of IBD (3-5). The incidence of IBD has steadily increased in industrialised countries over the 20th century. In developing countries, traditionally considered low-incidence areas, an increasing incidence has been described since the beginning of the 21st century (8-10,14). Studies on migrant populations moving from regions of low incidence to areas with a high IBD incidence point to early life as a key time for environmental triggers (18). In these populations, the second generation, i.e., those born in high-incidence areas, have been shown to have higher incidence rates compared to their parents. More recently, the fast evolving field of epigenetics has offered new explanations on the mechanism by which environmemntal changes induce pathological gene expressions and determine cell phenotype and function in IBD (9). The identification of IBD environmental risk factor remains a subject of intensive research, and diet is one of the best candidates. In fact, diet participates in the regulation of intestinal inflammation, either directly or indirectly by modifying the gut microbiota (6,7). The purpose of this review is to define the role of diet in the pathogenesis and management of IBD, explaining the potential functional role of the Mediterranean Diet in preventing and treating Inflammatory Bowel Disease. The anti-inflammatory potential of Mediterranean Diet is wellknown and established in several other health conditions associated with inflammation. However, the abundance in fibers of this dietary pattern could make it unsuitable for patients with gut inflammation. MD, with appropriate adjustments, is the better dietetic solution in IBD, as indicated by recent evidence.

Gut microbiota and IBD

The microbiota plays a vital role in the health of the host. In fact, it controls the proliferation of pathogenic bacteria present in the intestinal tract (such as *Clostridia* or *Colibacillacea*) (ref. 5,10), stimulates the immune system, regulates the absorption of nutrients (11), regulates the host metabolism and hysiology (12), intervenes in the production of vitamins and enzymes such as vitamin K and biotin6, and in the synthesis of compounds useful for the trophism of the colonic mucosa and required for cell renewal (5,10). Within the gut, the microbiota plays different roles, including the fermentation of amino acids and saccharides, the production of short-chain fatty acids (SCFAs), succinate, ethanol, H2, amines, lactate, phenols, thiolsand indoles, disposal of hydrogen (as acetate H2S andmethane), the degradation of undigested proteins and carbohydrates, and the transformation of bile acids (8). The production of SCFAs, including butyrate and propionateand acetate, plays an essential role in maintaining a healthy mucosa and in the production of anti-inflammatory interleukins (5,11). In brief, SCFAs are derived from the bacterial fermentation of dietary carbohydrates. This fermentation process in carried out in the colon by *Lactobacilli* and *Bifidobacteria* under anaerobic conditions (5). Moreover, butyric acid is the preferred source of energy for colonocytes. Butyric acid has an importantanti-inflammatory effect and controls the proliferation, differentiation and apoptosis of colonocytes. In addition, it strengthens the defensive barrier of the colon by increasing the production of mucin and antimicrobial peptides and by decreasing the intestinal epithelial permeability and increasing the expression of tight junction's proteins (5). Another important function of the microbiota is to keep the concentration of pathogens within limits. This action is accomplished via various mechanisms including direct competition for nutrients, increase of the mucus layer on the intestinal mucosa and through the development and the stimulation of the immune system, particularly of the gut associated lymphatic tissue (GALT) (ref.13). Targeted studies have shown that consumption of large amounts of fat and sugar in the long run, results in a degree of dysbiosis and in a change of microbiota, with increased numbers of *Bacterioides spp* and *Ruminococcus torques (*22).

Any alteration of the bowel eubiosis or in the composition of the microbiota is defined as dysbiosis. IBD is associated with alterations in the composition of the intestinal microbiota, characterised by decreased diversity, reduced proportions of Firmicutes, and increased proportions of Proteobacteria and Actinobacteria (6). Some of the bacterial species with pro-

inflammatory effect are enriched in patients with IBD (Escherichia, Fusobacterium), while anti-inflammatory species (Faecalibacterium, Roseburia) are largely reduced in IBD (6). For example, patients with active IBD have been shown to have a lower abundance of *Clostridium coccoides, Clostridium leptum, Faecalibacterium prausnitzii and Bifidobacterium* (24). Prospective studies investigating the role of microbiome changes on the disease course have been scarce. A Dutch study based on 10 CD and nine UC patients reported patient-specific shifts in the microbial composition, but could not demonstrate general changes in the microbial composition or diversity (25). A Spanish study followed up 18 UC patients over the course of one year; in those who remained in remission *Faecalibacterium prausnitzii* increased steadily, while in those who relapsed it did not (26).

Probiotic preparations contain living bacteria have been suggested to exert positive health effects on the human intestine, modulating mucosal permeability and strengthening the immune system and keeping away pathogens from the intestinal mucosa surface. In particular, animal research has suggested that *Lactobacillus and Bifidobacteria* produce substances which are harmful for Gram-positive and Gram-negative bacteria and compete with pathogenic bacteria (32,38). Moreover, human studies suggested that Clostridium coccodies and C. leptum exert a protective effect against IBD (32). Recently, a meta-analysis based on 22 randomised control trials compared the use of probiotics over 5-aminosalicylates (5-ASAs) and placebo in patients with IBD (40). Overall the meta-analysis suggested that probiotics may be as effective as 5-ASAs in preventing a relapse of quiescent UC. However, the overall efficacy of probiotics in IBD should be confirmed by further research. In addition, long-term benefits of probiotics may be limited without an overall modification of the patient's diet. Thus, single pre-/probiotic administration may not prove useful outside the context of switching to an overall healthy diet plan. Probiotics and other commercial interventions such as tea or berry extracts would be unlikely to counteract an unhealthy diet and, used alone, may, analogous to other medicinal products such as antioxidant supplements, fail to determine primary or secondary disease prevention (41). Dietary composition has shown to affect the microbiota balance; therefore, it is conceivable that altering the diet can have an impact on the inflammatory response (28). In contrast, high-fibre diet regimens increase short-chain fatty acid production by the microbiota and lead to an improved energy expenditure (31). More effort should be put into evaluating complex lifestyle and nutritional approaches for modulating the gut microbiome. Much work remains to be done before understanding whether the effect of dysbiosis in humans reaches that of mice; however, while definitive evidence may be lacking, current evidence strongly suggests that the gut microbiome is a major contributor to human health and the development of disease (32).

Immunity and IBD

The composition of the microbiota depends on several factors including the structure of the host's intestinal epithelium, peristalsis, dietary changes, age, genes, temperature, interaction between different bacterial species, response of the immune system in particular T and B cells, administration of antibiotics, radiations and chemotherapy drugs, and psycophisical stress (5,6,14). Consequently, dysbiosis causes an alteration of intercellular tight junctions that are responsible for keeping the integrity of the intestinal mucosa (15) and its permeability, which is crucial to prevent the access of pathogens (5). The entrance of pathogens determines an activation of the MALT (Mucosal Associated Lymphatic Tissue) and consequently of the inflammatory cascade (leukocytes, cytokines, TNF-α), leading to tissue damage (15). Dysbiosis is also related to the development of a number of diseases including type 2 diabetes mellitus, allergies, fatty liver disease, obesity and IBD (7). There is also discussion on the potential role of heat shock proteins (HSPs) in the pathogenesis of IBD. HSPs are involved in various processes such as folding, translocation and degradation of intracellular proteins under normal and stressful conditions. Being highly conserved molecules with similar sequences in bacterial and human orthologs (molecular mimicry), HSPs can stimulate an immune response, both innate and adaptive, therefore having a role in the autoimmune response (5,21). Several mi-

cronutrients are especially important for immunonutrition, in particular vitamins A, C, D and E, folic acid, beta carotene and trace elements such as zinc, selenium, manganese and iron have gained much research interest. Deficiencies in zinc and vitamins A and D may reduce the natural killer cell function, whereas supplemental zinc or vitamin C may enhance their activity (66,67). Vitamin D has been shown to play a role in the intestinal defence by suppressing the microbial invasion of the epithelium. Vitamin D deficiency has been identified in 82% of IBD patients, compared to the 31% national average, and has been linked to defective epithelial processes. Therapy targeting vitamin D3 signalling was suggested for the treatment of inflammatory diseases, affecting both innate and adaptive immune functions. The overall impact of vitamins on IBD is still not well understood. So far, only two randomised clinical trials were conducted to evaluate the effect of vitamin D supplementation on IBD outcomes. In a Danish study, 94 patients were randomised to receive oral vitamin D3 or a placebo; patients receiving vitamin D3 had a non-significant reduced risk of relapse (68). A more recent Iranian study conducted among 108 IBD patients reported that oral supplementation with vitamin D3 reduced serum TNF-alpha levels, though not substantially (69). In mice, Ananthakrishnan and colleagues demonstrated that a deficiency of vitamin D was associated with an increased risk of colitis, whereas Vitamin D supplementation had an anti-inflammatory effect in mice with colitis, due to the inhibition of pro inflammatory genes such as TNF genes (16). In addition, the intake of PUFA and conjugated linoleic acid (CLA) appears to have multiple benefits in IBD patients because they have anti-inflammatory effects (15). In fact, they decrease the production of interferon-γ and prostaglandin E2 (17) and modify the responsiveness of T cells (15). More studies with larger samples would be beneficial to assess the effect of vitamins supplementation in IBD. The role of trace elements in the prevention and management of inflammatory diseases represent another important field of research. Zinc is involved in the control of DNA replication and transcription and controls signal transduction during T-cell activation (70). Selenium deficiency decreases antibody production, while selenium supplementation enhances T-cell responses and increases antibody synthesis. Selenium is also known to exert antioxidative effects and protection against the deteriorating effects of reactive oxygen species (71). Iron deficiency leads to defective T-cell proliferative response and impaired cytokine production by lymphocytes. It should be noted that iron supports pathogen development and consequently that iron supplementation can also result in an increased susceptibility to infections (72). Of note, dietary iron has also been shown to enhance IBD and carcinogenesis by augmenting oxidative and nitrosative stress. In an experimental animal study, an iron-enriched diet significantly increased colorectal tumour incidence as compared with the control diet (73). Despite the fact that micronutrient deficiencies may theoretically influence the immune system and predispose to the onset and development of IBD, further research is needed to define optimal micronutrient levels and specific therapeutic implications (28). Beyond micronutrients, specific food compounds such as green tea (74-76) or Echinacea (77-79) have also been suggested to reduce or enhance immune stimulation and play a role in IBD prevention.

Epigenetic and IBD

In recent years research has contributed to an improved understanding of the role of epigenetic modifications – i.e., non-coding RNAs and DNA methylation – in defining the molecular basis of IBD (42,43). Such research has been largely driven by observations that genetics alone cannot explain the onset of IBD. Thus, a meta-analysis of GWAS studies estimated that susceptibility loci for UC explained only 16% of UC heritability (44). In this regard, gene-environment interactions have been suggested to play an important role in IBD pathogenesis and this is where epigenetics could offer new insights beyond genetic research (42,45). Epigenetic factors were therefore suggested to mediate interactions between the environment and the genome, thereby providing new insights into the pathogenesis of IBD (46). Earlier studies reported a differential expression of specific microRNAs in the colonic mucosa of IBD patients compared to the mucosa of control patients (43). miRNAs identified in

peripheral blood were additionally suggested as new biomarkers of disease development (47). Recent data showed the implication of miRNAs in the immune response to bacterial invasion and in the differential regulation of cytokines (48). miRNA dysregulation, especially in Th17 cells, has been implicated in IBD. miRNAs have also been shown to regulate the intestinal barrier integrity in UC. As previously reviewed, an increased expression of miR-21 is among the most consistently replicated novel therapeutic targets (47,48). More recently, DNA methylation signatures for UC and CD have been also described. However, whether changes in DNA methylation systematically correlate with gene expression is not clear (49). In addition, it remains a challenge to identify aetiologically significant epigenetic alterations since epigenetic modifications of DNA may differ between tissues, time of development within the same tissue, and environmental influences. Initial evidence arising from epigenetic research is sometimes hard to prove in clinical practice. An example is the identified role of cytokines and subsequent development of biological drugs that fail to prove an important role in disease control. Dysregulation of cytokine genes and increased mRNA levels of cytokines, including interleukin1-beta, interleukin-18 and tumour necrosis factor-alpha (TNFα), were reported in IBD patients compared with controls in the late 1990s (50-53). This led to the introduction of biologically based therapies (i.e., anti-TNFα) in IBD patients. However, predicting drug response and achieving adequate response levels is still difficult in many patients as stand-alone anti-TNFα therapies have not proven completely efficacious in preventing disease progression in many patients (54). Recently, animal models suggested that the lack of response to anti-TNFα could be related to differences in the gut microbiome composition. Thus, alternative strategies are needed to account for the interplay between immunity, epigenetics and dietary factors. Diet is known to influence epigenetic changes associated with various diseases and to modify gene expression patterns in a state of disturbed immunity (45). Poor dietary choices are encoded into the human gut and into the genetic make-up, and could be transferred to the offspring (55). A number of nutrients have been shown to modulate immune responses and may potentially counteract inflammatory processes (56). Recent research suggested secondary plant metabolites, such as polyphenols, may modulate gene expression, chromatin remodelling and DNA methylation (57). Polyphenols in green tea or soybean such as epigallocatechin-3-gallate or genistein have been demonstrated to inhibit DNA methyltransferases activity. Epigenetic effects have also been shown for other dietary components such as curcumin (58). Nutrition provides substrates necessary for DNA methylation and can regulate the activity of the enzymes involved in the one-carbon cycle. Thus, precursors of S-adenosylmethionine, such as methionine, folate, choline, betaine and vitamins B2, B6 and B12, have been suggested to influence DNA methylation patterns (59). Immune cells are rapidly dividing cells and have increased sensitivity to impaired DNA replication. Dietary factors appear to have the potential to modulate inflammation (60). Furthermore, active immunisation against the outer membrane protein of bacteria present in the gut was recently shown to enhance local and systemic immune control via apoE-mediated immune-modulation (61). Immunonutrition was therefore suggested as a less invasive alternative to immunotherapy in protecting against chronic inflammation predisposing to IBD (60). The gut microbiota may alter host histone acetylation and methylation in human colon tissues (62). Fermentation end products, especially short-chain fatty acids such as acetate, butyrate and propionate, which are mostly produced by microbial fermentation of fibres, may be particularly important for the epigenetic regulation of inflammatory reactions (62). A diet poor in fibre leads to a suppression in the microbiota-driven short-chain fatty acid production and to disturbed chromatin effects (62). Of note, the previously mentioned finding that butyrate-producing bacteria (Faecalibacterium) and SCFA-producing bacteria (Roseburia) are decreased in IBD (6). However, the therapeutic role of butyrate-producing bacteria as pharmabiotics in humans has been questioned because of the difficulty of growing them in vitro (63). The current state of research does not allow to make definitive statements on which exact changes in the diet affect epigenetics via the microbiota. Nevertheless, there is accumulating evidence that certain microbes communicate with their hosts by sending out metabolites, influencing gene transcrip-

tion in the colon and potentially driving disease development (see figure 1 online).

Diet and IBD

The Western Diet Pattern

The current diet is considerably different from the traditional diet of previous generations, when the prevalence of IBD was considerably lower. The Western diet pattern is dominated by increased consumption of refined sugar, omega-6 polyunsaturated fats and fast food, combined with a deficiency in fruit, vegetables, and fibers (72). Much of today's food supply has been processed, modified, stored and transported over great distances, in contrast to the traditional diet, where food that was produced locally was consumed shortly after harvest. This shift to the Western dietary pattern is hypothesized to increase pro-inflammatory cytokines, modulate intestinal permeability, and alter the intestinal microbiota promoting a low-grade chronic inflammation in the gut (73). A diet that contains pro-inflammatory foods is an important risk factor in the development of UC. A case-control study carried out in Iran with newly diagnosed UC patients (n=62 UC patients, 124 controls) found that subjects with a higher dietary inflammatory index (pro-inflammatory diet) had an increased risk of developing UC (Odds Ratio (OR): 1.55, 95% Confidence Interval (CI): 1.04-2.32) (23). The authors concluded that encouraging the intake of more anti-inflammatory dietary factors, such as plant-based foods rich in fibers and phytochemicals, and reducing the intake of pro-inflammatory factors, such as fried or processed foods rich in trans-fatty acids, could be a potential strategy for reducing the risk of UC. This was one of the first studies that examined the role of a dietary inflammatory index in the development of UC. Several large scale studies have attempted to elucidate the dietary components that are associated with the risk of developing IBD (22,24,26,27). Overall, these studies suggest that the Western diet pattern is a risk factor for IBD. If we compare the Western diet to Eastern diets based on carbohydrates derived from plants, vegetables, rice and fruits, we note that the Eastern population microbiota has a higher prevalence of *Prevotella spp.* rather than *Bacteriodes spp.* compared to the Western population (8,16). Furthermore, animal sources of protein and fat are associated with a greater number of *Bacteriodes spp.*, while simple carbohydrates and fibers are mostly associated with an increase of *Prevotella spp. (*8,16). On the other hand, while the bacterial fermentation of carbohydrates produces SCFAs that maintains a healthy intestine, the fermentation of protein residues produces metabolites such as organic acids, phenolic compounds, indoles and ammonia, which are deleterious and toxic for the intestine (8). It is demonstrated that diets high in fat and/or sugar destroy the intestinal microbiota, leading to dysbiosis and increased production of endotoxins (23). Dysbiosis modifies the intestinal mucosa, which becomes thinner and more permeable to pathogens and antigens with the consequent establishment a low-grade but persistent, inflammation (22). In contrast, a diet rich in vegetables and fibers reduces the intestinal pH and prevents the growth of potential pathogenic bacteria such as strains of *E. coli* and other *Enterobacteriaceae (*10). In brief, the literature is in support of the fact that the microbiota is intimately related to food quality and that diet influences the composition of the microbiota and represents a source of luminal antigens (15). In a review of 2015, Tomasello and colleagues noted that a Western-style diet may be a trigger for UC and CD (15).

Carbohydrate Intake as a Risk Factor for IBD

A systematic review (n=19 studies with 2609 IBD subjects) reported a negative association between dietary fiber (OR 0.12, 95% CI: 0.04-0.37) and fruit intake (OR: 0.2, 95% CI: 0.1-0.9) and CD risk (22). Soluble fiber from fruit may have a protective effect on CD (24). A high vegetable intake may be associated with a decreased risk of UC (OR range 0.32-0.75) (22). The European Investigation into Cancer and Nutrition study (n=366,351 with 256 incident cases of UC and 117 of CD, and four matched controls per case) reported that an increased consumption of sugar and soft drinks with a low vegetable intake was positively associated with the risk of UC (OR 1.31, 95% CI: 0.85-2.02; p=0.05) (25). An increased consumption of sweets is positively associated with CD (OR:

2.83, 95% CI: 1.38-5.83) and UC (OR: 2.86, 95% CI: 1.24-6.57) (30). Overall, these data suggest that while refined and processed carbohydrates and sweetened beverages are risk factors for IBD, complex carbohydrates including fruit, vegetables and fibers should be included in the diet to improve the management of IBD.

Protein Intake as a Risk Factor for IBD

Similarly, according to Agus and colleagues (22), an excessive consumption of animal proteins is associated with an increased risk of developing CD; while the consumption of fruit and vegetables was inversely related to the risk of CD (15). Patients with CD also showed a shift in the microbiota, with an increase of *Proteobacteria* and *Bifidobacteria* groups, and a decrease of *Firmicutes (*22). For UC, in addition to the large consumption of refined carbohydrates and simple sugars, the consumption of large amounts of fatty acids is also associated with an increased risk of the disease (17). A large prospective cohort study (n=67,581) completed over a 10.5 year period found that a high protein intake, specifically animal proteins (meat, not dairy products) was positively associated with an increased risk of IBD (31). A systematic review (n=2609 IBD patients; 19 studies) reported an association of a high total protein intake with the development of UC (OR range 0.2-3.7) and CD (OR range 0.45-3.34) (22). A high protein intake was associated with a 3.3-fold increased risk of IBD, suggesting that a diet high in animal proteins could be a major risk factor for the development of IBD.

Dairy Intake as Risk Factor for IBD

The European Investigation into Cancer and Nutrition study found that individuals that consumed milk had significantly reduced odds of developing CD (OR: 0.30, 95% CI: 0.13-0.65), suggesting a protective effect of dairy product consumption (28). Individual dairy products consisted of milk, yogurt, and cheese with variable fat content (e.g., full fat, skimmed, semi-skimmed, and unspecified). This is supported by a case-control study in children (n=130 CD patients andn=202 controls) that demonstrated that the consumption of dairy products was not associated with CD (OR: 0.86, 95% CI: 0.42-1.76, p=0.65) (29). Overall, the consumption of dairy products is not a risk factor for IBD.

Fat Intake as a Risk Factor for IBD

There have been conflicting data on the association between dietary fat intake and the development of IBD, as many of the studies are retrospective with small numbers. However, a very large, long-term, prospective study (n=170,805) completed over 26 years did not observe a significant association of total dietary fat intake, saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) intake with an increased risk of developing CD or UC (26). These findings have been supported by other research studies (74-76). A growing body of scientific evidence indicates that the Mediterranean diet pattern has been associated with significant improvements in health status (77,78) and decrease in inflammatory markers in humans (79). The protective effect is hypothesized to derive from the balance in fats, which includes incorporating MUFA, SFA and fish intake (80). While a few studies show that MUFAs are beneficial in colitis, studies on the effects of SFA and PUFAs on gut health are controversial. Dietary n-6 PUFA, in particular linoleic acid, has been implicated in the etiology of IBD. Dietary n-6 PUFAs are essential fatty acids present in high amounts in red meat, cooking oils (safflower and corn oil) and margarines. A prospective cohort study (n=203,193) conducted over four years found that intake of linoleic acid was associated with an increased risk of UC (OR: 2.49, 95% CI: 1.23 to5.07, p=0.01) (27). Further analysis of the European Investigation into Cancer and Nutrition study (n=260,686) over five years found an increased risk of UC with a higher total PUFA intake (trend across quartiles OR=1.19 (95% CI: 0.99-1.43) p=0.07) (74), which was also supported by a systematic review (n=2609 patients with IBD) that examined pre-illness intake of nutrients and subsequent development of UC (22). A case-control study in CD found that increased total PUFA consumption was positively associated with CD risk (OR: 2.31, 95% CI: 1.12-4.79) (30). The Nurses' Health Study cohorts (n=170,805 women with 269 incident cases

of CD and 338 incident cases of UC) reported that a high, long-term intake of trans-unsaturated fatty acids was associated with a trend towards an increased incidence of UC (HR 1.34, 95% CI: 0.94-1.92) but not CD (26). An increased relative risk of developing IBD has also been associated with a frequent intake of fast foods (fast foods are high in trans-unsaturated fatty acids) (81,82). The relative risk associated with the consumption of fast foods at least two times a week was estimated at 3.4 (95% CI: 1.3-9.3) for CD and 3.9 (95% CI: 1.4-10.6) for UC (82). Frequent fast food intake, defined as more than once a week, was significantly associated with a risk of UC (43%, OR: 5.78, 95% CI: 2.38-14.03) and CD (27%, OR: 2.84,95% CI: 1.21-6.64) (81). It has been speculated that the intake of long-chain n-3 PUFAs (docosapentaenoic acid, eicosapentaenoic acid, docosahexaenoic acid), known as omega-3s, may be of benefit in patients with IBD. The beneficial effects are believed to derive from the anti-inflammatory properties of n-3PUFAs; however, clinical and experimental studies have shown conflicting results (83). Various meta-analyses failed to show a benefit of the supplementation with fish oils in the maintenance of remission in CD and UC (84-86). The dietary intake of n-3 PUFAs was inversely associated with risk of UC, whereas no association has been found with CD (26). The European Investigation into Cancer and Nutrition study (n=203,193) found a negative association of increasing dietary intake of n-3 PUFA, specifically docosahexaenoic acid, with the development of UC (OR: 0.23, 95% CI: 0.06 to 0.97) (27). This is supported by the European Investigation into Cancer and Nutrition-Norfolk study findings (n=26,639)(OR: 0.43, 95% CI: 0.22-0.86) (57). Two case-control studies in CD report that a diet with a regular consumption of fish had a protective effect on the development of CD (OR 0.52, 95% CI: 0.33-0.80,p=0.003) and (OR 0.46, 95% CI: 0.20-1.06, p=0.02) (29,69). The total ratio of n-3 PUFA: n-6 PUFA found in the diet has been hypothesized to be an important issue. One prospective cohort (87) and one case-control study (29) report that a high n-3PUFA:n-6 PUFA ratio in the diet is inversely associated with the risk of IBD. In support of this finding, a dietary intervention trial that focused on increasing the n-3 PUFA: n-6 PUFA ratio was found to be effective in maintaining disease remission in patients with both UC and CD, through increasing n-3 PUFA intake (88). Overall, it does not appear that full fat diets should be avoided. Fat-containing diets rich in olive oil, dairy products and fish but not fish oil pills should be consumed while avoiding large intakes of vegetable oils rich in n-6 PUFA. In summary, several epidemiological studies provide compelling evidence on the role of food in the pathogenesis of IBD. Furthermore, the rise in incidence of IBD in countries that previously had a very low incidence suggests that industrialization and the adoption of a westernized diet may be risk factors for the development of IBD. A reduced consumption of fruits and possibly vegetables, resulting in a reduced overall intake of fibers, with high intake of meats, fast foods and trans-fatty acids appear to be associated with an overall increase in the risk of developing IBD (71).

Dietary Patterns for IBD Management and Prevention

Despite years of research, the role of diet in the prevention and management of IBD is not well understood (80-82). Overall, no effort has been made to provide evidence-based nutritional guidelines for IBD patients and the existing nutritional advice largely follows the principle "If it hurts, don't do it". Dietary recommendations include patient advice to self-monitor and avoid foods that may worsen symptoms, eating smaller meals at more frequent intervals, drinking adequate amounts of fluids, avoiding caffeine and alcohol, taking vitamin/mineral supplementations, eliminating dairy if lactose intolerant, limiting excess fat, reducing carbohydrates and reducing high-fibre foods during flares. Mixed advice exists regarding pre-/probiotics. Recommendations are different across regions/countries. For example, enteral nutrition is recommended for Crohn's disease patients in Japan, which differs from practice in the USA (82). A potential reason for the lack of solid dietary recommendations is the scarcity of studies evaluating the role of diet in IBD (83). So far, only one study has assessed nutritional factors and their influence on disease outcome in newly diagnosed IBD patients (84). In this inception cohort study, a high intake of caffeine

was associated with an increased risk of surgery, a severe disease course and need for higher treatment steps in CD patients; in UC patients, daily fast food intake was associated with an increased risk of surgery and high intake of caffeine was associated with a higher risk of extra-intestinal manifestations. In an attempt to fill this gap, in recent years more effort has been made into evaluating specific diets for the management of IBD, such as the Specific Carbohydrate Diet (SCD) and the low fermentable oligosaccharides, disaccharides, monosaccharides, and polyol (FODMAP) diet. Exclusive enteral nutrition is recommended as a first-line therapy to induce remission in children with active luminal CD (85). In adults, long-term diet interventions such as total parenteral nutrition or an elemental diet have also shown promise (86); however, their administration is complicated and does not allow patients to lead a normal life. The SCD is a dietary regime that aims at inducing and maintaining drug-free remission in patients with IBD, initially developed by gastroenterologist Sidney Haas in 1951 and later popularised by biochemist Elaine Gottschall in the book Breaking the Vicious Cycle: Intestinal Health Through Diet (87). The SCD diet is based on the hypothesis that patients with IBD and other intestinal diseases present a dysfunction of the disaccharidases, which are necessary to digest and absorb disaccharides and amylopectin; therefore, higher amounts of disaccharides would be present into the colon, which may lead to bacterial overgrowth and bowel injury with increased intestinal permeability. The diet allows carbohydrate foods consisting of monosaccharides only (fruit and honey) and excludes disaccharides and most polysaccharides; moreover, it includes vegetables with a high amylose-to-amylopectin ratio, fruits, nuts, solid proteins and fats (87). So far, several case-series studies have suggested an important potential of the SCD diet in the induction and maintenance of remission in IBD 65,88-90). The low FODMAP diet gained much attention in research as a mean for IBD treatment. A recent meta-analysis including two randomised control trials and four before-after studies with a total of 319 patients (96% in remission) reported an overall improvement in gastrointestinal symptoms such as diarrhea, abdominal bloating, fatigue and nausea (91). Recently, plant-based dietary patterns were suggested as valid means of long-term inflammation control (92). Semi-vegetarian diet (SVD) has been shown to exert a preventive effect against IBD relapse in patients who have achieved remission in a prospective, single-centre, two-year clinical trial (95). In particular, the Mediterranean diet has been suggested to exert a strong immunomodulatory effect.

Mediterranean diet in IBD

One diet pattern that is considered useful for the prevention and management of intestinal diseases is the Mediterranean diet. It is characterized by a high intake of fruit and vegetables (rich in fiber, antioxidants and vitamins), olive oil and oily fish (rich in mono and polyunsaturated fatty acids), and whole grains and nuts (15,24). It is based on the daily or weekly consumption of specific food groups according to the standardized food pyramid (24). Current research on probiotics as a food supplement in addition to a mediterranean diet, showed that probiotics (such as *Lactobacillus Rhamnosus*) change the composition of the microbiota, thus allowing the return to eubiosis (5). Prebiotic foods, which contains soluble fibers, have been shown to help in maintaining intestinal eubiosis (25). Prebiotics, for example inulin, are metabolized by the gut microbiota to form SCFAs including butyric acid. Tralongo et al. showed that butyrate had a positive effect on the physiological activity of colonocytes and that it had an anti-inflammatory effect (expressed by reducing the production of pro-inflammatory factors such as Nf-kb) making it a valuable ally in the treatment of IBD (5). Recent data from the Predimed study, a randomised, controlled, parallel trial in high cardiovascular risk volunteers, revealed that over five years the Mediterranean diet was associated with the methylation of genes related to inflammation and exerted high regulatory effects (93). Further intervention trials utilising transcriptomics analyses revealed the potential of the Mediterranean diet in the modulation of gene expression and in the normalization of the microbiota in IBD patients (94). Due to the high amount of fibres, MD can be unsuitable for patients during flares of the disease, but it is highly recommended after remission, with appropriate adjustments. In the following section, each main food of the Mediterranean

diet pattern is considered and adapted to be used in the daily diet of patients with IBD (see figure 2 online).

Pulses

Excellent source of vegetal proteins, minerals (calcium, iron, zinc, phosphate) and vitamins (B1, B9, B3), pulses can be consumed as skinned. Skinned pulses are free from the insoluble metanogenic fraction of their fiber and from anti-nutritive compounds such as phytates, but still contain soluble fibers, vitamins and minerals. Soluble fibers, such as pectin and inulin, do not irritate the gut lining, but still they have a prebiotic action, promoting the growth of microbial species that produce propionic and butirric acid. Propionic and butirric acid exert a high protective action on the gut mucosa, and are often supplemented in IBD patients. In particular, recent works show that butirric acid down-regulates the release of inflammatory cytokines and the activation of nuclear factor kB (34,35 MICI). Another way to consume legumes is to long cook them with their skin, then remove the skin using a sieve. Red decorticated lentils and peas are more digestible than other kind of beans, and are very suitable to recondition the gut.

Vegetables

During remission, vegetables poor of insoluble fibers can be consumed: zucchini, potatoes, carrots, eggplants without skin, green beans, chards. They must be cooked very weel, and consumed as a cream in the first period, then can be consumed whole. In a further stage, raw vegetables can be used: chopped carrots, the inner part of radicchio, fluffy lettuces with little leaves. Greens with a higher content of fermentable fibers, such as cabbage, broccoli, savoy cabbage, artichoke, tomatoes and peppers can be eaten only as cream. A juice extractor can be a very useful tool to eat even vegetables with insoluble fibers, as it is completely removed obtaining a whole raw juice containing enzymes, vitamins, minerals and antioxidant compounds. Recent evidence showed that the soluble fraction of fibers in broccoli prevents relapse in CD patients (36). Thereby a juice extractor can improve the consumption of vegetables in patients with gut diseases.

Fresh fruit

Many studies demonstrate that fruit consumption has a protective immune-modulating effect in IBD (37,38,39) preventing recurrence. The consumption of fruit, which is necessary for the content of natural vitamins, minerals and protective compounds, is possible at every stage of the disease by using a juice extractor. As reported previously, an extractor completely removes the fibres. After remission is achieved with medical treatment, whole fruits can be used: fruit with less fiber at first, such as apple and bananas, orange juice, later small amounts of other seasonal fruits.

Olive oil

Rising studies show that olive oil has a strong anti-inflammatory effect on the gut mucosa (40,41). This effect is due not to oleic acid per se, but to its synergic action with other antioxidants molecules, such as hydroxityrosol, squalen, oleuropein. These components are mainly present in some olive species, such as "coratina" olives, and they are preserved only if the oil is extra virgin obtained by cold extraction. A recent work showed that the synergic effect of dietary olive oil and fish reduces inflammation in IBD patients (42).

Cereals

Cereals, and wheat in particular, have been heavily modified by agricultural technology. Modern varieties of grains are very different from the original cultivar. The gene pool has been modified to select grains with a higher content of gluten, for a better use in panification. Treatment with gamma-rays occurred since 1950, to obtain stronger plants and to ameliorate cooking results. These changes increased the antigenic epitopes, and the related immuno-mediated reactions, with effects on the gastrointestinal tract (43). Ancient varieties of grains, such as Senatore Cappelli (Triticum turgidum durum) or as Enkir (Triticum monococcum, the most ancient cereal raised by man, 7500 b.C.), have not been genetically modified, and have a low immunogenic impact. These grains do not cause inflammation in the gut mucosa (44,45). Bread, pasta and related products made with ancient grains repre-

sent the best choice for patients with IBD. Rice is very indicated in IBD, as it is naturally gluten free, lowering gut inflammation. Whole rice can be consumed after a long cooking.

Bluefish

The grease from bluefish has a widely investigated anti-inflammatory activity, due to its content of omega-3 fats as EPA and DHA (46,47). This Mediterranean food is very appropriated for IBD patients at any stage of the disease.

Nuts and seeds

Less suitable for patients with active IBD, can be part of a daily use even during disease flares if assumed as almond milk. This beverage belongs to ancient tradition of Southern Italy. It can be prepared easily, using only local almonds and water: nuts are soaked over night, then blended. The smoothie is filtered, obtaining a tasty juice suitable for a daily consumption. Almond milk contains only 50 kCal for 100 g; its consumption does not substitutes cow milk, as the latter has a much higher content of proteins, but almond milk has re-mineralizing properties and a high content of unsaturated fats: the amount of oleic acid is similar to olive oil, and linoleic acid is also weel represented. This drink can help patients assuming vegetal omega-3 limiting the fiber intake.

Conclusions

The role of nutrition in the prevention and management of IBD symptoms has been widely demonstrated. There are clear benefits of the mediterranean or vegetarian diets over the western diet. The Mediterranean and vegetarian diets are rich in fruits, vegetables, fish oil, whole grains and olive oil, which provide nutrients such as vitamin D, essential fatty acids, minerals and fibers. These foods maintain a healthy intestinal microbiota preventing dysbiosis, which has been implicated in the pathogenesis of IBD. The role of diet and probiotics supplementation in restoring the balance of the intestinal microbiota and in improving IBD symptoms is well established. Targeted nutrition approaches which take into account the individual genetic make-up and microbiota composition may represent a novel strategy for the prevention and management of IBD.

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Review

Renal lithiasis and inflammatory bowel diseases, an update on pediatric population

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Summary. *Background and aim of the work:* Historical studies have demonstrated that the prevalence of symptomatic nephrolithiasis is higher in patients with inflammatory bowel disease (IBD), compared to general population. The aim of the review was to analyze literature data in order to identify the main risk conditions described in literature and the proposed treatment. *Methods:* A research on the databases PubMed, Medline, Embase and Google Scholar was performed by using the keywords "renal calculi/lithiasis/stones" and "inflammatory bowel diseases". A research on textbooks of reference for Pediatric Nephrology was also performed, with focus on secondary forms of nephrolithiasis. *Results:* Historical studies have demonstrated that the prevalence of symptomatic nephrolithiasis is higher in patients with inflammatory bowel disease (IBD), compared to general population, typically in patients who underwent extensive small bowel resection or in those with persistent severe small bowel inflammation. In IBD, kidney stones may arise from chronic inflammation, changes in intestinal absorption due to inflammation, surgery or intestinal malabsorption. Kidney stones are more closely associated with Crohn's Disease (CD) than Ulcerative Colitis (UC) in adult patients for multiple reasons: mainly for malabsorption, but in UC intestinal resection may be an additional risk. Nephrolithiasis is often under-diagnosed and might be a rare but noticeable extra-intestinal presentation of pediatric IBD. Secondary enteric hyperoxaluria the main risk factor of UL in IBD, this has been mainly studied in CD, whether in UC has not been completely explained. In the long course of CD recurrent urolithiasis and calcium-oxalate deposition may cause severe chronic interstitial nephritis and, as a consequence, chronic kidney disease. ESRD and systemic oxalosis often develop early, especially in those patients with multiple bowel resections. Even if we consider that many additional factors are present in IBD as hypomagnesuria, acidosis, hypocitraturia, and others, the secondary hyperoxaluria seems to finally have a central role. Some medications as parenteral vitamin D, long-term and high dose steroid treatment, sulfasalazine are reported as additional risk factors. Hydration status may also play an important role in this process. Intestinal surgery is a widely described independent risk factor. Patients with ileostomy post bowel resection may have relative dehydration from liquid stool, which, added to the acidic pH from bicarbonate loss, is responsible for this process. In this acidic pH, the urinary citrate level excretion reduces. The stones most commonly seen in these patients contain uric acid or are mixed. In addition, the risk of calcium containing stones also increases with ileostomy. The treatment of UL in IBD involves correction of the basic gastrointestinal tract inflammation, restricted dietary oxalate intake, and, at times, increased calcium intake. Citrate therapy that increases both urine pH and urinary citrate could also provide an additional therapeutic benefit. Finally, patients with IBD in a pediatric study had less urologic intervention for their calculosis compared with pediatric patients without IBD. (www.actabiomedica.it)

Key words: inflammatory bowel disease, secondary hyperoxaluria, urolithiasis

Background and aim of the work

Historical studies have demonstrated that the prevalence of symptomatic nephrolithiasis is higher in patients with inflammatory bowel disease (IBD), compared to general population. The aim of the review was to analyze literature data in order to identify the main risk conditions described in literature and the proposed treatment.

Methods

A research on the databases PubMed, Medline, Embase and Google Scholar was performed by using the keywords "renal calculi/lithiasis/stones" and "inflammatory bowel diseases". A research on textbooks of reference for Pediatric Nephrology was also performed, with focus on secondary forms of nephrolithiasis. Only full text papers in English were included.

Results

Urolithiasis (UL) consists in the formation of urinary stones and comprises those stones formed in the kidney (nephrolithiasis), or anywhere in urinary tract as well as in the bladder. Recurrent UL may lead to end-stage renal disease (ESRD) via multiple damages in the kidney, from obstructive uropathy to tubulo-interstitial nephritis, or renal scarring, or recurrent infection. The global incidence of urolithiasis in childhood is approximately 10% of that seen in adults, it appears at all ages, with moderate preponderance in males. Urolithiasis has become more common in children over the past few decades, mainly in adolescents, as a result of rapid variations in habits such as a high salt and a high protein content diet (1-5). Nephrolithiasis results from urine supersaturation of some components. In industrialized countries calcium-oxalate (CaOx) stones are prevalent in pediatric population. Hypercalciuria is recognized worldwide as the most frequent underlying factor in calcium oxalate stones in children. Another metabolic risk factor is hyperoxaluria, although it might be underestimated, as it is less frequently described. Struvite or infection-related stones, which were very common in children until the last century, are rarely seen nowadays in industrialized countries, possibly due to the improved management of both pediatric obstructive uropathy and urinary tract infections (3).

Historical studies have demonstrated that the prevalence of symptomatic nephrolithiasis is higher in patients with inflammatory bowel disease (IBD), compared to general population, typically in patients who underwent extensive small bowel resection or in those with persistent severe small bowel inflammation (6). In IBD, kidney stones may arise from chronic inflammation, changes in intestinal physiology due to inflammation, surgery or intestinal malabsorption. UL is quite rare in pediatric IBD patients (0,37-1%) compared to the incidence in adult cases (described as 9-18%), and the main studies and reports derive from adult experience. Renal calculi are more closely associated with Crohn's Disease (CD) than Ulcerative Colitis (UC) in adult patients for multiple reasons, mainly for malabsorption, but in UC intestinal resection may be an additional risk (6, 7). Nephrolithiasis is often under-diagnosed and might be a rare but noticeable extra-intestinal presentation of pediatric IBD (3).

Various pathophysiological mechanism at the basis of nephrolithiasis in IBD-patients are considered, and additional factors as surgery or infections may aggravate the situation (9). Principal factors are:

- intestinal malfunction unabsorbed fatty acids bind intraluminal calcium; therefore, less insoluble calcium oxalate is excreted in stools, resulting in higher oxalate reabsorption and higher oxalate concentration in urine.
- decolonization of the gastrointestinal tract of the oxalate fermenting bacterium Oxalobacter fromigenes was associated with hyperoxaluria and kidney stones in IBD patients
- due to intestinal malabsorption, patients have less urinary excretion of citrate and magnesium which can act as inhibitors of oxalate stone formation
- loss of water and salt in patients with ileostomy or pronounced diarrhea will lead to the production of more concentrated urine. Moreover, in patients with ileostomies, large amounts of alkaline fluids will be lost, and the urine of those patients will be acidic.

Analyzing the main risk factors for UL in general population, focusing to a pediatric population, several factors and their relative their role in IBD were considered (9, 10).

Hypercalciuria is the most common cause of UL in children, but secondary forms are less frequent than primary or idiopathic forms. In IBD secondary hypercalciuria is a rare mechanism and it may be principally related to high-dose and/or long-term steroid treatment, and rarely to vitamin-D intoxication (1-3, 10). Oral vitamin administration rarely leads to intoxication, while in IBD patients with intestinal failure the use of vitamin C-supplemented parenteral nutrition can cause hypercalciuria (11).

Secondary hyperoxaluria is a rare but emergent cause of UL in children, as dietary intake has increased in the last decades up to 50% in industrialized countries. Secondary enteric hyperoxaluria is the main risk factor of UL in IBD. This has been mainly studied in CD, whether in UC it has not been completely explained. In the long course of CD recurrent urolithiasis and calcium-oxalate deposition may cause severe chronic interstitial nephritis and, consequently, chronic kidney disease. ESRD and systemic oxalosis often develop early, especially in those patients with multiple bowel resections (2). Malabsorption associated with ileal disease, mainly of bile-salts and fatty acids, causes increased oxalate absorption by increasing oxalate solubility in the intestinal lumen and permeability of the colonic mucosa (9-12). Kumar et al. examined the association of intestinal oxalate degrading bacteria Oxalobacter formigenes with the development of hyperoxaluria in IBD. The investigators studied stool samples of IBD patients and controls respectively for the presence of O. formigenes using polymerase chain reaction. Only 10.4% of patients with IBD had positive for O. formigenes stool samples, compared to 56% of controls (13). Patients positive for O. formigenes had higher urinary oxalate than the negative ones. A recent study suggested that oxalate is not only absorbed but can also be secreted by the small intestine. Probably chronic inflammation may impair this function (10, 14). The most the urinary output of oxalate is high, the most the risk of UL is increased, and the symptoms are severe. In patients with Crohn's disease, ileocolonic disease was associated with a greater risk of nephrolithiasis than isolated ileal or colonic disease (6). Intestinal resections, including bariatric surgery, represent an additional risk factor. In some studies, urinary saturation index for calcium-oxalate was not statistically different in Crohn's patients with and without urolithiasis, but it was significantly higher in patients after bowel resection, compared with those who did not undergo resection (10).

Citrate is the main inhibitor of calcium and oxalate crystallization. Hypocitraturia is not always adequately recognized as a risk factor. It usually derives from low dietary intake and it has been demonstrated in patient with chronic acidosis or in case of intestinal malabsorption. A reduced citrate excretion is associated to mild acidosis due to the loss of bicarbonate in liquid stools. Hypocitraturia is commonly seen in patients with gastrointestinal malabsorption, both from small bowel disease and after small bowel resection. The levels of citrate excreted in the urine of patients with CD may be half or less of that in urine of healthy subjects (15-17).

Hypomagnesuria is also typical in patients with chronic diarrhea and malabsorption and may occasionally be accompanied by hypomagnesemia. In patients with small bowel resection, the degree of hypomagnesuria correlates with the length of resected bowel. Magnesium is also thought to confer some protection against CaOx crystal formation, by chelating oxalate and possibly by an effect on crystal growth (15-17).

Even if we consider that many additional factors are present in IBD as hypomagnesuria, acidosis, hypocitraturia, and others, the secondary hyperoxaluria seems to finally have a central role. A study comparing patients with UL, either affected by CD or otherwise healthy, did not demonstrate significant differences in the urinary excretion of other lithogenic or stone inhibitory parameters (9).

Uric acid stones are more common in adult patients, depending from high protein dietary intake. In UC, especially if an ileostomy is present, urine is scanty and concentrated, and urine pH falls, leading to uric acid or mixed stones formation. Patients with intestinal resections have a tendency to form uric acid stones, particularly in patients with colon resection (10, 15-17).

A single case of pediatric patient with UC had lithiasis of ammonium acid-urate is reported in a Japa-

nese patient, secondary to diarrhea and dehydration (18).

Struvite stones are commonly founded in patients with recurrent urinary infections. Patients with IBD are a risk of urinary tract infections, especially those who have fistulas (colon-bladder fistula) or in case of anal CD. Immunosuppressive treatments should also be considered possible infectious risks (1-3).

Dehydration intended as chronic or acute loss of fluid, is at high risk of UL. In IBD both might be observed. Furthermore, all patients with colon resection share a tendency to chronic volume contraction due to loss of water and salt in liquid stools, which leads to a decreased urine volume (15).

Medications should also be considered, also if they are responsible for only 1% of UL. Drug-induced stones may form by two mechanisms: the drug or its metabolites form the main stone component when they precipitate in urine or the drug induces metabolic alterations in urine that lead to the formation of calcium or less commonly uric acid stones (1-3). Corticosteroids and vitamin D parenteral administration have already been discussed as they can lead to hypercalciuria. Many antibiotics, such as Ceftriaxone, are included in lithogenic substances with the first mechanism, as well as other immunosuppressive or immunomodulating agents as sulfasalazine or cyclosporine. Sulfasalazine is converted by gut bacteria into sulfapyridine and the clinically active metabolite 5-aminosalicylic acid (5-ASA), and its efficacy is proportional to the 5-ASA concentration within the intestinal lumen. Renal complications are commonly reported for the chemically similar 5-ASA derivative mesalamine but are not well-known side effects of sulfasalazine therapy. In a case report in an adult patient, renal ultrasound revealed multiple stones, which, once excreted and analyzed, were composed of sulfasalazine metabolites. The patient recovered after fluid administration. The author conclude that hydration status may play an important role in this process (19).

Intestinal surgery is and independent risk factor for stones formation. Patients with ileostomy post bowel resection may have relative dehydration from liquid stool, which, added to the acidic pH from bicarbonate loss, is responsible for this process. In this acidic pH, the urinary citrate level excretion reduces. The stones most commonly seen in these patients contain uric acid or are of mixed composition. In addition, the risk of calcium containing stones also increases with ileostomy (9, 10, 15). Among patients with CD, UL increases in patients with ileal resection, whereas a study evidenced that UL risk is not increased in patients with total colectomy. In UC, the risk of UL is higher in case of colectomy with J-pouch or ileostomy, but J-pouch reduce the risk of calcium-stone formation (10).

The treatment of UL in IBD consists in the correction of the basic gastrointestinal tract inflammation, the restriction of dietary oxalate intake, and, sometimes, in the increase of calcium intake (1-3, 20). No study directly assessing the in vivo effect of a treatment to reduce urolithiasis in IBD patients was identified. In a study using computerized models, simulated urine compositions based on reported composition values of IBD patients were compared with those from normal individuals. The authors suggested that calcium supplements can help reducing stone formation in those patients, but initial efforts should be directed towards the reduction of urinary oxalate by reducing dietary oxalate. Citrate therapy that increases both urine pH and urinary citrate could also provide an additional therapeutic benefit (21). In UC patients who underwent total proctocolectomy with ileal pouch-anal anastomosis, close monitoring for renal stone formation and administration of prophylactic oral bicarbonate has been suggested. Urinary alkalinization along with increased hydration is also advocated in IBD patients receiving aminosalicylates (1-3, 19). Finally, a study conducted among pediatric patients showed that IBD patients had less urologic interventions for calculosis compared with non-IBD ones (17).

Conclusions

Urinary lithiasis in IBD patients is increased. The pathogenesis is multifactorial including inflammation, malabsorption and consequent alterations in the hydration status and electrolytes balance. In these patients, it is necessary to be aware of this increased risk, in order to set up an accurate follow up and prompt diagnosis, and avoid complications.

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Review

Inverse association between *Helicobacter pylori* and inflammatory bowel disease: myth or fact?

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Summary. *Background:* Inflammatory bowel diseases (IBD), are chronic, relapsing-remitting diseases of the gastrointestinal tract, including Crohn's disease (CD), Ulcerative colitis (UC) and Unclassified IBD (IBDU). Their pathogenesis involves genes and environment as cofactors in inducing autoimmunity; particularly the interactions between enteric pathogens and immunity is being studied. *Helicobacter pylori* (*HP*) is common pathogen causing gastric inflammation. Studies found an inverse prevalence association between HP and IBD, suggesting a potential protecting role of HP from IBD. *Methods:* A literature search of the PubMed database was performed using the key words "helicobacter pylori", "inflammatory bowel disease", "crohn disease", "ulcerative colitis". Embase, Medline (OvidSP), Web of Science, Scopus, PubMed publisher, Cochrane and Google Scholar were also searched. Prevalence rate-ratios among *HP* in IBD patients, *HP* in CD patients, *HP* in UC patients, *HP* in IBDU patients were extracted, each group was compared with controls, to verify the inverse association between *HP* and IBD prevalence. *Results:* In all groups the dispersion of data suggested an inverse association between IBD group and controls, even when the comparison was carried out separately between each group of newly diagnosed patients and controls, to rule out the possible bias of ongoing pharmacologic therapy. *Conclusions:* The results of this review show a striking inverse association between *HP* infection and the prevalence of IBD, independently from the type of IBD considered across distinct geographic regions. Anyway, data should be interpreted cautiously, as wider, prospective and more homogeneous research on this topic are awaited, which could open new scenarios about environmental etiology of IBD. (www.actabiomedica.it)

Key words: *Helicobacter pylori*, infection, inflammatory bowel disease, Crohn's disease, ulcerative colitis, prevalence, association

Introduction

Inflammatory bowel diseases (IBD), are chronic, relapsing-remitting diseases of the gastrointestinal tract, including Crohn's disease (CD), Ulcerative colitis (UC) and Unclassified IBD (IBDU). Their pathogenesis is still not completely understood; several environmental, immune and host (e.g., genetic, epithelial, immune and non-immune) factors are involved. Complex interactions between the immune system, enteric commensal bacteria/pathogens and host genotype are thought to underlie the development of IBD (1). Many factors have been examined as markers of environmental exposures in early life, including family size, sibship, birth order, country of birth, urban upbringing, socioeconomic status, and pet exposure (2).

Helicobacter pylori (*HP*) is a gram-negative, spiral-shaped pathogenic bacterium responsible for chronic gastritis. That mucosal inflammatory process is most likely driven by a cellular immune response to the ongoing stimulation of the host's immune system caused by the bacterium. This results in high production of interleukin (IL)-12, leading to a T helper type 1 (Th1)-polarized response and elevated levels of Th-1 cytokines (3, 4). Products of the local immune reactions may travel to extra-gastric sites, thus linking *HP* infection to the pathophysiology of a variety of extra-gastric diseases, including autoimmune disorder (5). Interestingly, however, *HP* has been proposed to play a protective role against the development of certain autoimmune disorders such as asthma and type 1 diabetes mellitus (6). The mechanisms underlying this protective role of *HP* infection is thought to be differential expression of an acute and/or chronic local mucosal inflammatory response, which may elicit a systemic release of cytokines, which in turn may down-regulate systemic immune responses and suppress autoimmunity (7).

In IBD, dysregulation of the immune response of the host to commensal bacteria has been proposed as an important underlying pathogenetic mechanism. Increased attachment of gut bacteria to the intestinal epithelium has been documented in IBD. Moreover, the secretion of pro-inflammatory cytokines, an up-regulation of cell signaling molecules and a Th1-driven immune reaction are characteristics implicated in the pathogenesis of IBD, especially CD (8).

The similarities between the immunobiology of IBD and that of *HP* infection provide background for the hypothesis that *HP* infection may be involved in the modulation of the pathogenesis of IBD.

Such speculation has been investigated in various studies. According to epidemiology data, IBD are more prevalent in developed countries than in developing countries, whereas *HP* infection shows higher prevalence in developing countries than in developed ones (9). Case control studies suggest an inverse association between *HP* and IBD.

In this review we analyze and evaluate a large volume of published data with the aim to clarify the proposed association between *HP* infection and IBD.

Materials and Methods

In order to verify the association between HP infection and IBD, we selected the studies focused on the evaluation of the prevalence of HP in IBD and in otherwise healthy controls.

A literature search of the PubMed database was performed using the key words "helicobacter pylori", "inflammatory bowel disease", "crohn disease", "ulcerative colitis". Embase, Medline (OvidSP), Web of Science, Scopus, PubMed publisher, Cochrane and Google Scholar were searched as well. Only English language publications were included.

Inclusion criteria among these papers were: case study papers, case report, clinical trial, retrospective study or journal article, published in the last 10 years, addressed to study the prevalence rate of *Helicobacter pylori* in IBD patients; in case the study did not include a control cohort, the prevalence was compared with a study about *HP* prevalence regarding otherwise healthy people in the same country in the same period (+/-5 years). Papers with topic not regarding the relationship between *HP* and IBD to obtain a prevalence rate, reviews of literature or meta-analyses were excluded. Two investigators (SK and FG) independently reviewed and extracted data from the papers according to the predetermined criteria. For each study the following data were extracted: year of the study, country, number of patients affected by IBD, number of patients affected by CD, number of patients affected by UC, number of patients affected by IBDU, number of controls, percentage of *HP* infection in IBD, CD UC, and IBDU affected patients. Details are shown in Table 1 and Table 2. Prevalence rate-ratios between *HP* in IBD patients and in controls, between *HP* in CD patients and in controls, between *HP* in UC patients and in controls, *HP* in IBDU patients and in controls were compared to verify the existence of an inverse association between *HP* and IBD prevalence. Since the results of the prevalence of *HP* infection in IBD affected patients could be theoretically influenced by the more frequent treatments with antibiotics, sulfasalazine, 5-aminosalicylic acid, corticosteroids, and immunosuppressants, we considered separately those studies dealing with newly diagnosed IBD patients (therefore not exposed to antibiotics, sulfasalazine, 5-aminosali-

Table 1. HP prevalence in IBD patients

Author	Country	Year	% HP in IBD	% HP in CD	% HP in UC	% HP in IBDU	% HP in Controls
So H. et al (10)	Korea	2016	23,4	23,4	/	/	52,5
Magalhaes MH et al (11)	Brazil	2014	57,9	54,5	62,5	/	50
Zhang S. et al (12)	China	2011	19,7	18,3	21,2	/	48,8
Sonnenberg A. Genta R. (13)	USA	2012	4,4	4	5	4	9
Rosania R. et al (14)	Germany	2018	11	12	11	/	25
Lahat A. et al (15)	Israel	2017	10,7	10,7	/	/	39
K.Farkas, H.Chan et al (16)	Hungary	2016	13,9	13,9	/	/	14,6
K.Farkas, H.Chan et al (16)	China	2016	4	4	/	/	15
Roka et al. (17)	Greece	2014	3,8	4,5	5,8	1,7	13,2
Annunziata et al (18)	Italy	2012	8,4	8,4	/	/	39
Ando T. et al (19)	Japan	2008	8	8	/	/	42
Ram M. et al (6)	Israel, Italy, Serbia	2012	2,5	/	/	/	39
Sonnenberg A et al (20)	USA	2011	5	4	6	8	7
Danelius M. et al (21)	Sweden	2009	10,8	5,8	9,5	25	44,4
Song M.J. Et al (22)	Korea	2009	25,3	17,7	32	/	52,5
Hong C.H. et al (23)	Korea	2009	32,5	27	37,2	/	53,2
Sakuraba A. et al (24)	Japan	2014	10,8	10,8		/	42

HP: stands for *Helicobacter pylori*; IBD: stands for Inflammatory Bowel Diseases; CD: stands for Crohn's Disease; UC: stands for Ulcerative Colitis; IBDU: stands for Unclassified Inflammatory Bowel Disease

Table 2. HP prevalence in newly diagnosed IBD

Author	Country	Year	% HP in IBD	% HP in CD	% HP in UC	% HP in IBDU	% HP in Controls
Park J.H. et al (25)	Korea	2017	5,8	5,8	/	/	52,5
Hansen R. et al (26)	UK	2013	2,3	0	7,7	0	14,3
Xiang Z. et al (27)	China	2013	27,1	27,1	/	/	47,9
Kaakoush et al (28)	Australia	2011	18,2	18,2	/	/	10,8
Jin X. et al (29)	China	2013	30,5	/	30,5	/	57
Horje et al (30)	The Netherlands	2016	6,7	6	8	/	30

HP: stands for *Helicobacter pylori*; IBD: stands for Inflammatory Bowel Diseases; CD: stands for Crohn's Disease; UC: stands for Ulcerative Colitis; IBDU: stands for Unclassified Inflammatory Bowel Disease

cylic acid, corticosteroids, and immunosuppressants) and compared the same *HP* prevalence rate-ratio (Table 2).

The techniques used to assess the *HP* infection in the selected studies were not uniform. Twelve studies based the *HP* infection diagnosis on the histopathologic analysis of gastric biopsies, 3 articles on Urease Breath Test positivity, 2 on the results of cultural tests, 1 on the research of fecal *HP* antigens and 2 on the result of the titer of serum anti-*HP* IgG (which was considered positive only if superior to 30 Enzyme Immune Unit (EIU)).

Results

The literature search retrieved 523 papers, among whom 286 were excluded since not consistent with the aim of the present review. Full texts of 237 articles were reviewed to assess eligibility. After the assessment of eligibility, 22 articles about *Helicobacter pylori* infection and IBD and meeting the inclusion criteria were included in the study (Figure 1).

The included studies report data from countries belonging to four different continents (China, Korea, Italy, Israel, USA, Germany, Hungary, Brazil, United

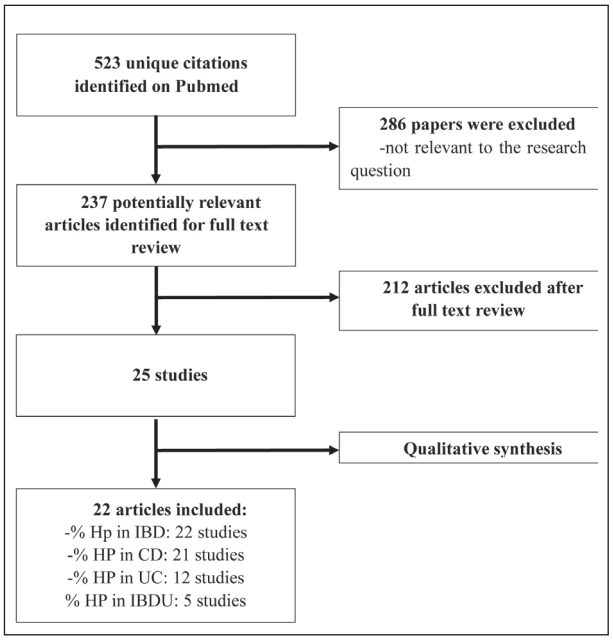

Figure 1. Flow diagram showing the selection of the included studies. HP: stands for *Helicobacter pylori*; IBD: stands for Inflammatory Bowel Diseases; CD: stands for Crohn's Disease; UC: stands for Ulcerative Colitis; IBDU: stands for Unclassified Inflammatory Bowel Disease

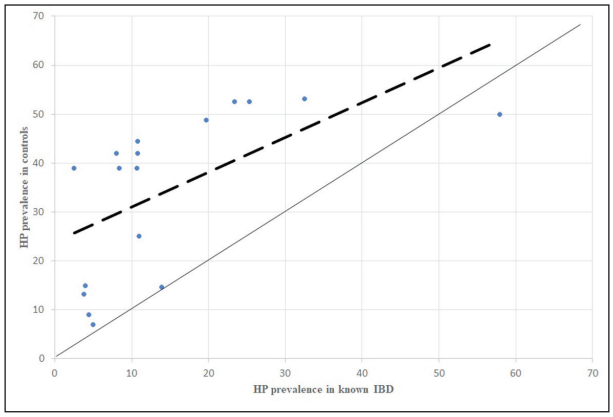

Figure 2. HP prevalence in newly diagnosed IBD patients, comparison between dispersion line and 1:1 line. HP: stands for *Helicobacter pylori*; IBD: stands for Inflammatory Bowel Diseases

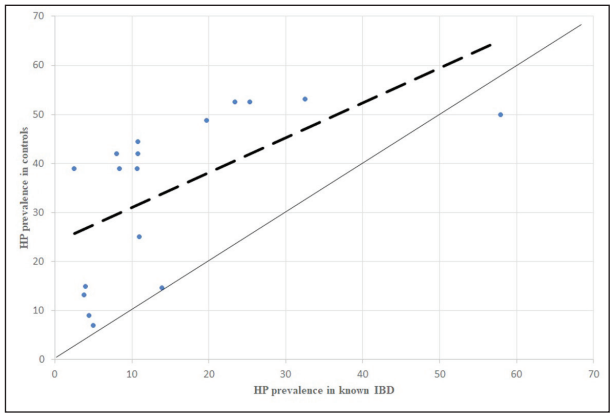

Figure 3. HP prevalence in known IBD patients, comparison between dispersion line and 1:1 line HP: stands for *Helicobacter pylori*; IBD: stands for Inflammatory Bowel Diseases

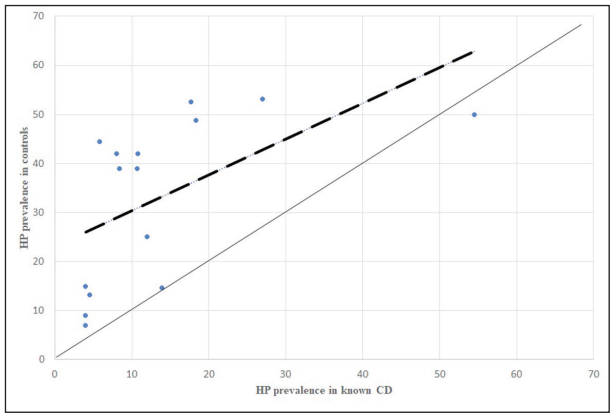

Figure 4. HP prevalence in known CD patients, comparison between dispersion line and 1:1 line. HP: stands for *Helicobacter pylori*; CD: stands for Crohn's Disease

Kingdom, Greece, Australia, The Netherlands, Japan and Sweden). There was considerable variability in the prevalence rates of *HP* infection both in IBD affected patients and in controls among the study populations. Prevalence rates of *HP* infection in IBD patients were the lowest in UK (2,3%) and the highest in Brazil (57.9%). Similarly, the highest prevalence rates for *HP* infection in controls were reported in Brazil and Korea (50 and 52,5% respectively), the lowest in USA (7%) as shown in Table 1 and Table 2.

Overall, the difference in prevalence of *HP* infection between IBD affected patients and controls was significative in 16/22 studies. As shown in Figure 2, 3, 4, 5, 6, 7 and 8, which were obtained by a comparison between the *HP* prevalence in IBD and controls in all the studies, the dispersion of data demonstrated higher prevalence in controls. This suggests the existence of an important inverse association between *HP* infection

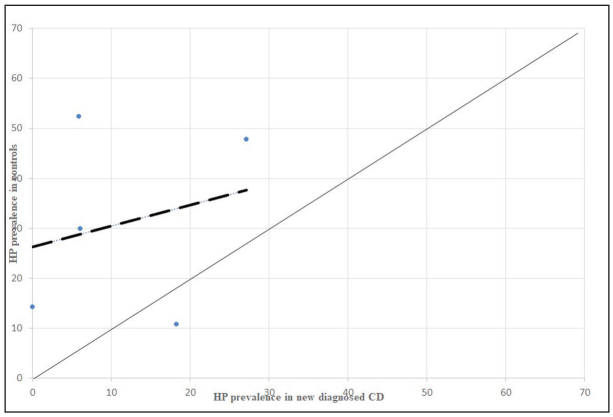

Figure 5. HP prevalence in newly diagnosed CD patients, comparison between dispersion line and 1:1 line. HP: stands for *Helicobacter pylori*; CD: stands for Crohn's Disease

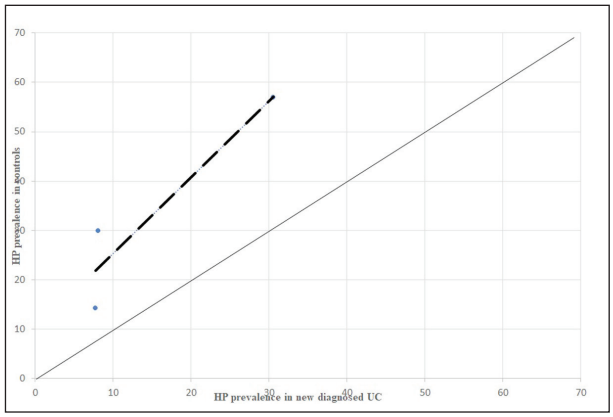

Figure 7. HP prevalence in newly diagnosed UC patients, comparison between dispersion line and 1:1 line HP: stands for *Helicobacter pylori*; UC: stands for Ulcerative Colitis

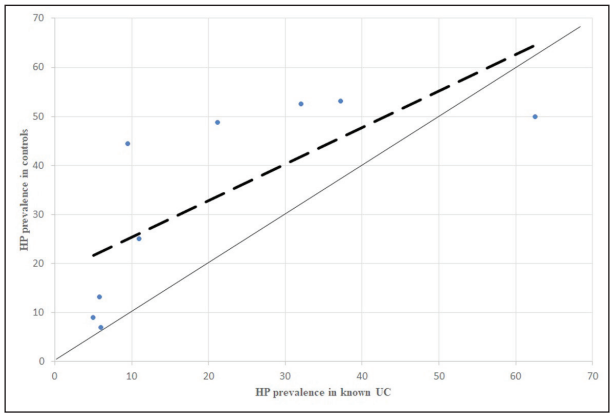

Figure 6. HP prevalence in known UC patients, comparison between dispersion line and 1:1 line. HP: stands for *Helicobacter pylori*; UC: stands for Ulcerative Colitis

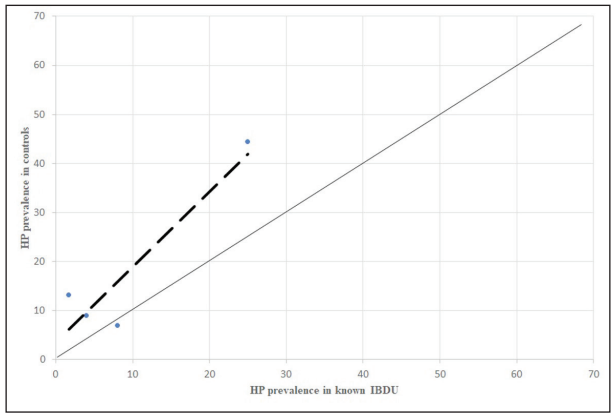

Figure 8. HP prevalence in known IBDU patients, comparison between dispersion line and 1:1 line. HP: stands for *Helicobacter pylori*; IBDU: stands for Unclassified Inflammatory Bowel Disease

and IBD (both in CD and UC, and even in IBDU).

From the analysis of HP prevalence carried out separately in studies considering newly diagnosed IBD patients (Figure 3, 5, 7) it is possible to assess that the inverse association previously reported is still confirmed in antibiotic- and immunosuppressant-free patients, even though less evident.

All the comparisons of HP infection prevalence carried out separately for CD, UC and IBDU patients showed the same inverse association between HP infection and the inflammatory bowel disease (Figures 4, 6, 8).

Conclusions

The key finding of this study is a striking inverse association between *HP* infection and the prevalence of IBD, independently from the type of IBD considered (CD, UC and IBDU) across distinct geographic regions.

The agreement among all the considered studies appears interesting from different points of view. Firstly, the same inverse association is confirmed by all the studies, although they considered different countries with different prevalence both of HP infection and

IBD. Moreover, the analysis conducted separately on newly diagnosed IBD patients tends to eliminate the possible bias of previous use of antibiotics or immunomodulators which are routinely prescribed to followed up IBD patients, and still this analysis showed the same inverse association, although less strongly than if considering the overall population. Therefore, antibiotics seem to have an effect on HP infection prevalence, but they represent non-important confounders. However, case control studies displayed substantial methodologic and population heterogeneity, as well as publication bias which could have influenced the results (10). Furthermore, it is of note that many studies relied on HP serology with variable sensitivity and specificity compared to histology (11).

Several theories have proposed that IBD develop as a result of the dysbiosis between harmful and protective bacteria and also the imbalance between pro- and anti-inflammatory immune responses. Lack of childhood exposure to enteric pathogens responsible for gastroenteritis may have a role in the development of IBD. In this context, *HP* infection may simply be a marker of infection that reflects a generally increased exposure to gastrointestinal microbes.

In summary, the results from our review should be interpreted with caution. In our opinion *HP* infection could be a marker for an increased likelihood of exposure to other gastrointestinal infections or bacteria that, all together, may have an immunomodulatory effect. Anyway, we strongly disagree with any proposal against eradication of *HP* in infected patients, since it has been recognized as an human group 1 carcinogen by the International Agency for Research on Cancer (IARC) (12), this means a certain potential carcinogenic agent for gastric mucosa.

Overall, these first studies of association highlight the need of wider, prospective and more homogeneous research on this topic, which could open new scenarios on the theme of environmental etiology of IBD.

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Review

Nutrition and lipidomic profile in colorectal cancers

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Summary. *Background:* Adherence to a healthy diet has been reported to be essential for the primary prevention of colorectal cancer, through a reduction of tissue inflammation, a low concentration of circulating lipoproteins and lower levels of serum cholesterol. Since an altered expression of the fatty acids pattern has been demonstrated to be a crucial event in colorectal carcinogenesis, lipidomic analysis is considered able to identify early diagnostic and prognostic biomarkers of complex diseases such as colorectal cancer. *Methods*: cell membrane fatty acid profile and serum lipoproteins pattern were evaluated by gas chromatography and electrophoresis method respectively. *Results:* There is a close association between diet and lipidomic profile in colorectal cancer, both in pre-clinical and clinical studies. A modified serum lipoproteins pattern has been demonstrated to be predominant in intestinal tumors. *Conclusions*: The study of fatty acids profile in cell membrane and the evaluation of serum lipoproteins subfractions could be useful to have an integrate vision on the interactions between lipids and the pathogenesis of colorectal cancer and to understand the mechanisms of action and the consequences of these interactions on human health status. (www.actabiomedica.it)

Key words: nutrition, colorectal cancer, lipidomic analysis, fatty acids, lipoproteins

Introduction

Nutrition and cancer

Increasing evidence reports an important and significant association between nutrition and cancer, in particular as nutrition is a potentially modifiable risk factor for cancer. However, interest in the association of diet/ nutrition and cancer first appeared in the early 1800s and probably even before. Starting from this date, progress in understanding this association has been made over the past two centuries, even if often not leading to conclusive statements (1).

In this regard, several works reported that a healthy diet is associated with a lower incidence and prevalence of cancer, particularly when the gastrointestinal system is involved. For example, a higher adherence to a Mediterranean diet has been associated with a lower incidence of breast cancer (2), lung cancer (3), and prostate cancer (4).

This beneficial effect seems to be attributable to some important effects of the Mediterranean diet, which includes foods with anti-inflammatory properties (5) and anti-oxidant effects (6). Since inflammation and oxidative stress seem to be the basis of several cancers, the Mediterranean diet could potentially lower the incidence of cancer through these mechanisms.

Moreover, the Mediterranean diet seems to have important effects on the genetic patrimony. For example, older people having a higher adherence to the Mediterranean diet have significant higher telomere length than those having a lower adherence, therefore

suggesting a potential role of this diet in maintaining a good genetic patrimony (7), protective for cancer.

Other studies have reported that also micronutrients may have a role in the prevention of cancer, both in terms of vitamins and minerals that have antioxidant and anti-inflammatory effects. For example, it has been reported that higher vitamin D levels are associated with a lower incidence of cancer and with better outcomes in people already affected by cancer (8), but the literature regarding this topic is still conflicting and not univocal. (9) Similarly, a higher magnesium intake seems to be associated with a lower incidence of pancreatic cancer in a large population followed-up for 7 years (10).

Altogether these findings suggest an important role of diet in cancer onset, that future research should better study.

Nutrition and colorectal cancer

Nutrition seems to play a pivotal role in the onset and in the progression of gastrointestinal cancers, particularly of colorectal cancer (CRC).

Regarding the Mediterranean diet, for example, it was reported in a large meta-analysis involving 11 cohort studies that a higher adherence to this dietary regimen significantly decreased the risk of CRC by 18%, independently from several potential confounders (11). In a recent umbrella review regarding Mediterranean diet and health outcomes, however, the strength of evidence was graded only as weak, mainly due to the presence of high heterogeneity of the studies available on the topic. (12) Therefore, other studies are needed in this direction in order to confirm these findings.

Among the food components present in the Mediterranean diet, some words should be spent on fibers and on meat.

It is widely known that a higher intake of dietary fibers is associated with a lower risk of CRC in several cohort longitudinal studies, and in people with pre-neoplastic lesion, such as adenomas (13, 14). In a recent umbrella review, a higher dietary fibers significantly lowered the risk of CRC of about 36%, but this evidence was, again, characterized by a high heterogeneity and therefore the strength of evidence was graded only a suggestive (15). In this regard, fibers seem to be able to decrease the risk of CRC by several mechanisms. Among them, the most important seems to be a cleaning effect on the colon, particularly of toxins (16). Another important effect seems to be associated with the viscosity of dietary fibers. Viscosity is a physicochemical property associated with dietary fibers, particularly soluble dietary fibers. Viscous dietary fibers thicken when mixed with fluids and include polysaccharides such as gums, pectins, psyllium, and βglucans. Viscous fibers have been associated with beneficial physiological responses in human, animal, and animal-alternative in vitro models (17).

On the other hand, the introduction of meat seems to be deleterious for the onset of CRC. Red meat intake was identified as a probable risk factor for CRC, with research supporting that this may especially be true for tumors of the traditional adenoma-carcinoma pathway. Dietary heme intake shows a strong association with KRAS-mutated tumors, such as CRC (18). It has been reported that heme can enhance the endogenous formation of carcinogenic N-nitrose compounds (18). In this regard, the way of cooking meats seems to be relevant, since N-nitrose compounds are mainly produced by processed meats (19).

Altogether these findings support the idea that diet plays an important role in CRC and that the amounts of some foods (especially processed meats) should be strictly limited.

Nutrition, colorectal cancer and lipid profile

Different pre-clinical and clinical studies have confirmed the anti-cancer and cancer preventing action of diet (20-22). In particular, colorectal cancer (CRC) is considered a metabolic pathology where tumor growth and progression are affected by the complex interactions between cancer cells and the surrounding microenvironment (23). Environmental factors such as smoking, physical inactivity, overweight and obesity have been related to an increased risk of CRC (24). In fact, adherence to a healthy diet has been reported to be essential for the primary prevention of CRC. The link between adherence to the Mediterranean diet and a lower risk of cancer is mediated by several mechanisms, including reduction of tissue inflam-

mation, low concentration of circulating lipoproteins and lower levels of serum cholesterol.

It is widely accepted that lipid and phospholipid metabolism plays a key role in cancer initiation, cellular invasion and tumor metastasis, and diet is considered as the major factor influencing fatty acid composition in tissue. The investigation of dietary intake can be useful to understand the relations between the patterns of fatty acid metabolism and specific diseases such as cancer (25,26).

Conflicting results have been reported on low-density (LDL-C) and high-density lipoprotein cholesterol (HDL-C) and serum triglyceride (TG) levels in different tumor types (27-29). Compared to subjects without cancer, in patients with malignancy LDL-C has been reported to be increased (27), normal (29) or decreased (30). The tumor types may explain the inconsistency in the results. Apparently, the effect on serum lipoprotein patterns differs among tumor types. However, the same type of tumor has been associated with different lipoprotein levels. The stage of disease is another possible contributory factor, although abnormal serum cholesterol levels have not consistently been linked to the progression of cancer (30,31). Studies of serum cholesterol levels in patients with gastrointestinal cancer have provided conflicting results, reporting either no (32), negative (33) or positive correlations (34). Recently, an association has been found between serum cholesterol and colorectal adenoma, a well-established precursor lesion of colorectal cancer (35). Moreover, experimental evidence of tumor cell accumulation in lesions caused by endothelial inflammation and injury has suggested an increased incidence of metastases in patients with increased LDL-C levels (36). In addition, recent studies showed that lovastatin, an 3-hydroxy-3- methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor, has anti-metastatic effects on human colon adenocarcinoma cell lines as well as on other cell lines (37).

Increased serum lipid levels are associated with the presence of metastases in patients with colorectal cancer (38). Although the exact reason is not clear, this is an interesting finding from an oncological point of view. In blood or interstitial fluids, most cholesterol or TG exists in a lipoprotein complex with apoproteins, and the major detrimental effect of serum cholesterol may be attributed to LDL, that can have various effects on tumor biology. There is much evidence to support the view that tumor cell growth is partially dependent on exogenous LDL-C, possibly through LDL receptors on tumor cells (39). However, experimental evidence shows that LDL receptors are frequently down-regulated in malignant tumors (36, 40-42). In these cases, neoplastic proliferation may be stimulated by de novo synthesis of endogenous cholesterol through the HMG-CoA reductase pathway (36, 40,42). Indeed, pharmacological inhibition of HMG-CoA reductase has been shown to prevent the growth and invasion of some tumors (43). These findings suggest that increased LDL-C might be beneficial for the proliferation and invasion step of carcinoma cells.

The relationship between a metastatic phenotype and hyperlipidemia in colorectal cancer patients supports this hypothesis and is in line with other previous studies. It has already been demonstrated that 63.3% of colon cancer patients are LDL receptor negative, and the absence of the LDL receptor is associated with a poor prognosis and with enhanced HMG-CoA reductase activity (41,42). High serum TC and LDL-C levels in patients with metastases might be explained by an increased demand for cholesterol from neoplastic cells, resulting in an increased endogenous cholesterol synthesis. Moreover, recent data from literature have attributed a role to LDLC in cell growth and differentiation, with a possible involvement of hypercholesterolemia in cancer progression and metastatic spread (36). LDL-C, indeed, has been reported to affect host immune functions. LDL is required for the optimal expression of the Fc receptor or CD14, which mediates the phagocytosis of human monocytes, and a high-fat diet has been shown to decrease the antitumor activity of macrophages in mice (44-46). Moreover, high LDL levels have been reported to inhibit T-cell proliferation (47). These facts suggest that stimulation of cancer cells and suppression of the immune system by high LDLC levels might facilitate tumor invasion and survival of colorectal cancer cells in lymph nodes and distant metastases. *In vivo* studies suggest that there may be an association between cholesterol and metastatic cancer, i.e. colon cancer induced in rats by dimethylhydrazine was associated with a reduced incidence of metastases after deprivation of dietary cholesterol (48). Moreover,

drugs that inhibit endogenous cholesterol synthesis, i.e. lovastatin or simvastatin, show anti-metastatic effects on colon, pancreatic and melanoma cancer cells in vitro and in vivo (37, 43, 49,50). Moreover, in addition to its greater growth-inhibitory effect on metastatic cancer cells, lovastatin appears to universally reduce trans endothelial migration by acting on tumor cells, quiescent endothelial cells and LDL-stimulated cells (37). Based on these findings, repeated monitoring of serum lipid profiles in colorectal cancer patients may facilitate to predict tumor aggressiveness. The reduction in serum lipid levels might help to prevent metastases in certain cancers. Further studies including larger patient cohorts are warranted to evaluate the role of serum LDL-C as a predictive marker of recurrence of neoplasia in colorectal cancer patients.

A new approach to study the relationship between nutrition, colorectal cancer and lipid profile is the lipidomic approach. This new approach, emphasized in this review, is in agreement with recent studies considering lipidomic platforms able to provide an invaluable window to novel pathogenic mechanisms as well as helping to identify early diagnostic and prognostic biomarkers of complex diseases, such as CRC. The attractiveness of lipidomics is that they are strictly connected with nutritional elements and lipid supplementation. This offers an opportunity for prevention and treatment: in prevention, it is important to have nutritional directions in order to maintain the membrane lipid balance in the optimal values; in therapy, it is important to follow nutritional directions that keep membrane receptors and functions at their best, in order to improve the effects of the medical intervention.

Improvement of the comprehension at molecular level of factors derived from nutrition, metabolism and stress that influence the functioning of the membrane compartment is certainly useful to identify and validate membrane profiles to gain a global picture of human metabolic states.

Methods

Human blood samples

Human blood samples collected in tubes containing ethylenediamine-tetraacetic acid (K-EDTA) anticoagulant were layered on a Ficoll–Paque solution and centrifuged at 400x g for 40 min at room temperature. The lymphocytes and plasma were then removed and the erythrocytes were recovered from the bottom layer and washed with phosphate-buffered saline. Isolated red blood cells were stored at -80 °C until they were assayed.

Tissue samples

For the pre-clinical phase of the study, mice treated with specific enriched diets were killed by cervical dislocation. The entire intestinal tract was immediately removed, washed and fresh tissue samples of intestine were collected and stored at -80°C until assayed. All animal experiments were carried out in strict accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

For the clinical phase of the study, patients with histologically proven colorectal cancer were enrolled in the study. At surgery, colorectal mucosa and cancer tissue were obtained from each patient and the specimens were stored at -80°C until assayed. Informed consent was obtained from each patient and the study was approved by the Ethics Committee of IRCCS "S. de Bellis".

To extract cell membrane fatty acids from tissue samples, about 20 mg of mice and human intestinal tissue were used and the method of Folch with slight modifications was performed for cell membrane fatty acids preparation (51,52).

Fatty Acids preparation and quantification

Samples of isolated red cells blood and intestinal tissue were treated with 0.9 mL of a salt solution acidified with sulfuric acid. All samples received 5.0 mL of chloroform:methanol (2:1, v/v) and the samples were centrifuged at 1000xg for 10 min. The lower layer, containing fatty acids, was removed with care, replaced in a new tube and dried by a centrifugal evaporator. The FAME were obtained by adding toluene and BF3 with MeOH 14% and incubating for 2 h at 80°C. After the addition of toluene and 5% aqueous sodium chloride solution, the samples were centrifuged and fatty acid methyl esters contained in the upper layer of the tubes

were collected, transferred into a vial and analyzed by gas chromatography equipment with auto-sampler, a split/splitless injector, FID detector and a hydrogen gas. A BPX 70 capillary column SGE Analytical Science, P/N SGE054623, 60 m x 0.25 mm ID, BPX70 0.25UM was used and the amount injected was 1 uL in splitless mode (split flow 50 mL x min^{-1}, splitlesstime 1 min). Quantification of fatty acid methyl esters was performed using a mixture of standards (Supelco 37-Component FAME Mix, Sigma-Aldrich, Milan, Italy).

Small dense LDL analysis

Blood samples were obtained by venous puncture, after 12 h fasting, and collected in tubes containing Ethylenediaminetetraacetic Acid (EDTA-K2) anticoagulant. The samples were then centrifuged at 2000x g for 10 min at 4 °C to obtain serum and stored at -80°C until use. Small dense Lipoproteins (sdLDL) were assayed using Lipoprint LDL System (Quantimetrix, USA). Each serum sample was applied on a high resolution polyacrylamide gel tube in order to separate LDL fractions and subfractions by electrophoresis. The resolved lipoproteins bands were scanned and analyzed.

Fatty acids profile and CRC

Lipidomic analysis aimed to identify and quantify cellular lipids and their interactions with other cellular components such as proteins and gene expression, as it is known to be a powerful tool to predict cancer progression and the development of metastases (53,54). Previous reports have shown that metabolic perturbation of phospholipids is associated with various cancer types (55-57), indicating that the composition of phospholipids may be critical for deciding the fate of tumor cells.

An altered expression of the fatty acids pattern has been demonstrated to be a crucial event in colorectal carcinogenesis (58-60). Fatty acid synthase (FAS) activity levels, the key enzyme in the fatty acids biosynthesis pathway, as well as its mRNA expression, are upregulated in colorectal cancer tissues (59). Recently, in Apc Min/+ mouse model, we showed that a possible molecular mechanism by which omega-3-polyunsaturated fatty acids (n-3-PUFAs) and olive oil in the diet were able to reduce intestinal cell proliferation was the reduction of lipogenic enzymes activity and gene expression, such as Fatty Acid Synthase (FAS) (61). Fatty acids and their polyunsaturated derivatives influence cell membranes fluidity and their physiological functions.

We demonstrated the presence of an altered fatty acid profile in patients with CRC compared to control subjects; a reduction of total n-3-PUFAs levels and consequently a higher n-6-PUFAs/n-3-PUFAs ratio was detected in cancer patients compared to control subjects (55). In addition, our recent study confirmed that not only several modifications in lipid metabolism occur in colorectal cancer, but that the presence of synchronous metastases was associated with a different tissue fatty acids profile, demonstrating the ability of tissue fatty acids analysis to identify lipid metabolism alterations associated with CRC and with synchronous metastasis (62).

Moreover, in an animal model of colon carcinogenesis, we demonstrated that cancer cell progression is affected by dietary natural compounds, which are able to control and to improve the environmental conditions in which tumors develop (63). The investigation of changes in the lipidomic profile of cell membrane is important to understand the complete scenario of the metabolic transformations which can occur in cancer. These changes may lead to alter the hydration levels and fluidity of cell membranes and to affect the proteins transduction involved in cell proliferation, apoptosis and differentiation (64).

In this context, we have demonstrated that olive oil and omega-3 PUFAs in the diet differently affect the fatty acid profile in intestinal tissue from ApcMin/+ mice, an animal model of CRC (65). Our data support evidences demonstrating the effects of dietary components, such as olive oil and omega-3 PUFAs, in counteracting intestinal carcinogenesis *in vitro* and *in vivo*. These protective effects seem to be due to the olive oil capacity to control the tissue inflammatory status, and to the omega-3 PUFAs ability to keep the cell membrane saturation index (known as stearic acid/oleic acid ratio) at high levels. Moreover, Eicosapentanoic acid (EPA), an omega-3-PUFA, has been demonstrated to affect cell proliferation through the regulation of lipogenic enzymes belonging to the cholesterol biosynthetic pathway (66,67). These enzymes

interact with both intracellular signaling pathways and extracellular microenvironmental stresses. Hypoxia, low pH and nutrient starvation could activate several intracellular signaling pathways to promote lipogenic enzymes expression (68-70).

Figure 1 summarizes the main steps of the lipidomic analysis performed in animals and clinical studies, as well as the effects of dietary intervention on CRC.

The anti-proliferative effects of omega-3 PUFAs seem to be also correlated with the over-expression of cell membrane receptors, which are considered negative modulators of cell proliferation, such as Low Density Lipoprotein (LDL) receptor and Cannabinoid type 1 (CB1) receptor (63,67,71). Different experimental evidences have reported that a CB1 receptor down-regulation is present in different types of cancer (72,73).

The expression of CB1-R seems to be modulated by bioactive natural components (74,75) such as flavonoids. The daily administration of quercetin in an animal model of induced colon cancer, exerted a protective effect against tumor formation by regulating the protein and gene expression level of CB1-R (76). The role of quercetin as an anti-cancer molecule has been confirmed in both *in vitro* and *in vivo* studies, demonstrating its ability to modulate p-STAT3 expression, a biomarker of cell proliferation and aggressiveness.

Low-density Lipoproteins and CRC

Low-density Lipoproteins are composed of subfractions that differ in particle size and density one from the other. Among these LDL subfractions, small dense LDL particles are more atherogenic than larger particles and are considered biochemical markers associated with metabolic syndrome (77). The increased prevalence of smaller LDL particles is certainly related to higher body mass indexes (BMIs), increased visceral adiposity and likely to an inflammatory status consistent with an altered metabolic profile. We have studied the small dense LDL levels in serum from subjects with CRC, demonstrating that a modified LDL pattern was associated with CRC and with the presence of metastasis. The smaller LDL subfractions (LDL-4) were observed in serum of CRC patients with synchronous metastasis whereas these particles were absent in CRC patients without metastasis (Figure 2).

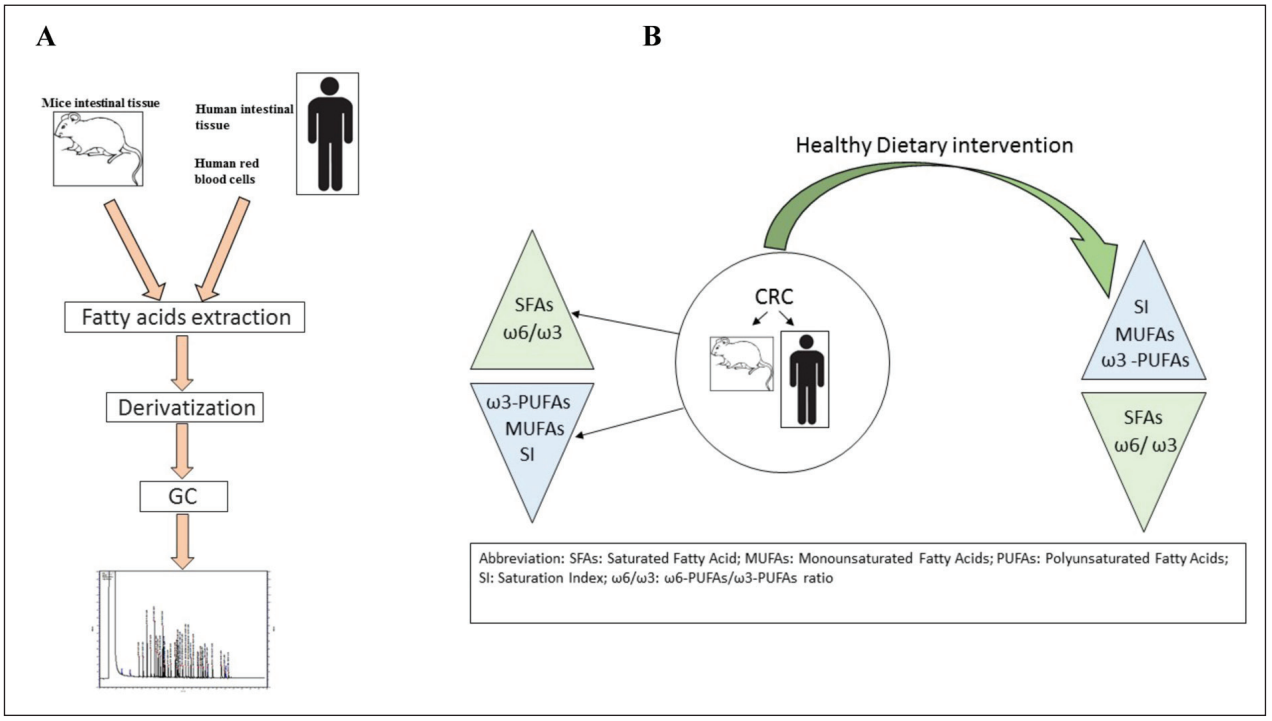

Figure 1. Panel A: Main steps of lipidomic analysis; Panel B: Dietary intervention effects

Literature data indicates that modified lipids, particularly oxidized and glycated low-density lipoprotein (ox-LDL and gly-LDL), are predominant in intestinal tumors, resulting in an increased pro-inflammatory cytokine expression (78-80). Inflammation and oxidative stress due to an increase in reactive oxygen species and a decrease of antioxidant defenses seem to be involved in the molecular mechanisms of colonic tissue carcinogenesis. Therefore, we investigated the levels of ox-LDL and gly-LDL in serum of CRC patients with and without synchronous metastasis. Figure 3 shows that higher levels of ox-LDL and gly-LDL were detected in CRC patients with metastasis in comparison with patients without metastasis, even if the difference was not statistically significant.

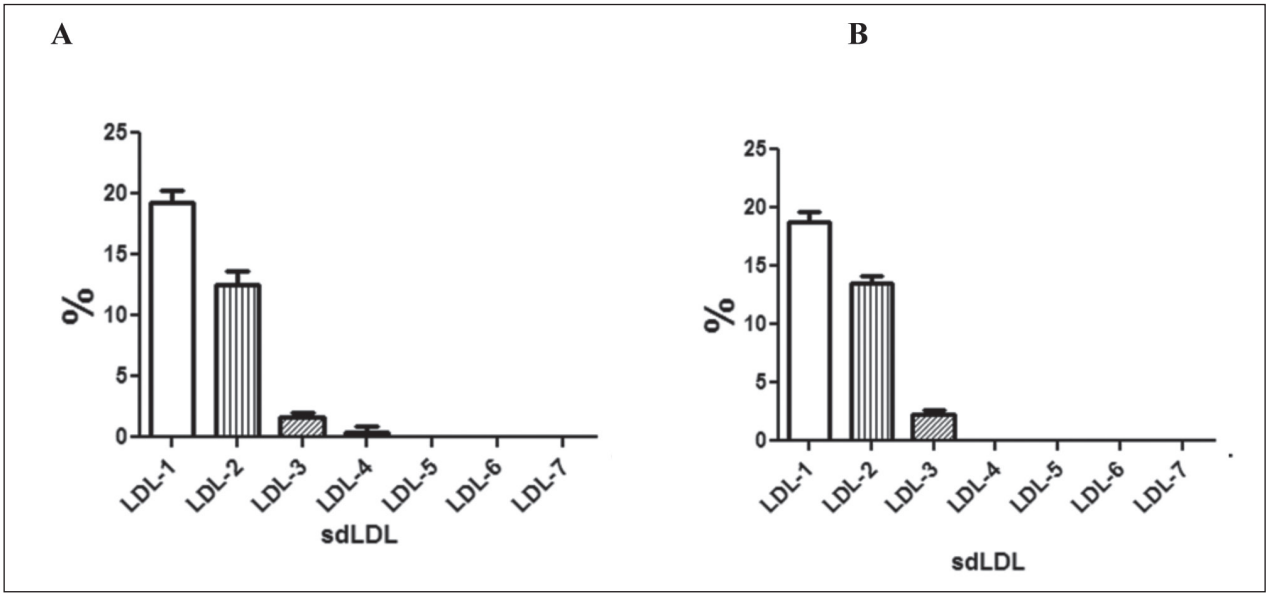

Figure 2. Serum levels of small dense LDL (sdLDL) of 51 cases of CRC patients with (panel A) and without (panel B) synchronous metastasis. All values are expressed as mean ± standard deviation

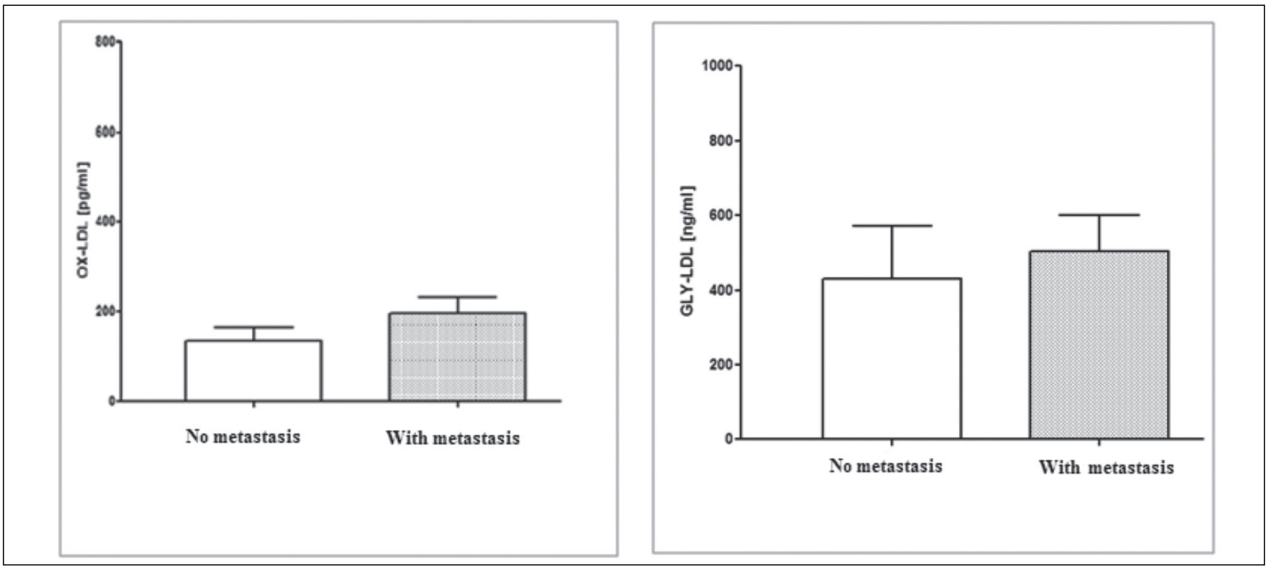

Figure 3. Serum ox-LDL and gly-LDL levels in 51 cases of CRC patients with and without synchronous metastasis. All values are expressed as mean ± standard deviation

Conclusions

We confirm the tight link between diet and lipidomic profile in CRC, as well as the therapeutic role of dietary components on human health. In fact, the marked effect of diet interventions in absence of toxicity can make some of the Mediterranean Diet components excellent candidates for the prevention and treatment of subjects with a high risk for metabolic diseases and CRC. The lipidomic approach could be useful to evaluate the onset and progression of CRC and for the development of nutraceutical lines aimed at membrane balance restoring. In order to connect the lipidomic analysis with the patients clinical status and with canonical biochemical parameters an integrate vision is needed. This vision could better clarify the role of cell membranes fatty acids profiles in the pathogenesis and treatments of CRC.

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Review

Microsatellite instability in colorectal cancer

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Summary. Microsatellites are short tandem repeat DNA sequences of one to tetra base pairs distributed throughout the human genome, both in coding and non-coding regions. Owing to their repeated structure, microsatellites are particularly prone to replication errors that are normally repaired by the Mismatch Repair (MMR) system. MMR is a very highly conserved cellular process, involving many proteins, resulting in the identification, and subsequent repair of mismatched bases, likely to have arisen during DNA replication, genetic recombination or chemical or physical damage. Proteins within the MMR system include MLH1, PMS2, MSH2, MSH6, MLH3, MSH3, PMS1, and Exo1. Deficient MMR (dMMR) results in a strong mutator phenotype known as microsatellite instability (MSI), characterized by widespread length polymorphisms of microsatellite sequences due to DNA polymerase slippage. MSI is recognized as one of the major carcinogenetic pathways of colorectal cancer (CRC): it represents a molecular hallmark of hereditary nonpolyposis colorectal cancer (HNPCC), also known as Lynch syndrome (LS); moreover it is detected in 15% of sporadic colorectal cancers, more often due to an epigenetic inactivation of MLH1. Identification of MSI CRC is important, as MSI may serve as a screening tool for detecting LS, a prognostic marker for patient outcome, and a predictive marker for response to chemotherapy and to immunotherapy. (www.actabiomedica.it)

Key words: microsatellite instability, colorectal cancer, mismatch repair, Lynch Syndrome, prognosis

Background

Microsatellites are short tandem repeat DNA sequences of one to tetra base pairs distributed throughout the human genome, both in coding and non-coding regions. Owing to their repeated structure, microsatellites are particularly prone to replication errors that are normally repaired by the Mismatch Repair (MMR) system (1). MMR is a very highly conserved cellular process, involving many proteins, resulting in the identification, and subsequent repair of mismatched bases, likely to have arisen during DNA replication, genetic recombination or chemical or physical damage. Proteins within the MMR system include MLH1, PMS2, MSH2, MSH6, MLH3, MSH3, PMS1, and Exo1. These proteins form heterodimers that repair DNA damage. During normal DNA replication with proficient MMR (pMMR), small DNA mismatch errors are initially detected and bound by MSH2/MSH6 and MSH2/MSH3 heterodimers. MLH1/PMS2 heterodimers are subsequently recruited for excision and resynthesis of a new, corrected strand. However, deficient MMR (dMMR) results in a strong mutator phenotype known as microsatellite instability (MSI), characterized by widespread length polymorphisms of microsatellite sequences due to DNA polymerase slippage (2).

MSI is recognized as one of the major carcinogenetic pathways of colorectal cancer (CRC): it represents a molecular hallmark of hereditary nonpolyposis

colorectal cancer (HNPCC), also known as Lynch syndrome (LS), usually linked to a germ-line mutation in one of MMR genes; moreover it is detected in 15% of sporadic colorectal cancers, more often due to an epigenetic inactivation of MLH1 (1, 3-4).

Clinicopathologic features of MSI CRC

Tumors with MSI are more common localized in the right colon and they are histopathologically characterized by mucinous features, tumor-infiltrating lymphocytes, poor differentiation with a medullary growth pattern, and a Crohn-like lymphocytic reaction. They are more frequent in stage II and relatively uncommon among metastatic tumors (5). Sporadic tumors typically occur in older female patients, whereas, CRC in the context of LS often occurs in younger patients (50 years of age or less). Finally, CRCs with MSI have a diploid DNA content with few losses or gains of chromosomal regions.

Screening for MSI

Two forms of testing are commonly used in screening patients and tumors for MSI or a deficiency in MMR: polymerase chain reaction (PCR) testing for MSI and immunohistochemical staining (IHC) for altered proteins.

MSI is detected through the comparison of the length of nucleotide repeats in tumor cells and normal cells. The standard diagnostic procedure recommended by the National Cancer Institute involves analyses of tumor and normal tissues using five microsatellite markers (Bethesda panel), including two for mononucleotide repeats (BAT26 and BAT25) and three for dinucleotide repeats (D2S123, D5S346, and D17S250) (6-7). In particular, frame shift mutations in microsatellites can be identified by extraction of DNA from healthy and tumor tissue, amplification of selective microsatellites by PCR, and analysis of fragment size by capillary electrophoresis on a automated sequencer. Samples can be classified into microsatellite instability-high (MSI-H), microsatellite instability-low (MSI-L) or microsatellite stable (MSS) according the percentage of loci with MSI. In particular, the phenotype is defined as MSI-H if two or more of the five microsatellite markers show instability (or >30% of unstable markers if a larger panel is used), as MSI-L if only one of five markers shows instability and MSS if none of the markers show instability (1, 6). A new expert consensus recommends the use of a panel of 5 quasi-monomorphic mononucleotide repeats (BAT-25, BAT-26, NR21, NR24 and NR27), characterized by a constant number of nucleotide repeats and an identical size between individuals, unlike most microsatellites are polymorphic, obviating the necessity to analyze simultaneously non-tumor DNA (8). With this method, two unstable markers are sufficient to classify tumors as MSI (9).

The use of IHC to test for the MMR proteins MLH1, MSH2, MSH6 and PMS2 can be used to indicate the presence or absence of a functional MMR system, and thus, indirectly MSI, and may allow identification of the defective protein, which can then be used to direct mutation analysis to the relevant gene (10). It should be considered that MMR proteins PMS2 and MSH6 cooperate with MLH1 and MSH2 respectively and their expression closely depends on the binding to the major partner (i.e. MLH1 and MSH2). Therefore, loss of expression of MSH2 is frequently associated with loss of expression of MSH6 and this pattern is highly suggestive of MSH2 germline mutation. Similarly, loss of expression of MLH1 is frequently associated with loss of expression of PMS2 and this pattern may result either from MLH1 germline mutation or from acquired somatic hypermethylation of the MLH1 gene promoter. Germ-line mutations of MSH6 and PMS2 are generally associated with isolated loss of expression of MSH6 and PMS2 protein respectively (11). Both IHC and PCR are sensitive and specific for dMMR and MSI, and the two tests show high concordance (>95%) (12).

Recently, several groups have evaluated new methods to assess MSI using next generation sequencing (NGS) technologies from tumor and/or normal tissue pairs (13-15). NGS refers to a group of technologies which have, in common, the ability to perform and capture data from millions of sequencing reactions simultaneously, also called massively parallel sequencing (16). Hause *et al.* developed the MOSAIC meth-

od for crosssectional MSI analysis in 18 cancer types including CRC using the cancer exomes from the Cancer Genome Atlas database. This method, based on weighted-tree microsatellite instability classifier (MOSAIC) for predicting MSI status using the most informative and independent features for classifying MSI, had a high sensitivity and specificity in identifying MSIH tumors (17). However, NGS remains restricted to highly specialized laboratories and requires high quality samples from both tumor and normal tissues. These strategies are generally more expensive, as higher throughput sequencing machines and complex data processing pipelines are required.

Clinical significance of MSI

MSI occurs in around 15% of all CRC tumors in white populations (18-19). It arises as a result of defective MMR caused by the failure of one of the four main MMR genes, MSH2, MLH1, MSH6, or PMS2. There are two different types of MMR gene failure: caused by an inherited germline mutation in one allele followed by somatic inactivation of the wild-type allele in a colonic mucosa cell (these individuals have Lynch syndrome and account for 3% to 5% of all CRCs), or failure caused by somatic inactivation of both alleles.

LS is an autosomal dominant disorder that increases the risk of developing CRC and endometrial adenocarcinoma, as well as tumors of the small intestine, stomach, ureter, renal pelvis, ovary, brain, prostate (20). Patients with LS benefit from increased surveillance; therefore, identification of patients as well as family members with this syndrome is very important. Since most (90%) CRC due to LS have MSI, MSI testing may serve as a screening tool for detecting LS.

Multiple retrospective and population-based studies have shown that patients with MSI-H CRCs have a more favorable stage-adjusted prognosis than those with MSS tumors (21-22). It has been suggested that the improved prognosis of MSI-H CRC may result from the pronounced antitumoral immune response of the host. In fact, lymphocytes infiltration, even with a Crohn's like reaction, is prominent in MSI CRCs. This is due to the lack of MMR system with the consequent accumulation of frame-shift mutations that causes the transcription and translation of peptides with altered amino acid sequences (neoantigens), that are presented by HLA class I and are recognized by cytotoxic T cells (23).

While it has been relatively well-established that the prognosis is better for patients with MSI-H CRC, whether MSI status predicts response to adjuvant chemotherapy has been more controversial. Numerous studies seem to suggest a lack of benefit of 5-FU chemotherapy in patients with MSI (24-26). On a molecular level, there is in vitro data supporting the fact that patients would need an intact MMR system to induce apoptosis of fluorouracil (FU)-modified DNA (27). Several studies supporting MSI-H as a predictive factor for improved response to irinotecan or irinotecan-based chemotherapy in CRC patients have been reported (28-29).

Recently, there has been an increased recognition of the host immune system importance in controlling tumor progression and new immunologic biomarkers have been included as a tool for the prediction of prognosis and response to therapy. MSI CRC selectively displays highly up-regulated expression of multiple immune checkpoints, including PD-1, Programmed Death-ligand 1 (PD-L1) and CTLA-4. It has been theorized that strategies involving the blockade of these immunoregulatory mechanisms might be selectively effective in this critical subset of CRC (30). Data from this study support the hypothesis that MSI tumors are more responsive to PD-1 blockade than are tumors with a proficient MMR system. Moreover, this data validates an approach for the management of a particular sub-set of tumors that is based solely on molecular status, without regard to the underlying tumor site. So, on May 2017, the Food and Drug Administration (FDA) approved pembrolizumab, a programmed death 1 (PD-1) inhibitor, for the treatment of adult and pediatric patients with unresectable or metastatic, MSI-H or dMMR solid tumors, regardless of tumor site or histology (31).

In conclusion, identification of MSI CRC is important, as MSI may serve as a screening tool for detecting LS, a prognostic marker for patient outcome, and a predictive marker for response to chemotherapy and to immunotherapy.

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Review

Potential predictive biomarkers in locally advanced rectal cancer treated with preoperative chemo-radiotherapy

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Summary. Fluorouracil-based preoperative chemoradiotherapy represents a standard option for the treatment of locally advanced rectal cancer. Randomized clinical trials have shown that fluorouracil concomitant to preoperative radiation enhances tumor shrinkage (with 10% to 15% of the patients showing a complete pathological tumor response) compared with preoperative radiation alone. A high response rate is of clinical importance in rectal cancer, since patients who achieve a complete pathological response may experience improved long-term survival. Adding oxaliplatin to fluorouracil-based preoperative chemoradiotherapy has no effect on response of the primary rectal tumor and single-agent fluoropyrimidine remains the standard chemotherapy in this setting. Despite novel biological insights and therapeutic advances, little is known about potential biological markers able to predict pathological tumor response before treatment and to subsequently impact patients' prognosis. This review focuses on the current available data on main molecular markers and molecular subtypes and the possible upcoming introduction of such analyses in the clinical setting. (www.actabiomedica.it)

Key words: rectal cancer, marker, chemo-radiotherapy, prognosis

Background

Preoperative radiation therapy alone (RT) or combined with chemotherapy (RCT) have improved the management of locally advanced rectal cancer patients (1, 2). With this approach, pathologic complete response (pCR), which is an important predictor for both local and disease-free survival, is achieved in up to 30% of patients (3). Furthermore, achieving a complete or near-complete pathologic response before surgery may increase the number of sphincter-sparing procedures (3). No benefit from adding oxaliplatin could be demonstrated on primary tumor response to preoperative chemoradiation (4-6) and chemotherapy with fluoropyrimidine remains the standard of care.

Only limited data are available regarding the role of biomarkers to predict complete pathological response to neoadjuvant chemoradiotherapy in locally advanced rectal cancer patients (Table 1). Subgroup analyses are ongoing to investigate if there are patients gaining a greater benefit from investigational treatment. The ability to predict the pathological tumor response before treatment may significantly affect the selection of patients for preoperative combined therapy and may potentially adapt the choice of post-operative treatments. There is, therefore, an unmet need to improve individual treatment approaches in this setting.

Complex molecular and clinical phenotypes trigger the development and progression of rectal cancer, thus yielding different pathological responses to treat-

Table 1. Potential predictive biomarkers

Glossary of molecular markers			
Marker	Abnormality or abnormal gene	Functions of wild-type gene product	Reported prognostic or predictive value in CRC
TS	Overexpression	Pyrimidine metabolism	Adverse prognostic marker, adverse predictive marker
P53	Overexpression	Control of DNA topology	Adverse prognostic marker
ERCC1 (9, 25)	Overexpression	Repair of platinum agents-DNA adducts	Adverse prognostic marker, adverse predictive marker
HER-2 (26-28)	Overexpression	Cellular signal transduction	Predictive marker
MSI	Consequence of abnormal genes in mismatch repair family	Repair of nucleotide mismatches	Favorable prognostic marker, adverse predictive marker
PD-L1 (29, 30)	Overexpression	Immune checkpoint	Adverse predictive marker
PTEN (31, 32)	Loss of expression	Phosphatase activity	Adverse prognostic marker, adverse predictive marker
CD3	Overexpression	Cellular signal transduction	Favorable prognostic marker
CD4	Overexpression	Cellular signal transduction	Favorable prognostic marker
CD8	Overexpression	Cellular signal transduction	Favorable prognostic marker

ERCC1: excision repair cross-complementing 1; HER2: human epidermal growth factor receptor 2; MSI: microsatellite instability; PD-L1: programmed death-ligand 1; PTEN: phosphatase and tensin homolog

ment (7). Recent molecular analyses uncover that tumors arising in the rectum may carry distinctive genetic alterations from other colon cancers (8). Compared to left colon cancers, rectal cancers display a higher frequency of *TP53* (71% vs. 57%, p=0.03) and a higher expression of excision repair cross-complementing 1 (ERCC1) (29% vs. 15%, p=0.03) (8), which is a marker of resistance to platinum drugs (9). Additionally, approximately 50% of rectal cancers express high levels of thymidylate synthase (TS), which is involved in pyrimidine nucleotide synthesis and it is an important target for 5-fluorouracil (5-FU) (10, 11).

Finally, some studies focus on the importance of immune infiltration to predict the clinical outcome of untreated patients but also to predict the response to treatment (12, 13). The presence of immune cells may reveal a distinct biology of the tumor, as gene expression profiling and other assays have unveiled.

This review focuses on the current available data on some of the molecular markers and on comparative analyses that showed molecular variations among rectal tumors that might contribute to differences in clinical behavior of rectal cancer tumors.

TS (thymidylate synthase)

TS is an enzyme that catalyzes the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) and is essential for 'de novo' DNA synthesis (14). The tissue expression of TS may affect tumor sensitivity to fluoropyrimidines, such as 5-FU (15). The role of TS in fluoropyrimidine cytotoxicity has been established in both preclinical and clinical studies (16, 17). Moreover, the association between TS levels and resistance to 5-FU could depend on the 5-FU schedules of treatment used and/or biochemical modulators and on the degree of incorporation into RNA (18). These may result in different mechanisms of cytotoxicity, potentially affecting the correlation between thymidylate synthase (TS) expression and the clinical response to the fluoropyrimidine.

Aschele and coll. (19) showed that TS levels predicted clinical response only for regimens involving continuous infusion with a higher response rate in patients with low and high TS levels compared with high TS levels (66% versus 24%, respectively). Conversely, TS expression failed to predict the clinical response within the group of patients treated bolus 5-FU.

To date, however, only a small number of retrospective heterogeneous studies have addressed the issue of TS expression levels and tumor response in rectal cancer patients, especially FU-based chemo-radiotherapy (20, 21). In rectal cancer, low TS gene expression has been found to correlate with pathological response to neoadjuvant FU-based CRT (20). In contrast, another study from our group showed a significant interaction between high TS level and the probability of achieving a pathological response (21). Several factors may account for these controversial results on the predictive role of TS expression. The first may be related to the different techniques used to assess TS levels. For example, a significant correlation between protein expression and tumor response in rectal cancer patients was seen only when both staining intensity and staining pattern were evaluated, with a significant association between high TS expression in tumor biopsies and resistance to therapy (20). Moreover, in contrast with previous data, in our study, FU was administered as continuous infusion and strong TS expression was found to be predictive of pathological tumor response to treatment. Therefore, the potential of TS expression levels to predict tumor response to preoperative combined-modality therapy remains to be proven.

p53

p53 mutations have been described in about 40% to 50 % of colorectal carcinomas and are associated with an aggressive behavior and resistance to chemoradiotherapy in several tumor models (22).

Microsatellite instability (MSI)

High microsatellite instability (MSI-H) status is a predictive marker for lack of response to 5-FU-based chemotherapy compared with microsatellite stable (MSS) disease (23). Moreover, MSI is a useful predictive criterion for irinotecan response in patients with colorectal cancer (24) (reviewed elsewhere).

Tumor infiltrating lymphocytes (TILs)

With the exclusion of MSI, which is limited to a small subgroup of rectal cancers, recent genetic and molecular studies did not identify any novel predictive biomarkers (33). One possible reason is that until recently research has been mainly focused on cell processes rather than on tumor microenvironment (34). Nowadays, a large body of data from retrospective cohorts of solid tumors has shown that the in situ immune infiltrate may have a strong impact on patients' outcome (35). The immune infiltrate has been shown to overcome the TNM scoring system in predicting survival and to influence the outcome also of colorectal cancer patients (36-38). To quantify the immune infiltrate, an "immunoscore" based on the enumeration of CD3 and CD8 lymphocytes within the core of the tumor and the invasive margin has been suggested (39). This applies also to rectal tumors, as an inverse relationship between tumor invasion and the extent of immune cell infiltration has been reported (40, 41). Moreover, the immunoscore seems to be a useful prognostic marker in rectal cancer patients treated by primary surgery (41). Studies on larger cohorts of patients are ongoing to validate the former results. In fact, a positive result could provide the rationale to assess the immune infiltrate in biopsies to predict potential responders to preoperative treatments and to select them for new strategies with minimal or even no surgery.

Conclusions

The overall landscape is multifaceted and our knowledge on this issue is still at the starting point.

Doubtlessly, analyzing and genotyping distinct tumor subtypes and setting apart patients with distinctive diseases represent the goal of future treatments to pave the way for precision medicine also in rectal can-

cer patients. Finally, accurate tools to predict response to therapies should probably consider both the genetic features and the immune components of the tumor.

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Review

Epidemiology and risk factors for diverticular disease

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Summary. Diverticulosis of the colon is the most frequent anatomical alteration diagnosed at colonoscopy. The prevalence of the disease is higher in elderly patients over 65 years old, recent studies show an increment also in youngers over 40 years old. Even its large prevalence in the population, its pathophysiology still remain poorly understood. It's widely accepted that diverticula are likely to be the result of complex interactions among genetic factors, alteration of colonic motility, lifestyle conditions such as smoking, obesity, alcohol consumption, fiber and meat intake with diet. Recently many authors considered also alterations in colonic microbiota composition, co-morbidity with diabetes and hypertension and the chronic assumption of certain medications like PPI, ARB and aspirin, as important risk factors for the development of diverticulosis. The aim of this narrative review is to summarise current knowledges on this topic. (www.actabiomedica.it)

Key words: diverticulosis, diverticular disease, risk factors, epidemiology

Introduction

Diverticulosis of the colon is an anatomic alteration of the colonic wall characterized by the presence of pockets (called diverticula) which occur when colonic mucosa and sub-mucosa herniate through defects in the muscle layer of the colon wall (1). Diverticulosis is merely the presence of colonic diverticula; these may, or may not, be symptomatic or complicated. 'Diverticular disease' is defined as clinically significant and symptomatic diverticulosis; this may be from true diverticulitis or from other less well-understood manifestations (e.g. visceral hypersensitivity in the absence of verifiable inflammation) (2). The overarching term 'diverticular disease' implies that the pathologic lesion (diverticulosis) rises to the level of an illness. Symptomatic Uncomplicated Diverticular Disease (SUDD) is a subtype of DD in which there are persistent abdominal symptoms attributed to diverticula in the absence of macroscopically overt colitis or diverticulitis. In contrast, 'diverticulitis' is the macroscopic inflammation of diverticula with related acute or chronic complications. Diverticulitis can be uncomplicated or complicated. The uncomplicated DD is characterised by colonic wall thickening with fat stranding at computerised tomography (CT); on the contrary complicated DD presents complicating features of abscess, peritonitis, obstruction, fistulas or haemorrhage. Segmental colitis associated with diverticulosis (SCAD) is a unique form of inflammation that occurs in areas marked by diverticulosis. Endoscopic and histological characteristics describe it as a forerunner of inflammatory bowel disease (IBD) (3).

Epidemiology

For many years it has been thought that this type of diverticulosis exclusively affected the westernized world and was due to a lack of fiber intake in the diet

and increased pressure in the colonic wall (4), however, recent data have revealed an increase in the prevalence of colonic diverticulosis throughout the world (5). Necroscopic studies from the first part of the 20th century show a colonic diverticular disease incidence between 2-10% and 5-20% in patients whom underwent a colonoscopy examination (6) being more often encountered in male patients at that time (7). This distribution model is now observed in developing countries. Later studies showed an incidence levelling between genders (8). Studies after the year 2000 showed an increasing incidence up 27% among patients that underwent colonoscopy, being more often encountered in elderly patients (9).

Worldwide incidence of diverticular disease

The anatomic distribution of diverticulosis in the colon also varies by geographic locations. In individuals that reside in western industrialized nations, diverticula are limited to just the sigmoid colon in 65%, sigmoid plus other colonic diverticula in 25%, pan colonic diverticula in 7%, and diverticula isolated to a segment proximal to the sigmoid colon in 4% of patients (10). In Asian population, the anatomic distribution is different and primarily involves the right colon with a rate of approximately 13 to 25% (11). Worldwide diverticular disease has the highest incidence in the United States, Western Europe and Australia (6, 12), reaching 50% in the population aged 60 and above; on the other hand, in sub-Saharan countries the disease is rare and encountered in the 4th decade (13). Nigeria reports an incidence as low as 9.4% among patients that underwent colonoscopy (5), and Calder finds a frequency of diverticular disease of 6.6% in Kenya (14).

The low incidence of diverticular disease in African countries can be due to limited access to healthcare in the general population, and to the low life expectancy in this area (15). On average, the prevalence of diverticular disease among Caucasian Western patients whom underwent barium enema is 15-35%, being equally distributed between genders, but more frequent in the elderly, affecting the left colon in 90-99% of the cases (15, 16). In South-Eastern Asia, the prevalence varies between 8 and 25% (17), reaching a peak in the 5th decade (18, 19) , affecting the right colon in 70-98% of the cases (19) .

Incidence of diverticular disease relative to age

Diverticular disease was first attributed to elderly patients, with a maximal incidence in patients above 70 years old (20). Recent medical literature shows however an increase in diverticulosis incidence in young patients. The most relevant increase was encountered in the group aged 18-44, where the incidence per 1,000 pop. rose from 0.151 to 0.251 in only 7 years. The incidence in patients aged 45- 64 knows

Incidence of diverticular disease around the globe (personal collection using GunnMap)

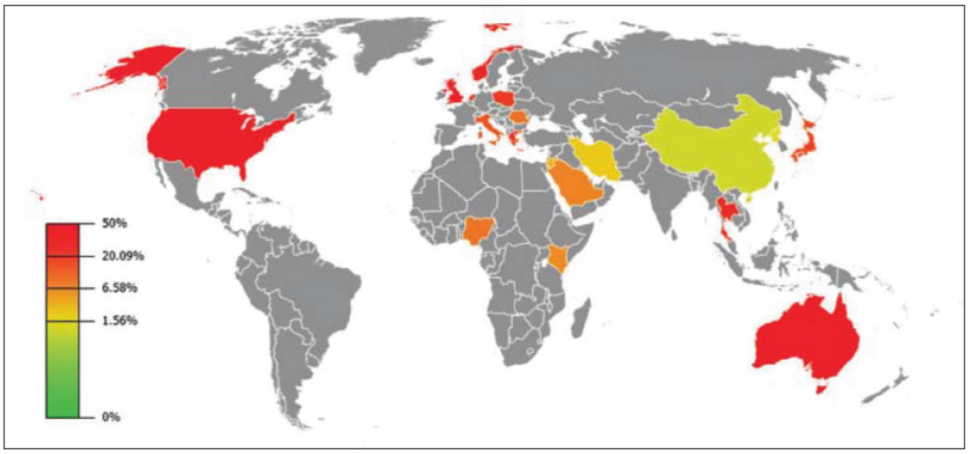

Figure 1. Worldwide incidence of diverticular disease

a lesser increase (from 0.650 to 0.777 over the same period) (21). Although it is well documented that prevalence of diverticulosis increases with age, multiple studies in the past decade have been looking at incidence and disease progression in younger patients who present with diverticulitis. A prospective analysis of 207 patients hospitalized at a single institution with diverticulitis between the ages of 27 and 92 years with mean age of patients being 61 years and found that 25 of the patients were younger than 45 years. The study found that diverticulitis in young patients has a male predominance and a more aggressive course with higher complication and recurrence rate (22).

Gender distribution of diverticular disease

Gender distribution has also changed over the years, initially the diverticular disease being encountered more often in males. However, recent data indicates that males under 50 have a higher incidence of diverticular disease, while after the 4th decade the illness is more frequent in females, as shown by a study from the United Kingdom between 1989 and 2000. Similar results were obtained in Canada, where males under 50 were more prone to diverticular disease (12).

Risk factors

In figure 2 are summarised the current knowledges that will be analysed in this focus on.

AGE: Diverticulosis was first observed in older patients, with a maximal incidence in patients over 70 years old (6), Although advancing age is obviously associated with diverticulosis, this association is not strong *per se*, because the prolonged time course during which the colonic wall is exposed and susceptible to other pathogenetic factors plays an important role (23).

GENETIC FACTORS: Heritability factors also seem to play a role in the development of DD. Some well-defined genetic diseases are associated with a higher incidence of DD. Patients with Ehlers–Danlos syndrome (24), Williams–Beuren (25), Coffin–Lowry (26) and renal polycystic disease (27) are prone to develop diverticula with colonic or other localization. The association with collagen disease can offer regarding the mechanisms that lead to diverticula formation. All these syndromes have in common extracellular matrix defects, suggesting that elastin and collagen accumulation in the smooth muscle may be a prerequisite to diverticula formation (3).

COLONIC MOTILITY: Neural degeneration with age may also contribute to diverticulosis occurrence, with several studies suggesting reduction in neurons in the myenteric plexus (28) and decreased myenteric glial cells and interstitial cells of Cajal (29). Denervation hypersensitivity has also been reported (30), and these abnormalities of enteric nerves might

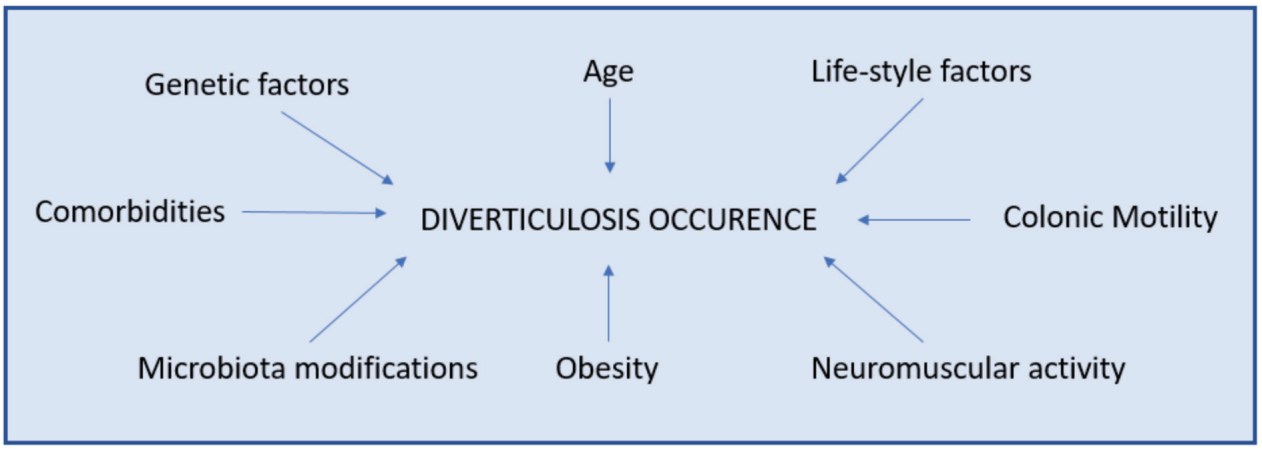

Figure 2. Current knowledges on possible risk factors for diverticulosis occurrence

lead to uncoordinated contractions and high pressure, producing diverticulosis.

NEUROMUSCULAR ACTIVITY: Serotonin is an important neuroendocrine transmitter participating in the control of colonic motor activity through neural and biochemical mechanisms in the enteric nervous system (31). Thus, a possible pathophysiological factor for diverticulosis occurrence has been hypothesized. However, a significant decrease in signalling including content, release, and 5-Hydroxytriptamine (5HT) transporter (SERT) transcript levels was present in the mucosa of patients with a history of diverticulitis compared with controls, but not in those with asymptomatic diverticulosis (32).

FIBRE INTAKE: The old study from Painter and Burkitt found a significantly different diverticulosis prevalence in a western population than in an African population, in which fiber intake is significantly different (6). Two recent colonoscopy-based studies raised the question of the role of fiber intake. Song and colleagues did not find differences in dietary fiber scores between patients with and without diverticulosis assessed by the Mini Dietary Assessment index (33). Moreover, two recent studies conducted in the USA and Japan surprisingly found that dietary fiber intake was positively associated with the presence of diverticulosis (34, 35).

RED MEAT INTAKE: The decrease in fiber intake typically seen with industrialization is paralleled by other dietary changes, including an increase in red meat intake. However, epidemiological studies have provided conflicting results (23). Aldoori and colleagues found significant association between red meat intake and increased risk of DD, even with no dose–response relationship (36). Peery and colleagues did not find any relationship between red meat intake and diverticulosis found at colonoscopy (37).

ALCOHOL INTAKE: There have been conflicting reports on the association of alcohol use and diverticular disease. One of the last meta-analysis found in literature shows that there is no clear association between alcohol consumption and diverticulosis neither diverticular bleeding (38).

SMOKING: The relation between smoking and diverticular disease is debate in literature. Aldoori found smoking was not appreciably associated with risk of symptomatic DD compared with non-smokers (RR=1.25; 95% CI 0.75-2.09) after adjustment for age, physical activity, and energy-adjusted intake of dietary fiber and total fat (39). The recent meta-analysis conducted by Aune on the other hand, provides evidence that tobacco smoking is associated with an increased incidence of diverticular disease and related complications (40).

OBESITY AND PHISICAL ACTIVITY: A relationship between body mass index (BMI) and DD was demonstrated; men with a BMI between 20 and 22.5 kg/m2 had the lowest risk. After adjustment for covariates, the risk increased linearly in men who had a BMI of 22.5-25 (multiple adjusted HR 2.3; 95% CI 0.9-6); 25–27.5 (HR 3; 95% CI 1.2-7.6); 27.5-30 (HR 3.2; 95% CI 1.2-8.6); and 30 or greater (HR 4.4; 95% CI 1.6-12.3) kg/m2 (p for linear trend=0.004) (41). Significantly, neither Strate (42) nor Song (33) found a significant relationship between BMI and diverticulosis detected at colonoscopy.

Physical activity also seems to show the same behaviour, with a significant relationship with reduction of DD complications but with less evidence of diverticulosis occurrence (37, 39, 43, 44).

MICROBIOTA: The detection of small intestinal bacterial overgrowth in patients with diverticulitis supported the hypothesis that bacterial imbalance could play a role in disease occurrence (45). Unfortunately, more recent studies seem inconclusive. Daniels and colleagues recently compared the fecal microbiota of patients with diverticulitis with control subjects from a general gastroenterological practice using a polymerase chain reaction based profiling technique on DNA isolates from fecal samples. They found that Firmicutes/Bacteroidetes ratios and Proteobacteria load were comparable among patients and controls ($p = 0.20$), while a higher diversity in diverticulitis for Proteobacteria ($p < 0.00002$) and all phyla combined ($p = 0.002$) was found (46). Tursi and colleagues recently found no differences in the numbers of rRNA gene copies either for total bacteria or in the different types

analysed in the stool samples comparing patients with Symptomatic Uncomplicated Diverticular Disease (SUDD), patients with acute diverticulitis and healthy controls (47).

MEDICATIONS: Multiple medications have been reported to be associated with diverticular disease. Regular use of nonsteroidal anti-inflammatory drugs and aspirin has been associated with increased risk of diverticular bleeding (48); an increased risk of diverticular disease is also suggested in patients taking steroids and opiates as shown in other studies (49). There is evidence to suggest that statins may have a protective effect against diverticular perforation in patients with diverticulosis (50).

COMORBIDITIES: In literature we can also find the association between diverticulosis and other disease like hypertension and diabetes. Sakuta found that the prevalence rates of type 2 diabetes and hypertension are higher among the middle-aged male subjects with asymptomatic colonic diverticulum (51); moreover Yang provided evidence that the correlation between hypertension and diverticular disease is higher in female patients (52).

Conclusions

Diverticular Disease is a worldwide condition that affect elderly people with an increasing incidence in younger patients as well as in developing countries that have started adopting western diets. Despite its prevalence, its pathophysiology still remains poorly understood and a complexity of factors may play a role in its pathogenesis. There is a significant need for more studies to improve our understanding about risk factors and complications.

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Review

Development and usefulness of the new endoscopic classification: DICA

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Summary. Colonic Diverticulosis is one of the most common anatomical findings during colonoscopy. This condition has 60% incidence in the population over 60 years old. About 20% of patients will develop Diverticular Disease, and 5% of them will evolve into Diverticulitis. Until the last years there weren't any approaches for the endoscopic classification of this pathology. In 2013, in Florence, the first endoscopic classification was developed: DICA (Diverticular Inflammation and Complication Assessment). The aim of this article is to focus on the process of the development and the validation of the classification by the pool of gastroenterology experts, and, as well, its usefulness during the clinical practice. (www.actabiomedica.it)

Key words: DICA classification, diverticular disease, endoscopy, colonoscopy, colonic diverticulosis, diverticulitis

Diverticular Disease

Colonic Diverticulosis is one of the most common clinical-anatomical alterations of the Western world.

Diverticular Disease involves multiple clinical conditions, where at the pathogenesis there is always the same lesion, the colonic diverticula, that goes from the asymptomatic form to the symptomatic not complicated one, including episodes of acute inflammation (1).

This pathology shows a 60% incidence in the population over 60 years old; about 20% of these patients will develop the symptomatic form of it, called Diverticular Disease (DD), and about 5% will develop to complications, such as acute Diverticulitis (2, 3).

It's important to be able to differentiate the different scenarios in which the diverticula could be identified, according to the current definitions (4): Colonic Diverticulosis, also known as simple presence of diverticula in the colon; Diverticular Disease which is the symptomatic form of Diverticulosis and could range from a mostly functional form to a really debilitating condition; the Uncomplicated Symptomatic Diverticular Disease is classified like a subtype of DD and it is characterized by abdominal pain (mostly in the left lower region) associated with the presence of diverticula, without any signs of inflammation; finally, Diverticulitis is characterized by macroscopic acute inflammation of the diverticulum (5).

The clinical classification of the Diverticula Disease is currently based on the EAES criteria (European Association for Endoscopic Surgery), dividing this condition in:

- Chronic Symptomatic Diverticular Disease;
- Symptomatic Uncomplicated Diverticula Disease (SUDD);
- Symptomatic Complicated Diverticular Disease (6).

EAES classifies as complicated all those conditions that go from the acute episode of Diverticulitis to the diverticular bleeding, stenosis or diverticular perforation (6).

The technique that, nowadays, has the major role in the diagnosis and in the management of DD is the endoscopic procedure. The benefit of the colonoscopy is that has a role in both diagnosis and treatment of some of the complications, such as diverticular bleeding when the localization is known (7).

Colonic Diverticulosis is one of the most common findings during routine colonoscopy, as well as during screening exams (8).

Moreover, the colonoscopy, when performed in early stages, is able to recognize the first signs of Diverticulitis; a recent study shows how premature findings of Diverticulitis with few accompanying symptoms could be individuated in the 2% of patients that made the endoscopic exam without any evident clinical complain (9). The most common endoscopic finding, in this case, would be the presence of hyperaemia and oedema (10).

However, the role of the colonoscopy is controversial and this technique is not indicated in cases where there are evident clinical signs of Diverticulitis because this exam could lead to some complications. The most convenient waiting period, before being able to make an endoscopic exam, is at least 6 weeks after the acute episode (11). On the other hand, there is a study that underlines that, after excluding with the CT-scan the presence of free air in the abdomen, the colonoscopy done before 6 weeks doesn't lead to any complications (12). When the Diverticulitis is not complicated, the episode should be resolved in a few days; but, if after 10 days there isn't an improvement of the conditions, the endoscopic examination should be performed in order to exclude some other possible conditions that could explain the persistence of the symptoms (13). Lahat et al., conducted a study that demonstrated that with an early colonoscopy performed to the patients that still had abdominal symptomatology, in 17% of the cases other comorbities in addition to Diverticular Disease were found (14).

DICA classification

Until the lasts years, there wasn't any approach for the classification of Diverticular Disease. Some of the classifications are based just on the manifestations of acute episodes of Diverticulitis showed by CT scan (like the Hinchey modified classification (15)), some others are just depending on the clinical picture of the pathology (like the Scientific Committee of European Association for Endoscopic Surgery); but, the majority of these classifications are mostly focused on the severity of the acute episode of on Diverticulitis instead of the comprehension of the whole spectrum of DD.

Until 2013 an endoscopic classification is missing, and as well it is known that some of the endoscopic findings are able to predict the evolution of the pathology (15). Is also known that patients with Diverticular Disease could present different clinical and endoscopic pictures of the same condition; for example, a patient with just some rare diverticula in the sigma is different from a patient with sigmoid rigidity and many diverticula but there is no evidence that these differences have any prognostic meaning (16). It's difficult to give a real prognostic value to conditions described like "diffuse diverticula of the colon" or just "inflammation of the diverticulum".

The aim of the DICA classification is to define, through the colonoscopy, a simply, validated and reproducible score of Diverticular Disease (15).

The acronym DICA means Diverticular Inflammation and Complication Assessment. DICA score is the sum of different parameters, like the extension of Colonic Diverticulosis, the number of Diverticula per region, the presence and the type of inflammation, the presence and the type of possible complications (15).

The elaboration of the DICA classification was made in Florence in the 2013, and the process was divided in three parts; in the first phase were identified the most common endoscopic findings, observed during the colonoscopy and to each of them were attributed the scores, during the second part the classification's reproducibility was verified and the set of items was modified in order to improve the simplicity, finally, in the third moment was measured the closeness of agreement (15).

The main steps in the implementation process of the development have been: the choice of the pool of endoscopic doctors and gastroenterologists, coming from different backgrounds such as university hospitals, endoscopic centers and first, second and third level hospitals (15); the choice of the endoscopic videos

that the doctors had to watch and the assignment of the different endoscopic items to take in consideration for the drafting of the classification (15).

In order to choose the entire different endoscopic elements to be included in the classification, the pool of experts watched 300 videos of colonoscopy where Diverticular Disease was presented in all of its forms (15). After the view of the videos, the most frequent findings were reported by the gastroenterologists. The identified items were: the extension of the Colonic Diverticulosis, the number of diverticula for each colonic segment, the type of inflammation and the possible complications (15). These items were the result of a careful review of all the colonoscopies, respecting the importance and the reproducibility of the most relevant characteristics of the pathology.

Then, the chosen items were further developed: when referring about the extension of Diverticulosis it was used the four anatomical parts of the colon (ascending, transverse and descending colon and the sigma); for the number of diverticula four different grades were established (Ist grade less than 5 diverticula, IInd grade from 5 to 10, IIIrd grade 10-20, IVth grade more than 20 diverticula); for the inflammation they identified the presence of oedema (congestion of the diverticula with loss of the vascular structure of the submucosae), hyperaemia (fig. 1), erosions (fibrinous ulcers with a diameter less than 0.5cm of the peri-diverticular and diverticular mucosae) and pus (purulent material coming from the diverticulum opening) (fig. 2); for the complications they focused on colonic rigidity, stenosis (fig. 3), Segmental Colitis associated with Diverticula (SCAD) and diverticular bleeding (15).

After detecting the items subjects to the classification, the next step was to attribute the score for each one of them, corresponding to their importance (15), meaning that a detection of an increase of severity in the inflammation and endoscopic complication leaded to higher points (15).

At the end of the process, 4 different scores were individuated: DICA 1, when the sum of the points was less than 4; DICA 2, between 5 and 7; DICA 3, between 8 and 12; DICA 4, more than 13 (15).

For the development of the classification, 70 patients were diagnosed with Diverticulosis and, from them, 30 videos were selected, based on the quality of the imaging (the endoscopic exploration of the colon had to be completed reaching the ileum and the bowel cleanse had to be adequate). All the videos were watched divided in 5 blocks and, at the end of each of them, a discussion was opened. From those discussions it resulted that the last score, DICA 4, was too complex to be used in the clinical practice, and that to differentiate a low and a medium number of diverticula (5 and 10 correspondingly, during the whole colonoscopy) was too pretentious, along with the discrimination of all the four colonic regions (15). The presence of pus coming from the opening of the diverticulum

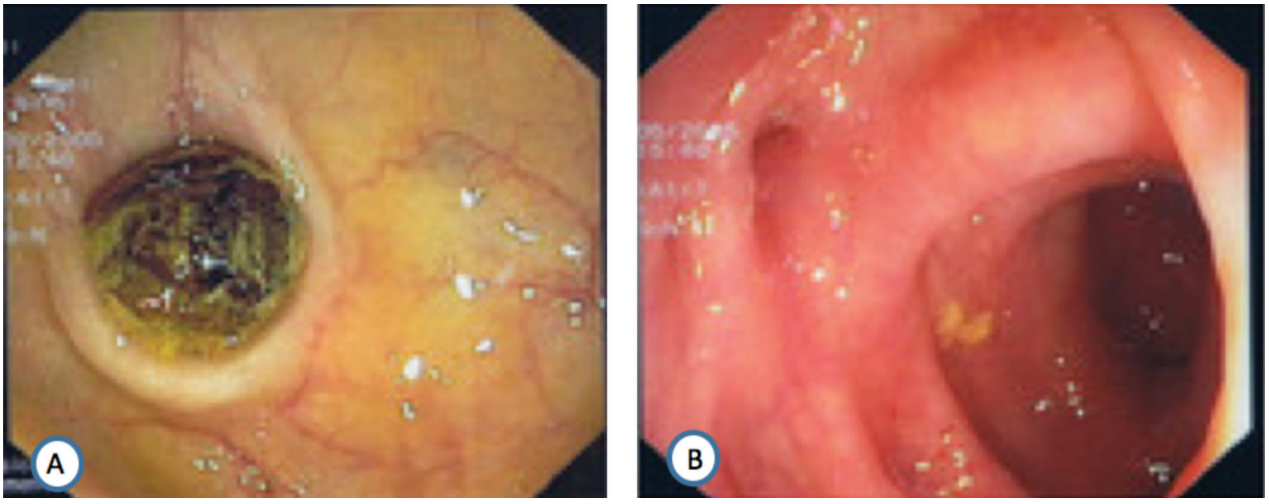

Figure 1. A: Oedema of the diverticular opening; B: Hyperaemia of the peri-diverticular colonic wall (15)

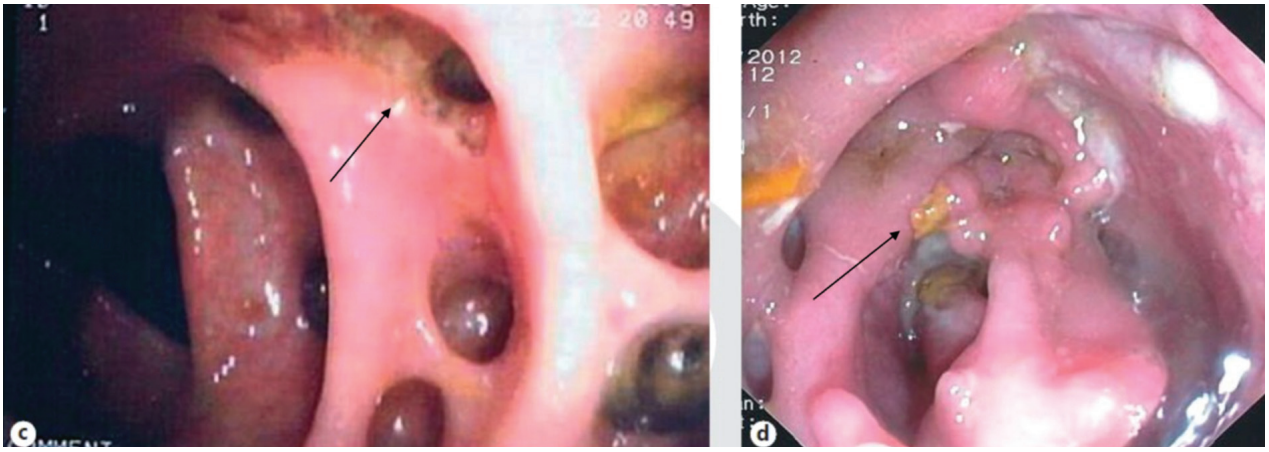

Figure 2. C: Erosions, small fibrinous ulcerations surrounding and involving the diverticular opening (arrow); D: Purulent material coming from diverticular opening (arrow) (15)

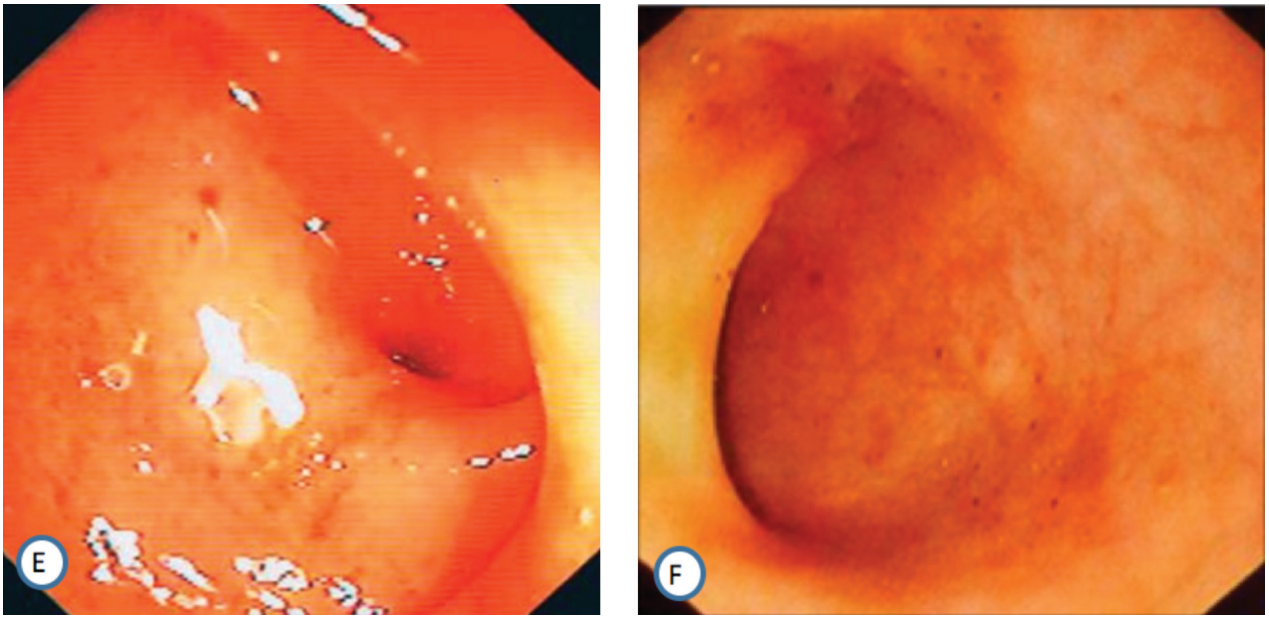

Figure 3. E: Stenosis of the colonic lumen; F: Rigidity (15)

was, as well, shifted from the inflammation category to the complication one; on the other hand, the presence of SCAD was moved to the inflammation group, because the gastroenterologists thought that it wasn't correct to define it like a complication of Diverticular Disease (15).

The final DICA classification includes:

- The extension of Diverticulosis: dividing the bowel in descendent and ascendant colon;
- The number of diverticula per region: choosing 15 like the cut-off (Grade I<15, Grade II>15);
- The presence of inflammation: including oedema and hyperaemia, erosions and SCAD; and in the case in which more signs of inflammation are found a higher point has to be indicated;
- The presence of endoscopic complications, such as rigidity, stenosis, diverticular bleeding and pus (15) (Table 1).

The DICA's score results:

- o DICA 1, when the sum of the points is less than 3, and it indicates Diverticulosis, the presence of diverticula without any endoscopic signs of inflammation and, mainly, without any probability of clinical complications;
- o DICA 2, when the sum of the points is between 4 and 7; this defines a moderate Diverticular Disease with low chance of clinical complications;
- o DICA 3, when the sum of the points is equal or greater than 8 that it determines a severe Diverticular Disease with a higher risk of complications (15) (table 2).

The closeness of agreement in the assessment of the endoscopic findings was defined by the statistic Kappa coefficient, that valued with a negative score the disagreement and with +1 the total agreement between the experts. Overall, 30 videos were assessed by 32 gastroenterologists, with a total of 960 judgements. After the modification of the classification, obtained in the second phase of the development, the K resulted for DICA 1 0.878, for DICA 2 0.765 and for DICA 3 0.891, detecting a positive closeness of agreement (15).

It's important to underline how this result came from a pool of varied experts (endoscopic doctors coming from University and community hospitals, etc.) indicative of a high grade of reproducibility.

Table 1. Numeric value of the items taken in consideration for the development of the DICA classification

	Left Colon (2pnt)	Right Colon (1pnt)
Diverticolulosis grade I	0	0
Diverticolulosis grade II	1	1
Absence of inflammation	0	0
Oedema- Hyperaemia	1	1
Erosions	2	2
SCAD	3	3
Rigidity	4	4
Stenosis	4	4
Pus	4	4
Diverticula bleeding	4	4

Table 2. Numerical DICA classification

DICA SCORE	Numeric values
DICA1	1-3 points
DICA 2	4-7 points
DICA 3	>7 points

Moreover, the link between the classification and the clinical findings was studied; some inflammation index, such as ESR and CRP, and it showed that both of them were correlated, with a statistical significance ($P<0.001$), to the different DICA scores (15).

DICA classification was, as well, validated choosing a group of 50 patients with a 1 year follow-up. Thirty of the 50 patients were classified with DICA 1 (68%), 20 with DICA 2 (32%). The onset and the recurrence of the complication occurred overall in 29 patients (58%), 10 of them classified with DICA 1 and 32 with DICA 2; in particular, SUDD occurred in 23 patients (9 DICA 1 and 14 DICA 2, $p=0.238$), Acute Diverticulitis in 6 patients (1 DICA 1 and 5 DICA 2, $p=0.083$) (15). The numeric classification resulted to simplify the classification of Diverticular Disease, being a user-friendly item in the clinical practice.

For the first time, the endoscopic classification of Diverticular Disease was developed, taking in consideration all the possible items identifiable with the colonoscopy and being able with three scores to have a clear definition of the extension and the severity of this pathology.

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Review

Conservative treatment of acute appendicitis

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Summary. Acute appendicitis has been considered by surgeons a progressive disease leading to perforation for more than 100 years. In the last decades the theories about this concept gained attention, especially in adults. However, appendectomy for acute appendicitis remains the most common urgent/emergent surgical procedure. At present, accumulating evidences are showing the changing in clinical practice towards the non-operative management of several cases of acute appendicitis either non-complicated or complicated. The present review aims to show the literature results regarding the non-operative management of acute appendicitis in non-complicated and in complicated cases. (www.actabiomedica.it)

Key words: appendicitis, conservative, complicated, uncomplicated, review, adult, children, treatment

Background

The acute appendicitis has been considered by surgeons a progressive disease leading to perforation for more than 100 years. In the last decade the theories challenging of this concept gained attention especially in adults. However appendectomy for acute appendicitis remains the most common urgent/emergent surgical procedure in children. The first report of operative treatment for AA has been reported in 1735 when Claudius Amyand treated an inflamed appendix during the course of a hernia operation in an 11-year-old boy. The perforated appendix was situated in the hernia sac. The abscess was opened, and the boy recovered and was discharged a month after the operation. Unfortunately, the hernia recurred (1). The first reported appendectomy for suspected AA was performed by the Scotsman Robert Lawson Tait in 1880 (2). His intervention precedes Charles McBurney who presented his first series in 1889 and Abraham Groves (3, 4). Five years later, McBurney published his article on the surgical procedure that bears his name. Some authors however suggested that the grid-iron incision was first performed by Louis L. McArthur (5, 6). The open appendectomy through a McBurney incision remained the method of choice to treat AA until Karl Semm in 1980 performed the first laparoscopic appendectomy (7). Successively Ure et al. published in 1991 the first series of laparoscopic appendectomies in children (8).

Conservative treatment of acute appendicitis during the years

Searching for the first case of nonoperative treatment of AA, it could be suggested that it is as old as man itself. The first report of a suspected spontaneous resolution of AA was published in 1910 by Smith and Wood Jones. They described the case of a young Nubian woman where the appendix was found attached with a thick adhesive band to the left pelvic wall suggesting that she had survived appendiceal rupture with abscess formation (9). The bizarre aspect of the report

is that at the time of diagnosis, she was an uneviscerated mummy from the Byzantine era. In 1930 Bailey proposed his non-operative treatment algorithm (10) and in 1959 Coldrey described for the first time a large series of 471 patients treated non-operatively with intravenous antibiotics (11). The recurrence rate was 48/470 (10.2%) with 1 death, 9 patients requiring abscess drainage, and 48 cases requiring a subsequent appendectomy. Another series from China reported 500 patients with the clinical diagnosis of AA (12). Of these, 425 had conservative treatment with Chinese traditional medicine and antibiotics were given to some. 7 recurrences have been reported. Conservative treatment has also been reported from doctors in service aboard of the U.S. Navy and the Soviet fishing fleet ships (13, 14). The Russian one is a review of conservative treatment in 252 patients with AA on vessels of the Kalingrad fishing industry from 1975 to 1987. In this series Gurin et al. (14) reported a recovery rate of 84.1% with only the use of antibiotics. The authors found no difference in outcomes based on presenting symptoms or age, but they suggested that conservative treatment was as more effective as earlier it starts. In fact it showed to be most effective if administered within 12 h from symptom onset, ideally within the first 6 h. Moreover they reported the best effect when early intervention with antibiotics was combined with antihistamines and spasmolytics. All these aforementioned trials however dealed with a non-confirmed diagnosis, a poor follow-up. For these reasons these experiences did not receive much attention (15).

Reason for treating conservatively acute appendicitis

It is general opinion that the appendix has no significant function in humans. De Coppi et al. in 2006 showed that the vermiform appendix was capable of producing mesenchymal stem cells (16). They found that appendix-derived mesenchymal stem cells are present in the vermiform appendix. These cells can differentiate into osteoblasts, lipoblasts, and myoblasts, depending on the stimulation. They suggested the possibility that vermiform appendix acts like a reservoir for stem cells capable of bowel repair trough life. From North Carolina many articles have been published on this topic (17). Some authors moreover suggested the possibility that appendix deserve as a reservoir for bacteria of the gut flora, and it is necessary to recolonize the bowel after bacterial infections. Appendectomy either open either laparoscopic is still associated with a significant morbidity and mortality despite advances in surgery and care. Blomqvist et al. analyzed a Swedish cohort of 117,424 patients who underwent appendectomy (1987-1996) analyzing the 30-day postoperative mortality ratio (18). They reported a 3.5-fold excess mortality after surgical intervention for non-perforated appendicitis and a 6.5-fold excess mortality in perforated ones. In patients with a discharge diagnosis of nonspecific abdominal pain the mortality rate after negative appendectomy was increased by 9.1-fold. This mortality rate may only partially be explained by an underlying condition that was concealed by the appendectomy. Also Flum and Koepsell found a threefold increase in mortality after negative appendectomy compared with appendectomy for AA (19). Different studies reported an increased SBO (small bowel obstruction) rate in the years after appendectomy. During a 30-year follow a Swedish report showed that 1.3% of patients subjected to an appendectomy had a SBO compared with 0.2% of controls (20). Others have reported rates of SBO between 0.16 and 10.7% after appendectomy (21, 22). Leung et al recently reported as the incidence of SBO after appendectomy at 2.8%, and the incidence of reoperation for SBO after appendectomy to be 1.1% during the 5-year follow-up (23). Sakorafas et al. recently reported reduced cost, morbidity, and abdominal pain associated with non-operative treatment (15). Svensson et al. described that centers with restrictive indications for exploration have fewer patients with non-perforated appendicitis, compared with centers with a more active attitude to exploration (24). This could suggest that many patients with AA would potentially have spontaneous resolution of their disease. The experience of a hospital in which patients with suspected appendicitis were admitted to five units on a strict 24-hours rotation, three with a conservative approach and two with an active approach to exploration by Howie et al. in 1964. On one hand the active units removed a significantly greater average number of inflamed appendices per unit (72 vs. 46, $p<0.0001$). On the other hand the conservative units removed fewer

normal appendices (16.7 compared with 34% for the radical units, p<0.0001). Luckman in 1989 suggested that perforated and non-perforated appendicitis could be two separate entities. Lastly a meta-analysis by Andersson et al showed as the incidence of perforated appendicitis did not correlate with the rate of negative appendectomy and as a counterpart that the incidence of uncomplicated appendicitis correlated directly with the rate of negative appendectomy and inversely with diagnostic accuracy.

Uncomplicated acute appendicitis

Definition:

Uncomplicated appendicitis is defined as appendicitis without neither perforation nor appendiceal abscess nor mass formation.

Literature evidence:

Randomized controlled trials: Many different prospective and retrospective observational trials comparing conservative treatment of uncomplicated AA have been published during the years. A few randomized controlled trials have also been published increasing the data level of evidence. For this reason the present review will focus on higher level of evidence data.

The first prospective randomized trial on acute appendicitis was presented in 1995.

Eriksson and Granström randomized 40 patients to either operation or conservative treatment (25).

Methods: Randomization of patients admitted with history and clinical signs of acute appendicitis. Ultrasonography and laboratory tests: white blood cell count and C reactive protein to identify patients with a high probability for acute appendicitis.

Participants: Patients with typical history and clinical signs, positive findings at ultrasound, and either increased white blood cell count and C reactive protein values or high C reactive protein or white blood cell count on two occasions within a four hour interval. Initial randomization of 20 patients in each group, but one patient from the antibiotic group developed increased abdominal pain and generalized peritonitis and had surgery, and subsequent data were discounted.

Interventions: The conservative group underwent cefotaxime 2 g 12 hourly and tinidazole 800 mg for two days. Patients were discharged after two days with oral ofloxacin 200 mg twice daily and tinidazole 500 mg twice daily for eight days. Patients were excluded from the study in the event of increased abdominal pain and generalized peritonitis and this case they underwent surgical intervention. The surgery arm underwent antibiotics for 24 hours only in the event of bowel perforation or in cases of abdominal spillage. They were discharged when conditions were satisfactory and/or when patients wished to return home. Histology were obtained for all specimens. All patients were seen for a follow-up visit at 6th, 10th, and 30th day after admission and underwent blood tests for white blood cell count and C reactive protein, pain scores and temperature were evaluated and recorded. Abdominal and rectal examination were performed on days 6th and 10th. Stools were examined for *Clostridium difficile* toxin at day 30th. Ultrasonography was performed on days 10th and 30th.

Outcomes: Pain scores (every six hours using a VAS), morphine consumption, white blood cell count and temperature, positive diagnosis at surgery, hospital stay, wound infection, and recurrent appendicitis were evaluated.

Results: One out of 20 patients needed operation due to failure of conservative treatment, 3 out of 20 appendectomies were negative and 7 out of 19 patients treated conservatively had recurrence of symptoms and surgical intervention within 1 year. Non-operatively treated patients had a faster decrease of C-reactive protein, a lower morphine consumption, and a lower pain score compared with the patients who underwent initial operation. The authors state that 40 out of 45 consecutive patients (27 men and 13 women between 18 and 75 years of age) were included in the trial, and only 5 declined participation. In the surgery group, two had mesenteric lymphadenitis and one had *Campylobacter* enteritis. All patients in this trial had an ultrasound diagnosis of appendicitis but, despite this 17 out of 20 patients who underwent operation had AA.

Styrud et al. randomized 252 men: 128 to conservative treatment and 124 to open appendectomy (26).

Methods: Patients were randomized to either surgery or antibiotic treatment. Patients were monitored at the end of the first and sixth week and of the first year.

Participants: Male patients, between 18 and 50 years of age, admitted to six different hospitals. No women were enrolled by decision of the local ethics committee. All patients with suspected appendicitis with a C-reactive protein concentration >10 mg/L and with no clinical signs of perforation.

Interventions: The antibiotics arm underwent intravenous cefotaxime 2 g 12 hourly and tinidazole 800 mg daily for two days. Patients were discharged after two days with oral ofloxacin 200 mg and tinidazole 500 mg twice daily for 10 days. If symptoms didn't improve within first the 24 hours, appendectomy was performed. All conservatively treated patients with a suspected recurrence of appendicitis underwent surgery. Patients randomized to surgery had open or laparoscopic operations at the surgeon's discretion. All removed specimens were sent for histology.

Outcomes: Hospital stay, sick leave, diagnosis at operation, recurrences, and complications were evaluated.

Results: Of the 128 patients treated non-operatively 18 required operation due to failure of antibiotic therapy. Of the 124 appendectomies, 4 were negative. 16 out of 110 conservatively treated patients had a recurrence within 1 year. 17 patients experienced complications in the open appendectomy group.

In 2009, Hansson et al. in a large randomized trial published the results of 369 patients where 202 were randomized to conservative treatment and 167 to surgical intervention (27). The trial protocol accepted a crossover after randomization, but before initiation of treatment. For this reason 119 patients were treated conservatively and 250 were operated.

Methods: Three centers participated to the study; one hospital enrolled patients to be used as a reference cohort for comparison and the other two centers enrolled patients into the study and control arms. Allocation were done by date of birth. Questionnaire was sent to all patients after one and 12 months. All patients who didn't answer to the questionnaire were contacted by telephone.

Participants: Patients were enrolled if they had positive history, clinical signs, laboratory tests, and, in some cases, ultrasonography, computed tomography, and gynecological examination.

Interventions: The conservative treatment arm underwent intravenous cefotaxime 1 g twice daily and metronidazole for at least 24 hours. Patients who improved were discharged 24 hours later with oral ciprofloxacin 500 mg twice a day and metronidazole 400 mg three times a day for 10 days. If there was no improvement the intravenous treatment was prolonged. The surgery arm underwent open or laparoscopic appendectomy with a single dose antibiotic prophylaxis, and postoperative antibiotic treatment when the appendix was gangrenous or perforated. All specimens were sent for histological examination.

Outcomes: Treatment efficacy, complications, recurrences and reoperations, length of antibiotic treatment, abdominal pain after discharge from hospital, length of hospital stay, and sick leave were evaluated. Moreover the total costs for the primary hospital stay were analyzed for each patient.

Results: Based on per-protocol analysis, 11 out of 119 patients in the conservative treatment arm needed early operation, 27 out of the 250 appendectomies were negative, and 15 out of the 108 conservatively treated patients had a recurrence within 1 year. Serious complications rate were three times more frequent in the surgery arm.

In 2011, a Vons et al. published the results of a randomized trial in which 239 adult patients were randomly assigned 120 to conservative treatment and 119 to surgical intervention (28).

Methods: The study is an open label, non-inferiority, randomized controlled trial to which participated six academic centers. Patients in both treatment groups were assessed twice a day after admission and were discharged after resolution of pain, fever, and any digestive symptoms. All patients were seen on days 15th, 30th, 90th, 180th, and 360th.

Participants: All included patients were adults over 18 years with suspected AA, who had diagnosis of uncomplicated appendicitis by computed tomography (CT). Included patients were randomized to appendectomy or antibiotic treatment. Patients who were allergic to antibiotics or iodine, had been on antibiotics before admission, were receiving steroid or anticoagulants, had a history of inflammatory bowel disease, were pregnant, had blood creatinine of ≥200 μmol/L, or were unable to understand the protocol or consent form were not included into the study.

Interventions: The patients included into the anti-

biotics arm underwent intravenous or oral amoxicillin plus clavulanic acid (3 g per day if <90 kg or 4 g for patients >90 kg) for 48 hours. If there was no resolution of symptoms after 48 hours patients underwent appendectomy. Patients were discharged with antibiotics and reviewed on day 8th if there was resolution of the symptoms. CT was done in presence of persistent pain or fever or if there was a suspicion for the necessity of appendectomy. If not, antibiotics continued for another 8 days. If symptoms persisted on day 15th, appendectomy was done. Patients enrolled in the surgery arm underwent open or laparoscopic appendectomy. Amoxicillin plus clavulanic acid 2 g was administered at the time of induction of general anesthesia. Antibiotics were given postoperatively only if the appendicitis was complicated. Histology was obtained for all specimens.

Outcomes: The primary endpoints was: occurrence of peritonitis within 30 days of initial treatment, diagnosed either at appendectomy or postoperatively by CT. The secondary endpoints were number of days with a post-intervention VAS pain score ≥4, length of stay, absence from work, incidence of complications other than peritonitis within one year, and recurrence of appendicitis after antibiotic treatment (considered as appendectomy done between 30 days and one year of follow-up, with a confirmed diagnosis of AA).

Results: There were 14 early failures and only 1 out of 119 negative appendectomy. Of 120 patients enrolled into the conservative treatment group 30 had an operation within the first year and 26 had appendicitis.

Malik and Bari published a trial where 80 patients were randomized 40 to conservative treatment and 40 to surgical intervention (29). This article was retracted from the Journal of Gastrointestinal Surgery in 2011 (31). The editors state that significant portions of the article were published earlier in other studies (25, 30).

Methods: This is a monocentric randomized controlled trial. Patients were evaluated during the follow-up at the 7th, 12th, 30th day, blood sample (WBC and CRP levels), pain (VAS) and oral temperature was registered. Patient with recurrent appendicitis within one year were readmitted.

Interventions: The patients enrolled to conservative treatment arm underwent intravenous ciprofloxacin 500 mg every 12 h and 500 mg of metronidazole administered intravenously every 8 h for a period of 2 days. After the discharge were administered a 7-day oral therapy with 500 mg of ciprofloxacin twice a day, and 600 mg of tinidazole twice a day. Patients randomized to the surgery arm received a preoperative antibiotic prophylaxis with cephalosporin and tinidazole that was protracted for 48 hours in the event of bowel perforation or abdominal spillage. cephalosporins and imidazole. For each patient the pain was registered every 6 hours using VAS and oral temperature was measured twice daily. Histology was obtained for all specimens. Patients from both groups were discharged once conditions were satisfactory.

Outcomes: hospital stay, complications, pain, analgesic consumption, inflammatory laboratory tests, and body temperature were evaluated.

Results: In the conservative treatment group the 85.0% and in the surgical treatment group 92.5% patients were successfully cured within two weeks without major complications. The mean duration of pain was 23 hours in antibiotic arm and 21.3 hours in surgery arm. The mean hospital stay was 2.3 days in antibiotic arm and 1.2 days in surgery arm. 2 out of 40 patients in the conservative treatment arm failed and undergone surgery during the first admission and 4 out of the remaining 38 undergone appendectomy during the first year.

Systematic reviews and meta-analyses:

There are several systematic reviews with meta-analysis published about the comparison between conservative and surgical treatment of AA.

Varadhan et al analyzed three trials (25-27, 32). Their analysis showed a trend toward a reduced risk of complications in the antibiotic-treated group [RR (95%CI): 0.43 (0.16, 1.18) p=0.10], without prolonging the length of hospital stay [mean difference (inverse variance, random, 95% CI): 0.11 (-0.22, 0.43) p=0.53]. In their analysis 350 patients were randomized to the antibiotic group, among them the 68% (238 patients) were treated successfully with antibiotics alone and the 15% (38 patients) were readmitted. The remaining 112 patients (32%) who were randomized to conservative treatment crossed over to surgery. At 1 year follow-up analysis, 200 patients in the conservative treatment group remained asymptomatic. Authors concluded

that "that although antibiotics may be used as primary treatment for selected patients with suspected uncomplicated appendicitis, this is unlikely to supersede appendectomy at present".

Ansaloni et al. included four trials in their study, including the discussed Malik and Bari's trial (25-29, 33). Efficacy was significantly higher for surgery (OR=6.01, 95% CI=4.27-8.46). No differences were found in the numbers of perforated appendix (OR=0.73, 95% CI=0.29-1.84) and patients treated with antibiotics (OR=0.04, 95% CI=0.00-3.27). Complication rates were significantly higher for surgery (OR=1.92, 95% CI=1.30-2.85). They conclude that "although a nonsurgical approach in AA can reduce the complications rate, the lower efficacy prevents antibiotic treatment from being a viable alternative to surgery".

Liu and Fogg included six trials in their meta-analysis (25-29, 33-35). They found a non-operative management failure rate of 6.9% and a 14.2% recurrence rate. They conclude that appears to be safe to treat AA with antibiotics. One appendectomy patient had a recurrence. A normal appendix was found in 7.3% of patients at appendectomy. Complications rate was lower with antibiotic treatment than with appendectomy (OR 0.31; 95% CI 0.19-0.49, p<0.05).

The Cochrane collaboration published its review in December 2011 (36). The authors included five trials (25, 26, 28, 29, 34) excluding the Hansson et al. trial as it was considered a low quality trial. The primary reason was the cross-over between the groups driven by patient or surgeon preference. Malik and Bari and Turhan et al trials were included in the review (34, 29). It should be mentioned that the Turhan et al trial is difficult to be considered a real randomized trial.

Authors found that the 73.4% (95% CI 62.7 and 81.9) of patients who underwent conservative treatment and the 97.4% (95% CI 94.4 and 98.8) of patients who underwent surgical intervention were successfully treated within two weeks and had no major complications (including recurrence) within the first year. Patients who undergone surgical intervention experienced a shorter hospital admission OR 0.66 (95% CI 0.44 to 0.87). However the duration of sick leave periods is significantly shorter in patients treated with antibiotics with an OR of 0,69 (95%CI -1.65 to 0.27). The authors concluded that appendectomy remains the gold standard, as a counterpart initial antibiotic therapy was not inferior to operation based on a 20% non-inferiority margin.

Fitzmaurice et al. published their systematic review with the aim to evaluate the evidence to challenge initial operation as the gold standard treatment for AA in adults (37). By searching in the literature they found 13 trials (1999-2009). Most of them were considered of low level of evidence. They included four randomized controlled trials (26-29, 38). Fitzmaurice et al did not find enough evidence to challenge initial operation as the gold standard treatment for AA in adults.

Mason et al. published their meta-analysis of five randomized trials (25-29, 39). In 2008 Mason has already published a review supporting the conservative treatment of AA showing as many of the treated patients (up to 70%) would not require surgical intervention (40). The aforementioned meta-analysis revaluate the evidence of the necessity of a blind assessment of the outcome. Authors proposed as the most important factor is the choice of treating AA with antibiotic is the safety of treatment. They focused on the lower complication rate of patients treated with antibiotics with an OR of 0.54 (95% CI 0.37-0.78, p=0.001). Patients treated with antibiotics experienced a reduction in sick leave/disability SMD -0.19 (95% CI -0.33, -0.06, p=0.005) and in pain medication utilization SMD -1.55 (95% CI -1.96, -1.14, p<0.0001). The failure rate is higher in conservative treatment with an OR of 6.72 (95% CI 0.08, 12.99, p<0.0001). Author conclude that "the conservative treatment is associated to fewer complication, better pain control and shorter sick leave disease, but has inferior efficacy because of the high rate of recurrence".

Varadhan et al published their meta-analysis as an update to their previous review (32, 41). They excluded the trial by Malik and Bari and Turhan et al included on an intention to treat basis data by Hasson et al. excluding the cross-over of patients (27, 29, 34). They showed as non operative management was associated with a significantly lower complication rate (RR 0.69; 95% CI 0.54-0.89; P=0.004). A secondary analysis, excluding the crossover of patients between the two interventions after randomization from Hasson et al,

confirmed the relative risk reduction RR 0.61 95% CI 0.40-0.92; P=0.02). The authors found no differences neither in the duration of hospital stay nor in the incidence of complicated appendicitis. This is the only of the published meta-analysis concluding that: "Antibiotics are both effective and safe as primary treatment for patients with uncomplicated acute appendicitis. Initial antibiotic treatment merits consideration as a primary treatment option for early uncomplicated appendicitis".

An interesting prospective non-randomized study recently published by Di Saverio et al. evaluate the question from a different point of view (95). Randomized trials that assign patients with suspected AA to either surgical or nonsurgical treatment group show a relapse rate of approximately 14% at 1 year. Authors suggested that would be useful to determine the relapse rate of patients treated according to the results of a thorough clinical evaluation, including physical examination and laboratory results (all characteristics used to determine the Alvarado score (101)) and radiological evaluation. Only clinical signs and symptoms and laboratory values, as included in the Alvarado and Appendicitis Inflammatory Response (AIR) (96) scores, were routinely evaluated in patients with suspected AA. If this clinical evaluation is effective, authors would expect patient selection to be better than chance and the relapse rate to be below 14%. Authors suggested that once established the utility of this evaluation, it would be possible to begin to identify those components that have predictive value. This would be a first step toward developing an accurate diagnostic-therapeutic algorithm, possibly functional for avoiding the risks and costs of needless surgery. Authors also suggest that observational studies have a role in research on the benefits and harms of medical interventions. Randomized trials cannot answer all important questions about a given intervention. For example, observational studies are more suitable for detecting rare or late adverse effects of treatments and are more likely to provide an indication of what is achieved in daily medical practice (97). This single-cohort, prospective, observational study has been registered on ClinicalTrials.gov database (identifier NCT01096927) (98) and published in the protocol form (99). All patients presented to the emergency department with right iliac fossa (RIF) pain and suspected AA had the following tests: complete blood cell count with differential and C-reactive protein. An attending/consultant surgeon conducted an assessment of the right lower quadrant pain suspected of being appendicitis and rule out the presence of acute appendicitis and need for operation; they eventually underwent additional abdominal US and eventual completion with an abdominal CT scan if requested by the attending/consultant surgeon. Those patients not needing immediate surgery were treated with a 5- to 7-day course of amoxicillin and clavulanate at dosage of 1 g orally thrice daily.

The aim of the study were to evaluate the outcome of patients treated non-operatively with antibiotics and to assess the reliability of the initial clinical evaluation in predicting which non-operatively treated patients should have been treated surgically. The primary outcomes were 1- Short-term efficacy of antibiotic treatment evaluated as failure of non-operative management with 7 days of amoxicillin and clavulanic acid therapy and defined as readmission due to lack of clinical improvement and/or worsening abdominal pain and/or localized/diffuse peritonitis. 2- Long term efficacy of antibiotic treatment defined as the efficacy of antibiotic therapy for right lower quadrant pain suspected of being AA defined as an incidence of recurrences of clinical episodes of appendicitis up to follow-up at 2 years (at 7 days, 15 days, 6 months, 1 year, and 2 years). 3- Long-term efficacy of antibiotic treatment (no need for surgery) defined as the efficacy of antibiotic therapy for right lower quadrant pain suspected of being AA defined as definite improvement without the need for surgery up to follow-up at 2 years (at 7 days, 15 days, 6 months, 1 year, and 2 years). 4- Safety of antibiotic treatment defined as the incidence of major side effects/drug- or treatment-related complications (i.e., allergy or other treatment related complications such as abscess formation).

Secondary outcomes were as follows:

1- Minor complications 2- Abdominal pain after discharge: assessed at 5, 7, and 15 days. 3- Length of hospital stay. 4- Outpatient clinic follow-up defined as the number of follow-up appointments scheduled in the outpatient clinic. 5- Sick leave. 6- Cost analysis. An additional objective was to identify clinical, labo-

ratory, and imaging findings that were predictive of failure of non operative management with antibiotics and/or relapse of appendicitis and need for appendectomy within 2 years.

The inclusion criteria were as follows: age more than 14 years, lower abdominal pain/RIF pain, clinical diagnosis/suspicion made by an attending general surgeon, of AA, confirmed by at least 1 validated score (Alvarado and/or AIR scores):

- Alvarado score 5 to 6 (equivocal for AA)
- Alvarado score 7 to 8 (probable AA)
- Alvarado score 9 (highly probable AA)
- AIR score 3 to 4 (low probability)
- AIR score 5 to 8 (indeterminate group)

Exclusion criteria: diffuse peritonitis, antibiotic (penicillin) documented allergy, ongoing/previously started antibiotic therapy, previous appendectomy, positive pregnancy test, inflammatory bowel disease history or suspicion of it recurrence.

Clinical diagnosis or clinical suspicion of non perforated AA not requiring immediate surgery was made by an attending surgeon and rigorously assessed and validated on the basis of routine use of clinical scores. Suspected AA was defined as patient presenting with RIF pain and the absence of a definite alternative diagnosis of a gastrointestinal disease, urinary tract disease or an obstetric-gynecological cause. Patients needing immediate surgery were defined as those with diffuse peritonitis and/or signs of severe abdominal sepsis and also those with clinic-radiological (US or CT scan) evidence of an intra-abdominal collection/abscess or free perforation. Sepsis was defined by the presence of systemic inflammatory response syndrome (100) in the presence of a known or strongly suspected intra-abdominal infection/collection or free perforation. Patients who did not undergo surgery were physically examined 5 days later. If their condition did not improve or worsened, they were admitted for surgical appendectomy. This study gave interesting results. In 2010, a total of 159 patients with a mean AIR (Appendicitis Inflammatory Response) score of 4.9 and a mean Alvarado score of 5.2. The follow-up period was 2 years. The study showed a short-term (7 days) non operative management failure rate of 11.9%. All patients with initial failures were operated within 7 days. At 15 days, no recurrences were recorded. After 2 years, the overall recurrence rate was 13.8% (22/159); 14 of 22 patients were successfully treated with further cycle of amoxicillin/clavulanate. No major side effects occurred. Abdominal pain was assessed by the Numeric Rating Scale and the visual analog scale with a median score of 3 at 5 days and 2 after 7 days. Mean length of stay of non operatively managed patients was 0.4 days, and mean sick leave period was 5.8 days. Long-term efficacy of non operative management was 83% (118 patients recurrence free and 14 patients with recurrence non operatively managed). None of the single factors forming the Alvarado or AIR score were independent predictors of failure of non operative management or long-term recurrence. Alvarado and AIR scores were the only independent predictive factors of non operative management failure after multivariate analysis, but both did not correlate with recurrences. Overall costs of non operative management and antibiotics were €316.20 per patient. Authors concluded that antibiotics for suspected AA are safe and effective and may avoid unnecessary appendectomy, reducing operation rate, surgical risks, and overall costs. After 2 years of follow-up, recurrences of non operatively treated right lower quarter abdominal pain are less than 14% and may be safely and effectively treated with further antibiotics.

Doubtful issues:

It has already been observed by Fitz that AA may takes various different clinical courses, mainly three: spontaneous resolution, persistent inflammation without perforation and perforation. With the advent of ultrasonography and CT, spontaneous resolution rate has been reported in the range of 3.6% to 20.0% in many cases reports, (42-44) and in large case series (45-50, 52). Case reported demonstrate as the typical symptoms of AA corroborated by imaging studies, appear to resolve completely in 24 to 48 hours without treatment. So on it could be speculated as the real challenge is to differentiate since the beginning those patients who are likely to resolve spontaneously the AA episode and those who will not. If an appendectomy results in a inflamed appendix that is considered sufficient to justify the surgical intervention (45). We must keep in mind however that the absence of inflammatory infiltrate extending into the *muscularis*

propria, with only mucosal or sub-mucosal involvement has no definitive significance. This inflammatory pattern in fact is commonly observed in the incidentally removed appendix (45, 53). So on the appendix reported as inflamed would comprise a lot of not "really inflamed" appendix (53) giving partial and incorrect results and leading sometimes to misinterpretation of data. Livingston et al showed that "there was a sudden reversal of the long term decreasing trend in the rate of nonperforating appendicitis coincident with more frequent use of CT imaging and laparoscopic appendectomy" (54). Anderson corroborated this statement by demonstrating that the increment in use of CT scan has leaded in last decade to an increase in the number of detected appendicitis (55). Moreover Petrosyan et al evaluated the direct correlation between appendectomy and CT scan. In fact at the increase of the number of patients with a CT scan increased also the number of patients who undergone appendectomy, and this phenomenon was especially pronounced in patients with low Alvarado scores (56). As a counterpart patients without CT scan are more likely to be treated without appendectomy. This confirm the trend toward overdiagnosis of AA by CT scan. Kirshenbaum et al. in fact reported an highest spontaneous resolution rate (up to 20%) if AA is diagnosed by CT scan (52).

As a consequence of all the aforementioned data, Liu et al suggested that the idea that appendicitis could be a condition that has a continuous spectrum from non-perforated to perforated, and from uncomplicated to complicated appendicitis, may be incorrect. They suggested the existence of several distinct types of appendicitis, each with varied pathophysiology and clinical courses (35).

Ongoing randomized trials:

The APPAC trial:

The APPAC trial aims to provide level I evidence to support the hypothesis that approximately 75-85% of patients with uncomplicated AA can be treated with effective antibiotic therapy avoiding unnecessary appendectomies and the related operative morbidity, also resulting in major cost savings (registration: Clinicaltrials.gov NCT01022567) (57). The APPAC trial is designed to be a randomized prospective controlled, open label, non-inferiority multicenter trial to compare antibiotic therapy (ertapenem) with emergency appendectomy in the treatment of uncomplicated AA.

Inclusion criteria are: signed informed consent, age between 18 and 60 years. CT scan diagnosis of uncomplicated AA. Exclusion criteria are: age <18 years or >60 years, pregnancy or lactating, allergy to contrast media or iodine, renal insufficiency, serum creatinine > 150 μmol/l, metformin medication, peritonitis, inability to co-operate and give informed consent, serious systemic illness, complicated AA in a CT scan (appendicolith, perforation, peri appendicular abscess or suspicion of a tumor).

The primary endpoint is the success of the randomized treatment. In the antibiotic treatment arm successful treatment is defined as the resolution of AA resulting in hospital discharge without the need for surgical intervention and no recurrent appendicitis during a minimum follow-up of one-year (treatment efficacy). Treatment efficacy in the operative treatment arm is defined as successful appendectomy evaluated to be 100%. Secondary endpoints are post-intervention complications, overall morbidity and mortality, the length of hospital stay and sick leave, treatment costs and pain scores (VAS, visual analogue scale). 610 adult patients (aged 18-60 years) with a CT scan confirmed uncomplicated AA will be enrolled from six hospitals and randomized by a closed envelope method in a 1:1 ratio either to undergo emergency appendectomy or to receive ertapenem (1 g per day) for three days continued by oral levofloxacin (500 mg per day) plus metronidazole (1.5 g per day) for seven days. Follow-up will be performed by a telephone interview at 1 week, 2 months and 1, 3, 5 and 10 years. Both the primary and secondary endpoints will be evaluated at each time point.

The ASAA trial:

The ASAA-Study (Antibiotics vs. Surgery in Acute Appendicitis) is an intention to treat prospective randomized controlled study which aims to compare the antibiotic therapy and the surgery in the treatment of uncomplicated acute appendicitis (registration: EudraCT 2011-002977-44). Preliminary agreement has been reached over Andersson's score as the most comprehensive diagnostic tool for patients

suspected to suffer of AA. According to the Andersson's score 3 groups have been individuated. Group 1: patients with very low probability to suffer from AA and group 3: patients with very high probability to suffer from AA. The group 2 includes patients with intermediate probability to suffer from AA; in this group we added ultrasound to better discern the presence of AA. Patients which require immediate surgery and group 1 or 2 patients with negative ultrasound and/or positive gynecological consultation are excluded. Of the remaining patients, those who meet the inclusion criteria are randomized. In order to perform a non-inferiority analysis between antibiotics and surgery the population size was calculated as 110 patients in each arm.

Inclusion criteria are: all the patients between 18 and 65 years old admitted to the hospital with a first episode of suspected AA diagnosed by Andersson's score combined with abdominal ultrasound (see below, at the population study section, for details). Exclusion criteria are: patients with any potential immunodeficiency status (diseases or syndromes, neoplasm in the last five years), diabetes, assumption of antibiotics for different infectious disease or surgery in the last 30 days, allergy to antibiotics established in the study protocol, no acceptance of study protocol, pregnancy or delivery in the last 6 months, ASA IV or V, no Italian or English fluently speakers.

The primary endpoints are: absence of symptoms and normalization of laboratory test after 2 weeks, no major complications or recurrence within 1 year. The secondary endpoints are: reintervention for bowel occlusion or intraperitoneal abscess, bowel occlusion longer than 48 hours, incisional hernia or wound dehiscence, recurrence of AA, wound infection, negative appendectomy, hospital stay, work absence and evaluation of pain (VAS at admission time, twice a day during the entire admission beginning since 24 hours from the intervention or the first antibiotic dose).

In the antibiotic arm will be administered to the patients Ertapenem e.v. infusion 1g for day for 3 consecutive days followed by Amoxicillin plus Clavulanic acid per os 1gr 3 times day for seven days. In the surgery arm will be administered Amoxicillin plus Clavulanic acid e.v. 2 gr followed by surgery.

Complicated acute appendicitis

Introduction and definition:

Complicated appendicitis is defined as appendicitis complicated by a local or contained perforation with an appendiceal abscess or mass formation.

Literature evidence:

Conservative treatment of complicated AA may include radiologic-guided drainage of a peri-appendiceal abscess. After successful conservative management, some centers are used to proceed with elective interval appendectomy. At present no consensus exists among surgeons regarding the optimal treatment for patients with complicated AA (58).

Randomized controlled trials: at the best of our knowledge no randomized trials exist comparing conservative and surgical treatment of complicated AA in adults.

Systematic review and meta-analysis: one systematic review with meta-analysis have been published by Similis et al. (58) **.** The following outcomes were evaluated to compare patients in the conservative treatment group and those in the surgery one: 1 - duration of hospital which means the mean duration of hospital stay during the first hospital admission and the overall duration of hospital stay. The overall duration of hospital stay included hospitalizations for interval appendectomy and eventual complications. 2 - duration of antibiotic therapy which means the average number of days the patient had intravenous antibiotic therapy as an inpatient but excluded any oral courses completed after discharge. 3 - complications rate divided into overall complications rate and wound infection rate. Wound infection is defined as superficial or deep after wound closure but excluded any abscess formation. Abdominal/pelvic abscess defined as a collection of fluid in the pelvis or abdomen diagnosed on radiologic imaging or at reoperation or at interval appendectomy, ileus or bowel obstruction diagnosed after CT or postoperatively, pneumonia, sepsis/diffuse peritonitis, deep venous thrombosis/pulmonary embolism, death, adhesions, and fistula formation. The authors choose these particular complications because they were the

most commonly reported by the different studies to compare the 2 groups. 4 – reoperation rate considers all the reoperations needed as a result of postoperative complications after interval appendectomy or acute appendicitis during the same and/or during any other hospital readmissions (58).

This review included a total of 17 studies published between 1969 and 2007 (59-75) considering the management either of adult either of pediatric patients. 16 non-randomized retrospective trials (59-75) and 1 non-randomized prospective trials (74). The analysis was performed on 1,572 patients, of which 847 (53.9%) patients received conservative treatment and 725 (46.1%) patients underwent acute appendectomy for complicated appendicitis. Of the 847 patients who received conservative treatment, 483 proceeded to have interval appendectomy. The duration of intravenous antibiotics given to patients, which was found to be similar between conservative treatment and acute appendectomy (WMD, 1.02; 95% CI, --1.30--3.34; P =.39). No difference was found in the duration of first hospitalization (WMD, 0.49; 95% CI, --2.70--3.69; P =.76). No difference was found in the overall duration of hospitalization (WMD, 0.04; 95%CI, --3.87--3.95; P = .98). Complications comparing the 2 treatment approaches were found to be more common in the acute appendectomy group compared with the conservative treatment group (OR, 0.24; 95% CI, 0.13--0.44; P<.001). A greater incidence of ileus/bowel obstruction was found in the acute appendectomy group (OR, 0.35; 95% CI, 0.17--0.71; P=.004). The acute appendectomy group was found to have a greater rate of abdominal/pelvic abscess formation (OR, 0.19; 95% CI, 0.07--0.58; P = .003). Wound infection was found to be more common in the acute appendectomy group (OR, 0.28; 95% CI, 0.13-- 0.60; P=.001). No difference was shown between the 2 groups when comparing pneumonia (OR, 1.11; P=.89), sepsis/diffuse peritonitis (OR, 0.54; P=.36), deep venous thrombosis/pulmonary embolism (OR, 0.37; P=.20), mortality (OR, 0.70; P=.67), adhesions (OR, 3.35; P=.39), and fistula formation (OR, 0.22; P=.07). Reoperation was found to be greater in the acute appendectomy group (OR 0.17; 95% CI, 0.04--0.75; P=.02).

This meta-analysis showed that conservative management of complicated AA, with or without interval appendectomy, is associated with a decreased complication and reoperation rate. Moreover the conservative treatment of AA has similar duration of hospital stay and duration of intravenous antibiotics. The authors however suggest the needing for subsequent studies (58).

Conservative treatment acute appendicitis in pediatric patients

Uncomplicated acute appendicitis

A different discussion should be reserved to the management of AA in pediatric patients. The vast majority of published data presented discussed almost exclusively about adult patients. Only one pilot randomized controlled trial exists (102) comparing appendectomy with non-operative treatment in children with uncomplicated AA. In this trial, 92% of patients treated with antibiotics had initial resolution of symptoms and only 1 patient (5%) had recurrence of AA during follow-up. These results suggested that non-operative treatment of AA in children is feasible and safe. Some other randomized controlled trial about this topics are in progress (103-106). Similarly, some meta-analysis and cohort studies (76, 77, 107-109) suggested the possibility to successfully use the non-operative treatment of uncomplicated AA with a reported success rate ranged from 74% to 97% and a recurrence rate of 14%. These studies reported the same complications rate in the surgery group and in the non-operative group. The reported long term efficacy of non-operative management ranged from 73 and 82%. Although scarce, present literature supports the feasibility of non-operative management of acute uncomplicated appendicitis in children. Higher quality prospective randomized controlled trials with larger sample sizes are required to establish its utility.

Complicated acute appendicitis

No consensus exists among pediatric surgeons regarding the optimal treatment complicated AA in children (78). The advent of broad-spectrum antibiotics leads some surgeons to suggest the possibility to try

to apply the non-operative management in cohort of children (79-87). As a counterpart a little evidence exists about the possibility to determine which children are most likely to benefit from this approach. In fact, the term "complicated acute appendicitis" includes different clinical entities: the gangrenous appendicitis, the perforated appendicitis, the phlegmon and the appendicular abscess.

The existing literature that try to determine the real efficacy of the non-operative management in patients with perforated AA has no possibility to reduce the heterogeneity of data and the incompleteness of them. For this it's impossible to differentiate the real clinical status of patients treated with conservative management and those treated with appendectomy and no definitive data could be obtained.

Literature reports that 30 to 60% of children with AA have already developed appendicular perforation at the moment of the child presentation to the surgeon (88, 89). The surgeon at that moment could choose between three main options: immediate appendectomy and non-operative management with or without drainage of a peri-appendiceal abscess. After successful conservative management, once the child is returned to normal activity many surgeons suggest interval appendectomy.. Several reports demonstrated good outcomes in series of children with perforated appendicitis, without abscess, phlegmon, or mass, treated non-operatively with intravenous broad-spectrum antibiotics (79, 80, 82, 84, 87, 90-92). As already stated before the majority of these series are affected by significant selection bias, most commonly due to the undefined clinical status at presentation which has determined the treatment choice. All the published series proposed retrospectively collected data where diagnosis was based on different combinations of clinical suspicion, abdominal US and CT scan. The success rate of non-operative is reported in 62 to 100% of cases. To overcome some of the limitations of these retrospective studies, Blakely et al. performed a prospective, randomized controlled trial comparing non-operative treatment to early appendectomy in 131 unselected children with perforated AA without evidence of abscess or mass (93). The protocol fixed the date of interval appendectomy following successful non-operative treatment after 6 to 8 weeks. The 89% of the children who underwent early appendectomy for suspected perforated appendicitis had this as a final diagnosis. The primary outcome was time away from normal activities. It has been demonstrated to be significantly shorter in the group who underwent early appendectomy compared with those who underwent non-operative management and who returned for interval appendectomy. The adverse events rate were significantly more common in the non-operative management arm. On the basis of these findings the authors propose a clear preference for early appendectomy for perforated AA. A subsequent paper with a cost-analysis based on this trial showed a significant cost-benefit to early appendectomy (94).

Another randomized controlled trial (110) focusing on children with appendicitis with abscess, didn't find advantages between initial laparoscopic appendectomy versus initial non-operative management and interval appendectomy in terms of total hospitalization, recurrent abscess rate or total charges. An analysis of the studies that included only pediatric patients in a meta-analysis about appendicitis complicated with abscess or phlegmon revealed that, compared with the non-operative group, the early appendectomy group had a greater rate of overall complications, wound infections and abdominal/pelvic abscess formation. No differences were found between the two groups in the duration of first hospitalization, ileus/bowel obstruction and reoperations. Similar results emerged in another recent meta-analysis (111) about pediatric patients with complicated appendicitis.

Finally a meta-analysis (112) of the two randomized controlled trials about complicated acute appendicitis found that for children with perforated appendicitis and no abscess at presentation, it appears that early appendectomy is favored, while for children with an intra-abdominal abscess at presentation, the controversial question of early versus interval appendectomy is still alive because there is no convincing evidence suggesting major differences between the two surgical approaches. More high quality randomized studies are needed to demonstrate the risks and benefits of operative and nonoperative approaches to complicated appendicitis.

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Review

Fluorescence guided surgery in liver tumors: applications and advantages

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Summary. The use of fluorescence-guided surgery for benign and malignant hepatobiliary (HPB) neoplasms has significantly increased and improved imaging methods creating new interesting perspectives. A major challenge in HPB surgery is performing radical resection with maximal preservation of the liver parenchyma and obtaining a low rate of complications. Despite the developments, visual inspection, palpation, and intraoperative ultrasound remain the most utilized tools during surgery today. In laparoscopic and robotic HPB surgery palpation is not possible. Fluorescence imaging enables identification of subcapsular liver tumors through accumulation of indocyanine green (ICG), after preoperative intravenous injection, in cancerous tissues of hepatocellular carcinoma and in noncancerous hepatic parenchyma, around intrahepatic cholangiocarcinoma and liver metastases, and it can also be used for visualizing extrahepatic bile duct anatomy and hepatic segmental borders, increasing the accuracy and the easiness of open and minimally invasive hepatectomy. (www.actabiomedica.it)

Key words: liver, fluorescence, indocyanine-green, surgery, tumors

Introduction

Since its approval (1954) by U.S. Food and Drug Administration, Indocyanine green (ICG) has been widely employed in many different clinical settings. At the beginning, it has been helpful to evaluate cardiac output and liver function.

In the 1970s, protein-bound ICG was found to emit fluorescence, peaking at about 840 nm, under illumination with near-infrared light (750–810 nm) (1).

Then ICG was initially clinically used for ocular fundus angiography in the early 1990s (2). With the advances in technology over the recent years, ICG fluorescence imaging has received significant interest for use in various surgical procedures, i.e. to detect lymphatic flow in the extremities (3), sentinel lymph nodes in patients with breast (4) and gastrointestinal cancers (5), to evaluate blood flow during coronary artery bypass grafting (6) and clipping of cerebral artery aneurysms (7).

Primary liver cancer and liver metastases of colorectal cancer are among the most common leading causes of cancer-related death worldwide (8) and surgery represents one of the main treatments to obtain the best results in overall and disease free survival.

Over the last few decades, imaging technologies in hepatobiliary (HPB) surgery such as ultrasonography (US), computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET) have become indispensable tools for preoperative planning in surgical procedures. During interventions, in liver surgery, the surgeon must recog-

nize vital anatomical structures and, in case of tumor, discriminate between lump and healthy tissue by visual inspection and palpation. Although in some cases intraoperative imaging modalities such as US or cholangiography can be applied. Irradical (R1) oncologic resections and iatrogenic surgical injuries are still major issues in hepatobiliary surgery.

Real-time visualization of the liver and localization of hepatic tumors can help surgeons to perform therapeutic liver resections even with sparing parenchyma hepatectomies, thereby reducing post-operative complications.

During development of fluorescence cholangiography, it has been observed that ICG accumulates in primary tumor liver cells and around adenocarcinoma mass, thereafter a new imaging technique has taken place.

ICG fluorescence imaging (FI) technique helps to guide the hepatic surgical procedures and provides the surgeon with real-time visualization of the fluorescent structures of interest that would be invisible under conventional white light. The extrahepatic bile duct anatomy and liver tumors can be emphasized, and hepatic segments highlighted, based on the fluorescence property of ICG and its biliary excretion.

The aim of this paper is to report useful aspects in the practice about this imaging technique in HPB surgery, and showing the concept and the technical bases of fluorescence imaging, by summarizing its practical and technical aspects, and describe the features of the images that can be obtained, and the limitations of its use in hepatic surgery.

Useful indocyanine green features

Indocyanine green, is a hydrosoluble molecule (disulfonated heptamethine indocyanine) with the characteristics of fluorescent dye. Its metabolic properties of intravascular confinement, binding plasma and bile high molecular weight proteins (eg. albumin, lipoproteins), that are not metabolized, and do not alter protein structure, avoid the toxicity of intravenous administration and maintain efficacy at low doses (9,10). Furthermore, thanks to its rapid and biliary excretion, via an active transport system, to its spectral proprieties, and to the development of suitable imaging systems, application of ICG fluorescence has been extended to several surgical fields.

Characteristically, under the action of light, depending on wavelengths, the level of energy of the molecules rises; as soon as the level returns to its basal state, light is emitted. The difference between excitation and emission wavelengths is exploited thanks to cameras equipped with interferential filters to obtain the images.

Fluorescent light is largely attenuated by hemoglobin and water as it traverses biological tissues. Hemoglobin strongly attenuates all wavelengths less than 700 nm (which corresponds in fact to the entire visible spectrum excepting deep red). Water is transparent in visible and near-infrared light but attenuates wavelengths over 900 nm. Therefore, there is a "window" of wavelengths at the limit between deep red and near infrared (700–900 nm) where tissue transparency is maximal. This is one of the reasons why ICG fluorescence can be detected in the near-infrared zone from as deep as 10 mm from the surface of tissues (11).

In hepatic surgery, the ICG dye has been used to evaluate hepatic function and more recently to outline hepatectomy strategies for oncologic resections (12,13) and to plan hepatectomy in living donor hepatic transplantation (14).

After its intravenous or direct intrabiliary injection (15), imaging techniques based on near-infrared ICG fluorescence, allow the visualization of bile ducts (16-18), as well as primary and metastatic liver tumors during surgery (19,20).

Basically, ICG is administered intravenously before, or even during surgery, in a variable interval time, and lights up the liver surface when illuminated with a near-infrared source intraoperatively. Several studies have reported that an intravenous preoperative ICG administration of 0.25–0.5 mg/kg from 12 hours to 14 days helps to identify tumors by intraoperative fluorescence (21,22). After the injection, tumoral and non-tumoral hepatocytes rapidly take up ICG. Normally ICG is excreted in the bile and disappears from healthy liver parenchyma within a few hours (23). On the other hand, ICG remains fixed in tumoral hepatocytes and in pathological areas of the liver, particularly around non-hepatocellular tumors, where hepatocytes are underactive. The features of the camera allow the detection of

hepatocellular (tumor fluorescence) and non-hepatocellular tumors (peri-tumoral fluorescence), thanks to fluorescent light emitted by withheld ICG.

Apparatus Fluorescence imaging system

Apparatus for fluorescence imaging system (FIS) is a mobile system, which provides real time quantitative fluorescent imaging (Fig. 1). The system includes an infrared camera and an amplifier. The camera simultaneously provides the functions of fluorescence excitation with a laser (LED emitting an infrared radiance) over the operative field, and fluorescence image acquisition is ensured by a captor, which filters the light, so that only near infrared wavelengths can be seen.

The camera and cable do not need to be sterilized. The screen and amplifier are placed sufficiently far away, thanks to the length of the cable; so that a non-sterile person can hold the infrared camera above the sterile operative field.

ICG based imaging

ICG most important uses during hepatobiliary surgery for tumors are: cholangiography, liver mapping, and intra and post-operative tumor detection.

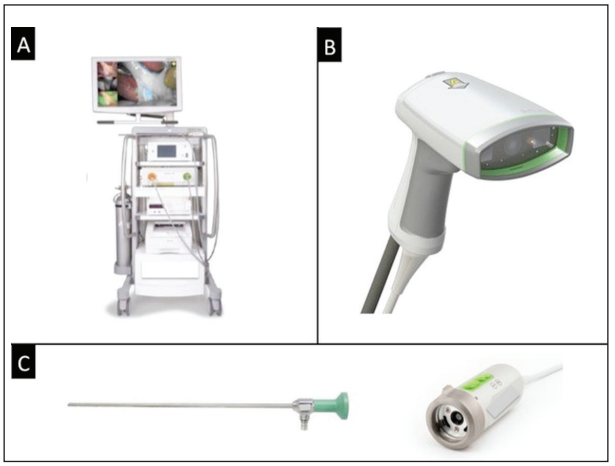

Figure 1. Fluorescence imaging system. The camera includes a laser and a captor: the laser emits a radiance that induces the excitation of fluorescence of the ICG molecules, while the captor filters the light so that near-infrared wavelengths can be seen on the screen. 1A: The system is integrated into a laparoscopic column. 1B: open surgery bundle. 1C: laparoscopic surgery bundle

The main application of fluorescence imaging (FI) ICG-based is the visualization and the study of biliary anatomy. Because of its biliary excretion starting approximately 30 min after intravenous injection, ICG biliary imaging allows a clear visualization of the biliary anatomy, which is useful during difficult cholecystectomies and during resections of centrally located liver tumors and hilar cholangiocarcinoma (17,24). In case of intra-hepatic cholangiography FI-ICG based is limited by depth of the tissue (5–10 mm) (25), nevertheless ICG cholangiography can detect bile duct leakages during hepatectomies, that are missed by other routine tests, like shown by Keiburi et al. in a controlled trial (26).

Anatomic segmentectomy is an essential surgical technique in hepatectomy, balancing cancer curability and postoperative hepatic function. Delineation of liver segments can help surgeons to perform resections based on the exact segmental liver anatomy (27). For this purpose, intra-operative contrast-enhanced ultrasound remains the gold standard for liver mapping (28). Hepatic segments can be identified prior to resection by a dye-staining technique, in which indigo-carmine solution is injected into the corresponding portal branch under US guidance, and segments boundaries are defined as blue staining of hepatic surfaces. However, portal hypertension, in the case of liver cirrhosis, might obstruct conventional liver mapping by US. In contrast, FI allows accurate visualization even in the case of liver cirrhosis (29).

One of the disadvantages to ICG-FI is that tracking the stained plane during dissection of the liver parenchyma is difficult. The fluorescent dye within the targeted segment gradually disappears and is necessary a repeated injection of ICG or temporally clamping the hepatic artery for reducing washout of the dye and permanently visualize the segment. Additionally, a small amount of ICG circulates through the body after injection into the portal vein branch, which eventually stains the entire liver. To avoid these problems, intermittent periods of inflow clamping using the Pringle maneuver are recommended (30). This obtains continuous fluorescence imaging during the operation and allows persistent visualization of the segmental boundaries (31).

Detection of lesions by fluorescence is based on the contrast between tumoral or peri-tumoral fluores-

cent tissues and the rest of the non-fluorescent liver tissue. The observation that hepatocellular carcinoma (HCC) and colorectal metastases (CLL) were detectable by infrared light after ICG was administered intravenously as part of a routine pre-surgical liver function test has been employed in the clinical setting of intraoperative detection of liver tumors (20,32). Moreover, fluorescence patterns are related to the type of cancer and its grade of differentiation (22). Moreover, fluorescence patterns are related to the type of cancer and its grade of differentiation. Injured bile excretion in HCC cells means that ICG is retained, therefore well-differentiated HCCs can be detected by strong, homogenous fluorescence emissions. In contrast, in poorly-differentiated HCCs and metastases ICG is retained in the parenchyma cytoplasm. This means that poorly-differentiated HCCs and CLL produce rim type fluorescence patterns (Fig. 2).

A single dose of ICG (generally 0.5 mg/kg) for routine liver function tests, administered within 14 days prior to surgery, is sufficient to identify tumors by fluorescence imaging.

Normal liver tissue can rapidly uptake ICG, which it is usually eliminated in bile; however, severely cirrhotic liver tissue may not be able to eliminate ICG.

Among limits for viewing tumors using FIS-ICG, there's limited depth of infrared light penetration and tissue thickness. Both affect the fluorescence intensity. Infrared light can only penetrate 5–10 mm of tissue and deeper lesions cannot be visualized. Kudo et al. (33) showed that tumors located 8 mm or more from the liver surface could not be identified, both resected liver metastasis and HCCs. Kudo in his study, evidenced that only tumors that were 5 mm or closer to the liver surface were observable by ICG-FIS.

The study from Peloso A., et al. in 2013 (34) gives preliminary clinical evidence that the intraoperative use of ICG fluorescence can improve detection of CLL, particularly in case of very small lesions, which are frequently missed with conventional imaging modalities.

Literature data highlight the usefulness of fluorescence guided surgery to detect new tumors not diagnosed by preoperative imaging. Handgraaf et al., in their retrospective analysis (35), observed that the percentage of patients in whom additional lesions were identified during surgery was significantly higher by near infrared fluorescence imaging in addition to inspection, palpation or intraoperative ultrasound and the diameter of the lesions identified by fluorescence were smaller than lesions detected by conventional diagnostic procedures.

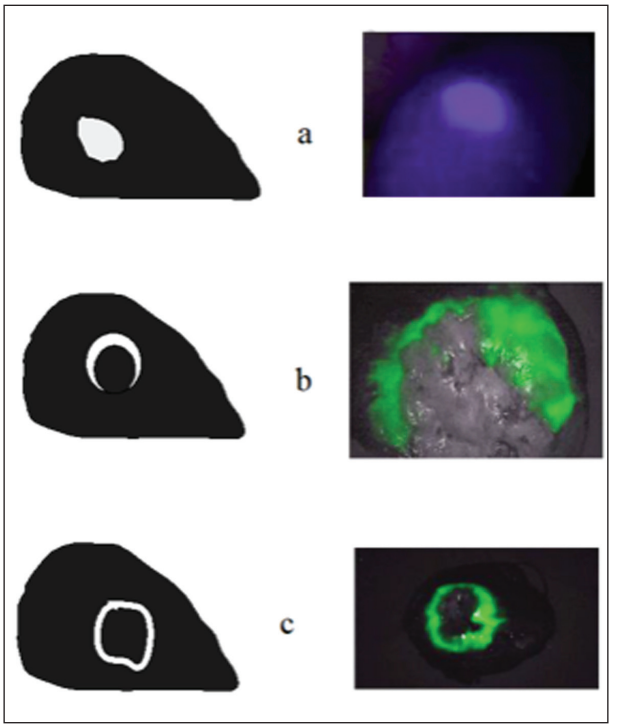

Figure 2. Schematic visualization of liver lesions by FIS. A - homogeneous fluorescence (tipical aspect of well differentiated HCC); B - partial fluorescence (visualized in moderately differentiated HCC); C - peri-tumoral fluorescence (fluorescent ring in poorly differentiated HCC, colorectal liver metastasis, cholangiocarcinomas)

On the other hand, malignant tumors cannot be distinguished from benign tumors, thus leading to a high false positive rate.

Conclusions

The development of more specific molecular tracers should help finding new indications for the application of fluorescence guided surgery. Molecular information provided by this technique could present a paradigm change in decision-taking during resection.

On the other side there has been a steady growth in commercially marketed systems, each with their

own differentiated performance characteristics and specifications (36).

Intraoperative fluorescence imaging will develop into an essential navigation tool, particularly in laparoscopic and robotic liver surgery. This trend will allow surgeons to personalize the procedures according to cancer spread, anatomical variations, and risks of complications.

In these perspectives, procedural protocols should be uniformed for different fields of application. Timing of injection and optimal dosage of ICG are important issues for the standardization of the technique. Some authors have suggested to inject ICG before surgery, and time interval ranged from 1 to 14 days, while others proposed an intraoperative injection (37,38). Most authors prefer to inject ICG 24 hours before surgery, in order to consistently reduce physiological hepatic uptake and to allow the drug to concentrate in the tumor, and 0.5 mg/kg represents the most commonly used dose.

Even administration routes of ICG were differently applied, related to preoperative or intraoperative ICG use (e.g. portal vein or right vein of the stomach, central venous catheter, or peripheral vein) (39).

ICG-fluorescence imaging can be used safely and easily to identify liver tumors, hepatic segments, and extrahepatic bile ducts, in real time during open and minimally invasive surgery.

Despite the various benefits of FI-ICG based in hepatobiliary surgery, there are some drawbacks; these include limited tissue penetration and poor specificity. Intraoperative US remains the goal standard for detection of deeper tumors and FI-ICG based is complemental.

In the literature the high incidence of false positives in tumors detection (about 40%) (19,40) is evident, mostly in liver cirrhosis.

Further clinical studies are required to assess the sensitivity and specificity of FI-ICG based during hepatobiliary surgery.

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Review

Epidemiology and risk factors of pancreatic cancer

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Summary. The most frequent pancreatic cancer is pancreatic adenocarcinoma. It has high and early locally and distant invasiveness; this is the reason why it often shows little sign or symptoms in early stage and poor prognosis after the diagnosis, frequently in advanced stage. Although it is possible to detect this tumor in early stage because of its neoplastic precursor (PanINs). Epidemiological data shows that pancreatic cancer is not very common but obvious it is one of the most neoplastic death-cause in the world. The trend of incidence is quite increasing through years, proportionally to the increase of risk factors. About risk factors, it is not easy to detect in all the cases but it is known the role of some of that: there are hereditary risk factors, such as genetic pattern like HBOC, HNPCC, FAP, PJS, FAMMM, HP and CF and environmental ones (modifiable) such as smoke, alcohol consumption, chronic pancreatitis, obesity and diabetes mellitus. This narrative review aims to analyze the epidemiological data of pancreatic cancer and associated risk factors. (www.actabiomedica.it)

Key words: pancreatic cancer, pancreatic adenocarcinoma, epidemiology, risk factors

Introduction

All the pancreatic epithelial tumors are classified by WHO as benign (we will not consider these tumors in this review) and malignant ones; they are split out considering some macroscopic and microscopic features: on a macroscopic level they are solid, cystic or intraductal, while on a microscopic level they are ductal, acinar or endocrine tumors.

The most frequent pancreatic malignant tumor is the solid one represented by ductal cells and it is called Pancreatic Ductal Adenocarcinoma with his variants (1).

In this review we're only considering pancreatic adenocarcinoma referring to it as "pancreatic cancer".

About its behavior, pancreatic cancer has high invasiveness and the tendency to infiltrate peri-pancreatic tissues, in addiction to that, it has high trend to show nodal metastasis (peri-pancreatic, gastric, mesenteric, omental and peri-portal nodes) and hepatic, bone or pulmonary metastasis.

Rarely pancreatic cancer manifests specific signs or symptoms and because of its poorness of symptoms, it is often diagnosed in an advanced stage; frequently, when patients have symptoms like asthenia, jaundice, abdominal pain and weight loss, they already have a local advanced pancreatic neoplasia.

On a biological level pancreatic cancer has a clear multistep carcinogenesis; just as colo-rectal cancer presents the adenoma-carcinoma sequence, pancreatic cancer shows a similar one starting from Pancreatic Intraductal Neoplasia (PanINs IA, IB, II and III) and ending with an invasive neoplastic lesion. PanINs' progression is supported by a succession of a lot of gene mutations (2).

This specific multistep carcinogenesis suggests that if we will diagnose an early stage lesion, by means of PanINs' detection, we could stop the progression

mentioned above, treating early lesions. This is the safer way to change the natural history of pancreatic cancer. Unfortunately, early-stage pancreatic cancer is usually clinically silent, highlighting the need for improved methods of early detection of precursor.

All the above-mentioned aspects show that pancreatic cancer is one of the most big killing neoplasia loaded by a poor prognosis after diagnosis; in this context, the key to solve this problem is the primary prevention knowing the epidemiology and the risk factors associated.

In this review, the purpose is to define the epidemiology of pancreatic cancer by means of descriptive data coming from the literature and identify risk factors associated with this cancer.

Epidemiology

In 1999 Parkin D et al. had compared cancer registries to obtain the incidences and mortality data bank to obtain information on cancer death in 23 world areas. The result was that pancreatic cancer was responsible for 168,000 deaths per year and it was the 9th most common cause of death from cancer in both sexes combined and about incidence it was the 13th. Unfortunately the mortality to incidence ratio was 98% because of the poor prognosis, worse in developed countries than in developing ones (3).

To investigate the most recent worldwide epidemiology, we are basing our research on the GLOBOCAN estimates. The Global Cancer Observatory (GCO) is an interactive web-based platform presenting global cancer statistics to inform cancer control and cancer research.

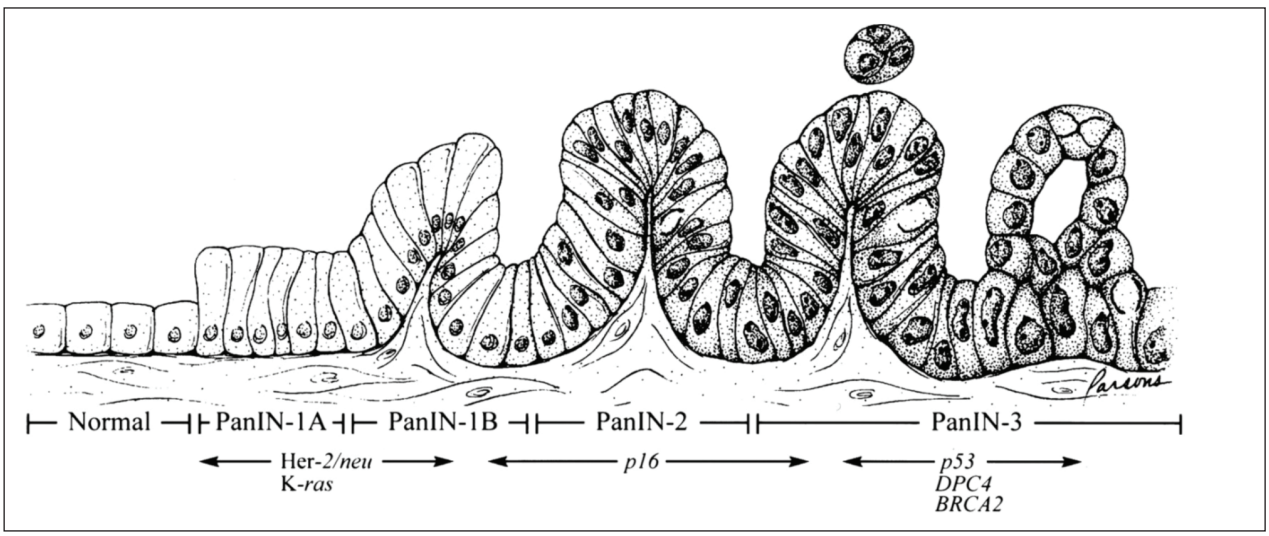
Incidence and mortality rates were estimated using GLOBOCAN by country, using the most recently available data collected by the IARC or available in routine reports from the registries themselves.

GLOBOCAN 2012 estimates demonstrate different results of incidence and mortality, both lower than 350,000 (338,000 were new cases and 331,000 were the deaths); but already 6 years ago pancreatic cancer was the 7th leading cause of cancer death in both sexes and it was more frequent in developed countries (4).

GLOBOCAN 2018 estimates show that, worldwide, pancreatic cancer is the 14th neoplasia sorting by new cases per year. There is little difference between male and female incidence rate. Because of its poor prognosis, with almost as many deaths (n = 432,000) as cases (n = 459,000), pancreatic cancer is the 7th leading cause of cancer death in both males and females (5).

The trend of incidence of pancreatic cancer through years is increasing as reported by the recent Cancer Statistics Review (CSR) (6).

Several authors, agree with that result, such as in Taiwan Tseng CM et al., analyzing Taiwan National Cancer Registry and the National Cause of Death



Figure

Registry, support the evidence we mentioned above and they formulate that presumably the incidence and mortality will continue to rise in Taiwan (7). In Germany, Quante AS et al. have noted a constant increase in the incidence of pancreatic cancer, which will surpass colorectal and breast cancer to rank as the second most common cause of cancer-related deaths by 2030 (8); this trend is the same we can observe in US, where current projections suggest that pancreatic cancer will become the second-highest cause of cancer death (9). Finally we show the epidemiological data in Italy: in 2017 there was over 13,000 new cases and in 2015 over 11,000 deaths; in 2016 the incidence was 22/100,000 new cases per year and it is raising in both sexes, even if more in male population (10).

Pancreatic cancer is mostly frequent in elderly people, the risk of developing pancreatic cancer goes up as people age: about 80% are at least 60 years old and 71 is the average age at the time of diagnosis (11). A population-based epidemiological sudy (12) conclude that the secular trends in the incidence of pancreatic cancer match trends in the prevalence in known risk factors for pancreatic adenocarcinoma such as smoking, overweight and obesity, and diabetes.

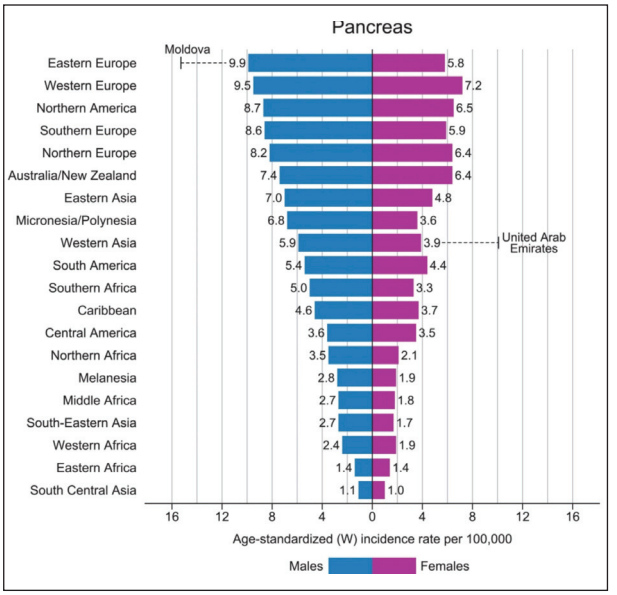
Risk factors

Despite other gastrointestinal tumors, evidences of risk factors for development of pancreatic cancer are poor and they don't explain the whole pancreatic cancer world: we can identify risks factor only in 40% of cases. We have genetics factors (10%) and environmental (modifiable) factors (13).

Curiously, it is difficult to understand the effect-cause relationship of some risk factors, such as diabetes mellitus: in literature some authors tried to clarify the question (14).

About unmodifiable (hereditary) risk factors, we can remember:

- HBOC (Hereditary breast and ovarian cancer syndrome): the BRCA1 and BRCA2 mutations can cause early-onset malignant tumors, most of all breast and ovarian cancer, and including pancreatic cancer, especially if in BRCA2 mutation. This pathway can explain 17-19% of hereditary pancreatic cancer (15).
- HNPCC (Hereditary Non Polyposic Colorectal Cancer or Lynch syndrome): people who have Lynch syndrome, because of the microsatellite instability (MSH2, MSH6, MLH1, PMS2 and EPCAM genes), are predisposed to early-onset colorectal cancer without polyposic lesions and other-site neoplasia, including pancreatic cancer (RR=8.6) (16).
- FAP (Familial Adenomatous polyposis): this syndrome, caused by a mutation in the APC gene, is characterized of early-onset polyps in gastrointestinal tract that can be develop in malignant neoplasia. If FAP is involved in an increased risk of pancreatic cancer is uncertain because it may reflects a misdiagnosed of ampulla carcinomas (17).
- PJS (Peutz-Jeghers Syndrome): The STK11/LKB1 genes mutation characterize an hamartomatous polyposis syndrome and this condition can determine gastrointestinal neoplasia and other tumors like pancreatic cancer (RR=132) (18).
- FAMMM (Familial Athypical Multiple Mole Melanoma syndrome): this syndrome is characterized by malignant melanoma in one or more



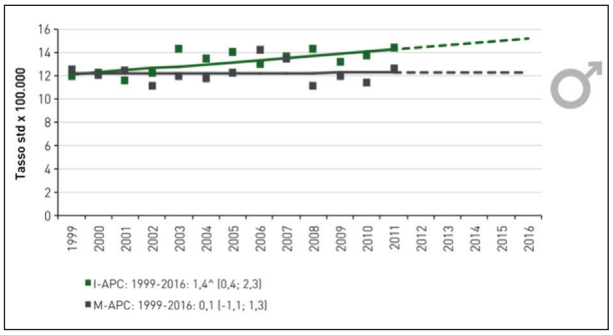
Figure

first-degree or second-degree relatives. In 38% of cases this pathology is caused by a p16INK4a gene mutation, dysregulates the normal cellular cycle. These people have an higher relative risk for pancreatic cancer (13 to 22-fold increased risk of developing pancreatic cancer) (19).

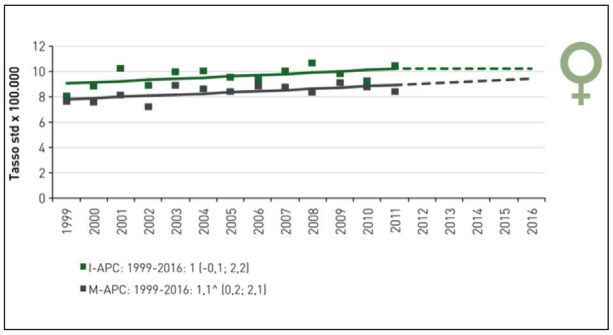
- HP (Hereditary Pancreatitis): In 80% of cases of hereditary pancreatitis it is possible to identify a PRSS1 gene mutation: this is a condition characterized by recurrent acute pancreatitis starting in childhood, that can be evolve in a praecox pancreatic failure (20); the pathogenetic mechanisms, involved in pancreatic cancer onset, are triggered by the pancreatic chronic inflammation (21). Some authors found an high relative risk (RR= 69) for pancreatic cancer for patients with HP compared to the general population (22).
- CF (Cystic Fibrosis): this pathology, caused by CFTR gene mutation, has the same pathogenetic mechanisms explained in HP, because recurrent acute pancreatitis can be involved in pancreatic cancer onset (23).

More interesting is the acknowledgment about environmental risk factors:

- Tobacco use: smoking is the main demonstrated environmental risk factor for the development of pancreatic cancer; the pathogenetic mechanisms include genes mutations inducement (KRAS, p53) and, on the other hand, a chronic inflammation and these two factors can induce cytokines and growth factors output, providing the right pathway to cellular transformation. Tobacco smoking habits in considered responsible of 20-35% of pancreatic cancer cases (24).
- Alcohol consumption: the evidence of this association is limited to heavy alcohol assumption: more than three drinks consumption per day has a relative risk from 1.22 to 1.36 of developing pancreatic cancer, with a dose-response relationship. Alcohol and its metabolites make a pro-carcinogenetic pathway through chronic inflammation (considering alcohol consumption is responsible of 60-90% of chronic pancreatitis) and cellular gene instability (25).
- Chronic pancreatitis: The morphological and functional modification in chronic pancreatitis are the same we can found in pancreatic cancer; this similarity is represented also on a molecular level. The chronic inflammation causes the production of TNFα, IL-6, IL-8, PDGF, TGFβ and other cytokines that can induce cellular proliferation and reduce immune-surveillance (26). The main damage on DNA is caused by ROS production, promoting the progression to cellular transformation (27). The risk of pancreatic cancer is significantly elevated in subjects with chronic pancreatitis and appears to be independent from sex, country, and type of pancreatitis (28). In a 2010 meta-analysis, Raimondi S. et al. identify the association between chronic pancreatitis and pancreatic cancer with a 13.3 relative risk (22).
- Obesity: Some studies had demonstrated a relative risk increase of 1.12 for each increase in 5 kg/m^2 in the BMI (29). In obese people the pathogenesis is characterized by adiposopathy, a chronic adipose disease in which macrophages product pro-inflammatory cytokines and there is a dysregulation of hormonal level: in particular we can find high level of leptin and low level of adiponectin (30). It is interesting to observe that there is a temporal relationship between BMI and the neoplasia: obesity since childhood have a higher risk relative for pancreatic cancer development (31). On a dietetic level, high consumption of red meat and fatty diet may have a role in the pathogenesis (32).
- Diabetes mellitus: About 80% of people with pancreatic cancer have also glucose intolerance or diabetes. The association between these two diseases is clear but it is important to define the relationship. The majority of patients with pancreatic cancer has diabetes in close up the diagnosis of the tumor validating the hypothesis which support that this diabetes is a consequence of the neoplasia (33). But there is a relevant association also with diabetes mellitus type 2 with OR=1.8 (34); the pathogenetic mechanisms sustain this relationship are the hyperinsulinemia, often detected in DMT2, and high level of IGF1: that modification can induce pancreatic glandular proliferation and specific



Figure



Figure

cellular interaction (35). The cellular interaction is between Pancreatic Stellate Cells (PaSCs) and Tumor-associated Macrophages (TAMs): while their dysregulation, because of hyperinsulinemia and hyperglycemia, can induce fibrosis, cellular proliferation and apoptosis inhibition, they are the actors of desmoplastic reaction and hyperplasia frequently detected in pancreatic neoplastic tissues (36).

- There are some others factors pointed to have a role in pancreatic carcinogenesis, but there isn't enough evidence in literature, e.g. biliary obstructive diseases. Some studies underline the role of cholecystectomy: the pivot of this association is the CCK levels, often high in cholecystectomized patients, CCK is responsible for pancreatic glandular hyperplasia (RR=1.23) (37). Some other studies support the hypothesis according to which the risk factor isn't the surgery but the history of gallstones (RR=1.70) (38). We have to consider that risk factors associated with gallstones, like obesity, diabetes, alcohol consumption and fatty diet are the same involved in the pathogenesis of pancreatic cancer; for this reason some studies clear the role of biliary obstructive diseases (39).

Conclusions

Pancreatic cancer is one of the most oncological big killer; in few years it will become a non-marginal healthy problem, because of its increasing trend of incidence and poor prognosis after diagnosis. In several cases it is difficult to identify certain risk factors and the hereditary ones is clear just in small portion; the large majority of pancreatic cancer results from environmental factors: smoke, alcohol consumption, chronic pancreatitis, obesity and diabetes mellitus.

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Review

Diagnosis and management of intraductal papillary mucinous neoplasms of the pancreas

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Summary. *Background and aim of the work:* Intraductal papillary mucinous neoplasms (IPMN) of the pancreas are cystic lesions with malignant potential. Given their increasing incidence in the latest years, a precise characterization and management of these lesions have become more and more crucial: even though the majority of IPMN has a benign and indolent course, it is fundamental to early recognize and stratify patients in order to accurately plan a tailored follow-up and to individuate those that would benefit of surgical treatment. The aim of this paper is to highlight the most recent evidence on IPMN available in the current literature. *Methods:* We performed a review of the recent literature and of the recent guidelines about pancreatic cystic lesions, especially IPMN. *Results:* The incidence of IPMN is now on the rise: an increasing number of patients, possibly because of the increasing diagnostic yield of imaging techniques, is being diagnosed with pancreatic cystic lesions, a great part of which are IPMN. The possibility of malignant transformation requires a careful approach to these patients, in the need of tailoring the follow-up and the therapy. *Conclusion:* A detailed diagnosis, the determination of risk factors for malignant transformation and a multisciplinary approach are of foremost importance for an effective management of IPMN. (www.actabiomedica.it)

Key words: intraductal papillary mucinous neoplasms

Background and aim of the work

Intraductal papillary mucinous neoplasms (IPMN) of the pancreas are mucin-producing cystic tumors, classified as premalignant lesions (1), originating from the pancreatic epithelium of the main pancreatic duct (MPD) or its side branches (2).

The overgrowth of mucin-producing cells usually forms intraductal papillae, along with the production of thick mucus in a variable extension, with a subsequent dilation of the ducts in a grossly visible manner (3). Although the behavior of IPMN can be considered benign, "borderline" and malignant in consideration of the different grade of dysplasia of the involved cells, they always have to be considered as potential precursors of pancreatic ductal adenocarcinoma (PDAC) (4,5).

The reported incidence of IPMN, slightly predominant in males, varies from 0.31 to 4.35/100000, with an average age of 64 at diagnosis (6,7). The marked increase of the incidence in the recent years is probably due to the enhanced accuracy of imaging techniques and to the progress in recognition of the disease (8): up to 15% of patients undergoing abdominal magnetic resonance imaging (MRI) for other reasons are diagnosed with previously unknown pancreatic cystic lesions (9), which can be IPMN in a large number of cases (up to 82%) (10).

Based on the site of the involved pancreatic ducts, IPMN can be differentiated in 3 groups: branch duct (BD)-IPMN, characterized by cyst-forming dilation of lateral branches in communication with a normal MPD; main duct (MD)-IPMN, characterized by dif-

fuse or segmental dilation of the MPD; mixed type (MT)-IPMN, which includes characteristics of both types (1,11). Pancreatic ducts can be involved in a unifocal or multifocal fashion, the latter being more frequent in the elderly (11,12).

Methods

A research of the literature was performed by using Pubmed, Medline, Embase databases.

Cochrane database and google Scholar were searched as well. All types of papers in English, including abstracts and reviews, were included. Considering the year of publication, the research was limited to the last 15 years.

All recent articles were taken into consideration, then a manual search was performed in order to identify all relevant reports. We also considered the reference list from the most relevant articles and guidelines. Articles published as abstracts were also included.

Results

After a detailed evaluation, we included 37 articles in total: 22 were original articles, 6 were reviews, 4 were guidelines, 1 was a consensus of experts, 1 was a case report, 1 was an abstract, 1 was an editorial and 1 was the WHO classification of gastrointestinal tumors.

Clinical appearance

Most patients with IPMN are clinically silent: symptoms such as abdominal pain, weight loss, steatorrhea, new-onset diabetes or jaundice generally occur in the setting of an obstruction of the ductal system or of a complication such as pancreatitis, perforation, hemorrhage or fistulation (13-15).

Diagnosis

Imaging (MRI and CT)

The diagnosis is often incidental. MRI with magnetic resonance cholangio-pancreatography (MRCP) is considered the imaging technique of choice, being more accurate than computed tomography (CT) in the evaluation of pancreatic cysts (11): its sensitivity and specificity in assessing the presence of communication with the MPD are 91-100% and 89%, respectively (7). Indications for CT include the presence of calcifications, assessment of vascular involvement or metastatic disease, suspicion of post-operative recurrence of PDAC (16).

Endoscopic UltraSound (EUS)

EUS, with its high accuracy in the evaluation of the cystic component and the pancreatic parenchyma, should be used in case of suspicious morphological features: based on morphology, EUS is more sensitive (76%) than MRI or CT (34% and 48%) in differentiating neoplastic from non-neoplastic cysts (16,17).

Contrast harmonic enhanced EUS (CH-EUS) can be considered in the evaluation of mural nodules, as in this setting it seems superior than CT or standard EUS, with a sensitivity of 100% and a specificity of 80% (18).

EUS-guided fine needle aspiration (FNA) is of great value, as it allows the possibility of cytological sampling any solid component and aspirating cyst fluid for analysis (19).

Cystic biomarkers

CEA, CA 19.9 and amylase should be tested whenever cystic fluid is available.

CEA values ≥192 ng/mL can distinguish mucinous from non-mucinous cysts; high amylase levels confirm communication with the MPD (as in IPMN) and CA 19.9 helps distinguishing cases in which CEA is indeterminate (7). In a little cohort of patients, glucose outperformed the accuracy of CEA in differentiating mucinous from non-mucinous cysts (20). Next generation sequencing on cystic fluid for KRAS/GNAS mutations is extremely useful in the differential diagnosis of IPMN with other pancreatic cystic lesions, although not widely available yet (7,16).

Serum biomarkers

Serum CA 19.9 values correlate with the presence of malignant IPMN, being therefore considered

a feature of concern (11). Its sensitivity is 79-100%, although a normal value does not exclude malignancy (7).

Natural history

Adequate and tailored surveillance of IPMN patients is fundamental, as it allows the early detection of potentially resectable pancreatic cancer that may develop on this premalignant condition (21). Invasive carcinoma derived from IPMN does not have to be confused with concomitant PDAC arising in a different site from IPMN, which is considered a separate entity (22).

As premalignant lesions, IPMN harbor the potential for progression towards cancer. Although the time for progression is limited in the elderly, IPMN diagnosed in older patients are more prone to degeneration (12). Multifocal cysts correlate with the incidence of PDAC concomitant with IPMN, thus being a possible risk factor for cancer (23).

Risk factors for degeneration are defined, in the Japanese guideline, as "worrisome features" (cyst ≥3 cm, enhancing mural nodule <5 mm, thickened enhanced cyst walls, MPD size of 5-9 mm, abrupt change in the MPD caliber, lymphadenopathy, cyst growth >5 mm/2 years, increased serum levels of CA 19.9) and as "high risk stigmatas" (obstructive jaundice with a cyst in the pancreatic head, enhanced mural nodule >5 mm, MPD size ≥10 mm), the latter being strong indications for surgery (11).

Although it is known that the risk of malignancy in main duct- or mixed type IPMN is higher than in BD-IPMN (1,24), the rate of progression hasn't been clearly defined yet (25). In a recent study of Han and colleagues, a population of 1369 patient diagnosed with BD-IPMN was followed-up for at least 3 years resulting in the detection of high-grade dysplasia (HGD) or invasive IPMN in 13 patients (0.9%): a relation with progression was found with the initial cyst size and a faster cyst growth rate (26). In another study, Pergolini and colleagues highlighted an overall risk of malignancy (including concomitant PDAC) of 8% in a cohort of patients followed up >10 years, supporting the idea of continued surveillance, as long as the patient is fit for surgery (27): this is in contrast with the previous recommendations of the American guidelines, which suggest stopping surveillance after 5 years in cysts without worrisome features if no significant changes are detected (9).

IPMN in liver transplant recipients haven't shown any accelerated pattern of progression, compared to the general population in a large cohort of patients (28).

At this moment, current literature is inconclusive about increased incidence of extrapancreatic neoplasm in IPMN patients, suggesting that standard surveillance should be advised (29).

Treatment

Surgery

The frequency of HGD and cancer in MD-IPMN and MT-IPMN is high: these high rates justify the indication for surgical resection in all patients fit for surgery (11,16).

European guidelines recommend surgery in MPD dilation >5 mm, while Japanese guidelines strongly recommend surgical resection in IPMN with obvious "high risk stigmata": MPD ≥10 mm, jaundice or enhancing mural nodules > 5 mm (11). Enhancing mural nodules < 5 mm, MPD dilation of 5 to 9 mm are considered "worrisome features" with a recommendation of surgical evaluation but not to immediate resection.

If the MPD dilatation affects the entire gland, a pancreatoduodenectomy with frozen section analysis is recommended (16).

A total pancreatectomy is otherwise indicated in case of increased risk for malignancy: familial pancreatic cancer, a mural nodule, involvement of the entire MPD (30-32).

A partial pancreatectomy is indicated in localized IPMN together with frozen section analysis on the resection margins, useful for detecting spread of dysplasia or cancerous lesions and guiding an extended resection, especially in young fit-for-surgery patients.

In cases of multifocal IPMN each lesion should be evaluated as a single entity and a tailored surgical approach is indicated according to the presence of "high risk stigmata" or "worrisome features" (33,34).

Since small BD-IPMN can evolve into HGD or cancer, it is suggested to monitor the presence of rela-

tive criteria for resection and, if multiple, to evaluate for surgery.

Relative surgical criteria, proposed by European guidelines, are: growth rate ≥5 mm/year, CA 19.9 level >37 U/ mL in the absence of jaundice, MPD diameter > 5 and < 9.9 mm, cyst diameter ≥40 mm, symptoms (new-onset of diabetes mellitus or acute pancreatitis), contrast-enhancing mural nodules <5 mm.

Literature reports an increased risk of malignancy from 12% to 47% in cases of a cyst ≥30 mm; European guidelines propose a cut-off point for resection of IPMN, regardless of the absence of clinical symptoms or (other) risk factors, of > 40 mm (16,26,35).

In young patients (< 65 years), surgery has to be evaluated against the burden of life-long imaging follow-up owing to the cumulative risk of HGD and malignancy. Resection can be indicated in young fit-for-surgery patients even in cyst > 2cm without "worrisome features" (11,36).

Post-surgical follow-up is required until the patient is fit for surgery, also because a significant number of recurrences can develop over 5 years after the index operation (37). In the post-surgical setting: patients with PDAC associated to IPMN have to be followed-up as those with PDAC; patients with HGD or MD-IPMN need MRI or EUS monitoring every 6 months for the first 2 years then yearly; patients with IPMN in the remnant pancreas (no HGD left) and patients with low grade dysplasia (LGD) need the same monitoring as the unresected ones (16).

Margin positivity after resection for non-invasive IPMNs is primarily due to LGD and is not associated with developing recurrence in the remnant pancreas or at the resection margin (37).

"Watchful waiting" and follow-up timing

Watchful waiting is justified in patients with asymptomatic BD-IPMN without high risk stigmata (7,9,11,16). In these patients an aggressive approach is not justified, as the low rate of progression of these lesions should be compared with the risk of surgery itself and post-operative mortality (15).

The perfect timing of follow-up is still a matter of debate. European guidelines indicate repetition of imaging every 6 months in the first year after diagnosis and then yearly, in patients with no current indications for surgery and independently of the bigger cyst size. MRI is the preferred imaging modality, whereas EUS has to be used if features of concern show up (16). On the other hand, Japanese guidelines propose a variable timing based on the size of the main cyst; Italian guidelines also take into consideration the possibility to lengthen the intervals if the IPMN is stable in time (7,11). Conversely, patients with a relative indication for surgery need a tighter follow-up, with MRI or EUS scheduled every 6 months (16).

Regarding the possibility of progression even after 5 years, the follow-up should be life-long until the patient is fit for surgery: in this setting, stopping the follow-up after 5 years of stable disease, as suggested by the American guideline, seems too risky (9,25,27).

Conclusions

Due to the increasing incidence and aging of the population, IPMN management is an uprising problem. Given their natural history and the possibility of progression towards malignancy, a life-long surveillance seems the most appropriate management to advise, in a multidisciplinary setting. Follow-up of these lesions is recommended until the patient is fit for surgery, as surgery is the only therapeutic option in patient with high-risk features. The perfect timing for follow-up is still a matter of debate and should be discussed in a tailored manner based on patient's and tumor's characteristics. Further studies are required in order to better assess the behavior of IPMN and to highlight early predictors of malignancy. The molecular profile determination has given, until now, promising results.

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Review

Hemolytic uremic syndrome: differential diagnosis with the onset of inflammatory bowel diseases

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Summary. *Background:* Shiga-toxin Escherichia coli productor (STEC) provokes frequently an important intestinal damage that may be considered in differential diagnosis with the onset of Inflammatory Bowel Disease (IBD). The aim of this work is to review in the current literature about Hemolytic Uremic Syndrome (HUS) and IBD symptoms at the onset, comparing the clinical presentation and symptoms, as the timing of diagnosis and of the correct treatment of both these conditions is a fundamental prognostic factor. A focus is made about the association between typical or atypical HUS and IBD and a possible renal involvement in patient with IBD (IgA-nephropathy). *Methods:* A systematic review of scientific articles was performed consulting the databases PubMed, Medline, Google Scholar, and consulting most recent textbooks of Pediatric Nephrology. *Results:* In STEC-associated HUS, that accounts for 90% of cases of HUS in children, the microangiopathic manifestations are usually preceded by gastrointestinal symptoms. Initial presentation may be considered in differential diagnosis with IBD onset. The transverse and ascending colon are the segments most commonly affected, but any area from the esophagus to the perianal area can be involved. The more serious manifestations include severe hemorrhagic colitis, bowel necrosis and perforation, rectal prolapse, peritonitis and intussusception. Severe gastrointestinal involvement may result in life-threatening complications as toxic megacolon and transmural necrosis of the colon with perforation, as in Ulcerative Colitis (UC). Transmural necrosis of the colon may lead to subsequent colonic stricture, as in Crohn Disease (CD). Perianal lesions and strictures are described. In some studies, intestinal biopsies were performed to exclude IBD. Elevation of pancreatic enzymes is common. Liver damage and cholecystitis are other described complications. There is no specific form of therapy for STEC HUS, but appropriate fluid and electrolyte management (better hyperhydration when possible), avoiding antidiarrheal drugs, and possibly avoiding antibiotic therapy, are recommended as the best practice. In atypical HUS (aHUS) gastrointestinal manifestation are rare, but recently a study evidenced that gastrointestinal complications are common in aHUS in presence of factor-H autoantibodies. Some report of patients with IBD and contemporary atypical-HUS were found, both for CD and UC. The authors conclude that deregulation of the alternative complement pathway may manifest in other organs besides the kidney. Finally, searching for STEC-infection, or broadly for Escherichia coli (E. coli) infection, and IBD onset, some reviews suggest a possible role of adherent invasive E. coli (AIEC) on the pathogenesis of IBD. *Conclusions:* The current literature shows that gastrointestinal complications of HUS are quite exclusive of STEC-associated HUS, whereas aHUS have usually mild or absent intestinal involvement. Severe presentation as toxic megacolon, perforation, ulcerative colitis, peritonitis is similar to IBD at the onset. Moreover, some types of E. coli (AIEC) have been considered a risk factor for IBD. Recent literature on aHUS shows that intestinal complications are more common than described before, particularly for patients with anti-H factor antibodies. Moreover, we found some report of patient with both aHUS and IBD, who benefit from anti-C5 antibodies injection (Eculizumab). (www.actabiomedica.it)

Key words: Hemolytic Uremic Syndrome (HUS), Inflammatory Bowel Disease (IBD), Shiga-Toxin E. Coli (STEC)

Background

Shiga-toxin E.coli productor (STEC) provokes frequently an important intestinal damage that may be considered in differential diagnosis with the onset of Inflammatory Bowel Disease (IBD). The aim of this work is to review in the current literature the reported similarities and differences between Hemolytic Uremic Syndrome (HUS) and IBD symptoms at the begin, as the timing of diagnosis and of the correct treatments of both these conditions is a fundamental prognostic factor. An association between typical or atypical HUS (aHUS) and IBD is searched in literature and case reports, as it has already been established a possible renal involvement in patient with IBD (IgA-nephropaty).

Methods

A review of scientific articles was performed consulting the databases PubMed, Medline, Google Scholar, and consulting most recent textbooks of Pediatric Nephrology.

Results

The HUS is a clinical diagnosis at first, defined by simultaneous occurrence of microangiopathy (MAT) with hemolytic anemia, thrombocytopenia and acute kidney injury. In the past HUS has been divided in diarrhea-positive HUS also called "typical", and diarrhea negative HUS, or "atypical" HUS (aHUS) (1-3). Shiga-toxin productor E. coli (STEC)-associated HUS is considered at first. In 70% of cases in North America and Western Europe the most frequent serotype is O157:H7, but other serotypes are reported (O111:H8, O103:H2, O121, O145, O26, and O113 (4, 5). In STEC-associated HUS, that accounts 90% of cases of HUS in children, the microangiopathic manifestations are usually preceded by gastrointestinal symptoms lasting about 2 weeks, with symptoms including abdominal pain, vomiting, diarrhea, bloody stools. However about 25% of cases of STEC-associated HUS do not present with diarrhea (1-5). The Shiga-toxin like (Stx) produced by E. coli is responsible to direct damage and to complement alternative-pathway activation. E. coli strains that produced Stx-2 were most commonly associated with HUS. Stx are picked up by polarized gastrointestinal cells via transcellular pathways and translocate into the circulation. Once the endothelial cell internalizes the toxin, it can inhibit protein synthesis, induce the apoptosis to start, and induce endothelial changes in a thrombogenic phenotype (1, 5-9). In a primate model of HUS, it resulted that the rate of gastrointestinal absorption plays an important role (4). After the bacteria colonize the colon, they cause a severe colitis. Thereafter, based on the presence of specific pathogen-associated molecular patterns which interfere with the host response, SEU may appear presenting with renal failure and neurological symptoms (6, 7). In vitro studies have demonstrated that several cytokines are involved, TNF-alfa seems to play an important role in the cellular damage. However STEC associated HUS is finally characterized by the activation of the alternative pathway of the complement, which results in microangiopathic vasculitis (1, 6, 7).

Initial presentation of STEC associated HUS may be considered in differential diagnosis with IBD onset.

IBD include Crohn's Disease (CD) and Ulcerative Colitis (UD), that are both chronic inflammatory diseases characterized intestinal inflammation with variable extent and a possible systemic involvement. The onset of CD is variable from abdominal symptoms (abdominal pain, bloody stool, vomit), perianal manifestations (fistulas, tags, strictures), and extra-intestinal symptoms (cutaneous lesions, growth failure, anemia, uveitis, etc.). Any area in the gastrointestinal tract can be involved (10, 11). UC is usually segmental, but it can present even with severe pancolitis. Usually, it presents with bloody diarrhea and abdominal pain. Possible life-threatening complications include perforation or toxic megacolon, and surgery may be required. While the inflammation and injury in UC is limited to the mucosa, CD is a transmural process (10, 11).

Once a person is exposed to STEC, diarrhea typically occurs after 3-7 days and contains blood in about 85% of children. When the diarrhea starts resolving, about only 15 % of infected patients develop HUS (1, Figure 1). The kidney and gastrointestinal tract are the

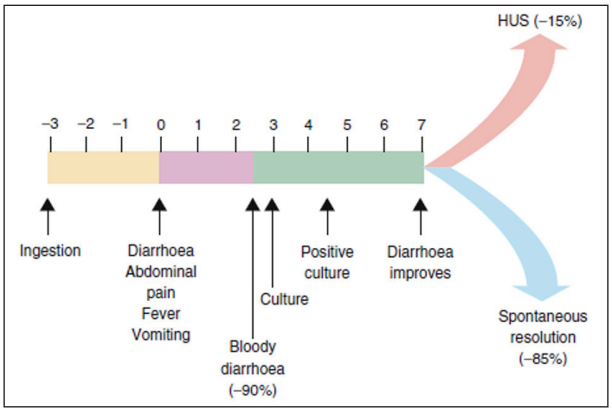

Figure 1.

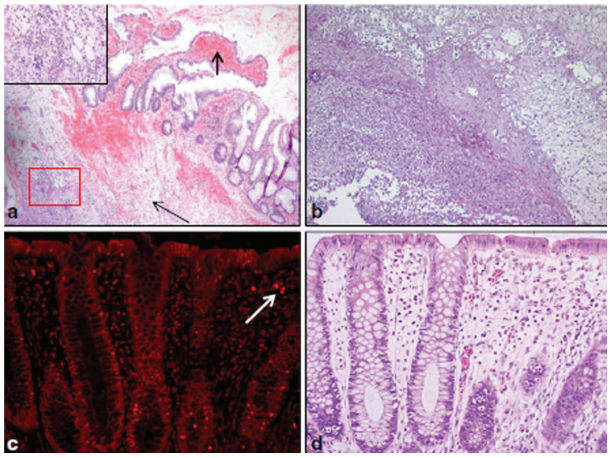

Figure 2.

organs most commonly affected, but an involvement of central nervous system, pancreatic, and myocardial involvement may also be present (1, 2, 8). Gastrointestinal symptoms with STEC-associated infection lead to a worse renal prognosis in comparison to patient with scarce intestinal symptoms (12). The transverse and ascending colon are the segments most commonly affected, but any area from the esophagus to the perianal area can be involved. The more serious manifestations include severe hemorrhagic colitis, which may be misdiagnosed as UC, bowel necrosis and perforation, rectal prolapse, peritonitis and intussusception. Severe gastrointestinal involvement can result in life-threatening complication as toxic megacolon and transmural necrosis of the colon with perforation. Transmural necrosis of the colon may lead to subsequent colonic stricture, as in CD. Perianal lesions and anal strictures are described (13-18). It is reported that for some patient intestinal sigmoidectomy was needed for severe complications (perforation, ulceration); in other patients, biopsies were performed in order to exclude IBD. In all cases specific histological findings (TUNEL-cells) suggest that apoptosis is the main mechanism of cell injury (15, Figure 2). Gastrointestinal complications can be lethal, and early surgery may sometimes be necessary. However, no correlation has been demonstrated between the severity of the gastrointestinal manifestations and clinical or biological signs (16).

The incidence of colonic perforation and stricture secondary to HUS is estimated to 1% and 3%, respectively (16, 17). Two peaks in the diagnosis of colonic stricture are described: the first from one to two months, and the second peak over 1 year after the acute event. Histological findings in stricture areas are characterized by granuloma formation and edema in the submucosal layer, and/or fibrosis in all the layers of the stricture. Both the vascular injury (MAT) and chronic inflammation secondary to an acute phase are hypothesized as possible pathogenetic mechanisms (17). Elevation of pancreatic enzymes, liver damage and cholecystitis are other described complications (1, 2, 19). Severe gastrointestinal complications are associated with a poor renal outcome (20).

There is no specific therapy for STEC HUS and the standard of care remains supportive. General management of acute kidney injury includes appropriate fluid and electrolyte management (hyperhydration when possible), often antihypertensive therapy, and initiation of renal replacement therapy when appropriate, to treat anemia and to avoid antidiarrheal drugs (1-3, 21, 22). Some studies have demonstrated that children who received antibiotic therapy were more likely to develop HUS. In vitro studies have shown that some antibiotics promote production and release of Stx from bacteria. Other studies and metanalyses have not demonstrated such an association, but antibiotic administration remains controversial and finally it is considered not safe in the clinical practice (1-3, 22).

Most of the literature affirm that aHUS rarely is in differential diagnosis with IBD at the onset, as the gastrointestinal manifestations are often absent or mild (1-3, 23). Underlying causes of aHUS are now better understood as genetic causes or secondary ones

(autoimmunity, drugs). It may manifest at all ages but is more frequent in adults (1-3). aHUS often presents with nonspecific symptoms, before the onset of the renal involvement, which is typically nephrotic or nephritic syndrome. A preceding illness, particularly a respiratory or gastrointestinal infection, is often reported as a trigger. Gastrointestinal symptoms and diseases have been described in the form of vomiting, hepatitis, pancreatitis, and rarely intestinal bleeding (1-3, 23). A recent study has evidenced that gastrointestinal complications and symptoms, as well as pancreatitis, are more common in aHUS with anti-factor-H autoantibodies (24). In other papers is also reported that some patients develop ischemic colitis and may be misdiagnosed as acute appendicitis or acute ulcerative colitis (1, 23). Regarding the direct associations between IBD and HUS, Peraldi et al. hypothesized the relationship between thrombogenic status in IBD and HUS development, reporting two cases of HUS in patient with CD, one of which was non-STEC associated (25). Another recent case report has described the development of diarrhea and non-STEC associated HUS with a concomitant diagnosis of CD in an adult patient (26). An association with aHUS and UC is also described. In a report, a young adult patient with UC recovered after Eculizumab treatment after developing aHUS with anti-factor H antibodies (27). In a second report, a 16 years old patient with UC developed aHUS (without anti-H factor antibodies) and received anti-C5 injection with benefit for both his renal and gastrointestinal disease (28). The authors conclude that deregulation of the alternative complement pathway may manifest in other organs besides the kidney and maybe hyperactivity of the alternative complement cascade plays a role in the pathogenesis of IBD (27). However, this affirmation is based only in in-vitro experimentations and probably requires further investigations. Recently, some authors conclude that while a direct causal relationship cannot always be established, improvement in IBD symptoms has been demonstrated after treatment with complement blockade (27-29). Finally, searching for STEC-infection, or broadly for E. coli infection, and IBD onset, some reviews suggest a possible role of adherent invasive E. coli (AIEC) on the pathogenesis of IBD (30).

Conclusions

The current literature shows that gastrointestinal complications of HUS are quite exclusive of STEC-associated HUS, whereas aHUS have usually mild or absent intestinal involvement. Gastrointestinal complications are mostly related to the Stx action for its apoptotic effect. When the gastrointestinal involvement is important, the clinical presentation is similar to IBD at the onset, therefore differential diagnosis may take a few days, several laboratory and imaging exams. Colonic strictures are possible described complications, as in CD. For these similarities, some patients underwent endoscopy with intestinal biopsies. Early differential diagnosis is important to start a correct and prompt treatment. Laboratory exams showing renal involvement, thrombocytopenia and hemolytic anemia are the first elements that can help differentiating the two conditions, although they often need to be repeated. HUS and IBD have other points in common. Whereas no case of IBD after STEC-associated HUS are reported, some type of E. coli (AIEC) are considered as risk factor for IBD onset. Histological findings on intestinal stricture after STEC-associated HUS are similar to CD. Recent literature on aHUS shows that intestinal complications are more common than described before, particularly for patients with anti-H factor antibodies. Moreover, a few reports of patients with both aHUS and UC were found, who benefited from anti-C5 antibodies injection (Eculizumab). Other reports of patient with CD who developed non-STEC associated HUS, support the hypothesis of a possible common pathogenesis.

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Review

Is the risk of contrast-induced nephropathy a real contraindication to perform intravenous contrast enhanced Computed Tomography for non-traumatic acute abdomen in Emergency Surgery Department?

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Summary. *Background:* Contrast enhanced Computed Tomography (CCT) is the most used imaging test to investigate acute abdominal clinical conditions, because of its high sensitivity and specificity. It is mandatory to make a correct and prompt diagnosis when life threatening abdominal diseases as mesenteric ischemia are suspected. Contrast medium administration was linked to acute renal failure, therefore radiologist often prefer to perform CCT without contrast in patients needing to undergo the exam with increased serum creatinine. The aim of the review was to focus on the incidence of contrast induced nephropathy in patients presenting non-traumatic acute abdominal clinical conditions, who underwent CCT with intravenous contrast agent administration in emergency setting. *Materials and Methods:* The systematic review protocol was guided by the Preferred Reporting Items for Systematic Reviews and Meta-analyses Protocol (PRISMA-P). Quality of the evidence will be evaluated using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) methodology. *Results:* The strongest currently available evidence on the incidence of post-contrast acute kidney injury (AKI) following intravenous contrast agent administration consists in a meta-analysis of observational studies. Data extracted from meta-analyses demonstrate that, compared with non-contrast CT, CCT was not significantly associated with AKI. Moreover, the risk of AKI (RR=0.79; 95% confidence interval [CI]: 0.62, 1.02; P=.07), death (RR=0.95; 95% CI: 0.55, 1.67; P=.87), and dialysis (RR=0.88; 95% CI: 0.23, 3.43; P=.85) is similar, compared with the risk of AKI in the non-contrast medium group. Furthermore, intravenous low-osmolality iodinated contrast material is a nephrotoxic risk factor, but not in patients with a stable SCr level less than 1.5 mg/dL, therefore many factors other than contrast material could affect PC-AKI rates. *Discussion and conclusions:* The benefits of diagnostic information gained from contrast enhanced TC in assessing AA are fundamental in some clinical scenarios. The risk of contrast induced nephropathy (CIN) is negligible in patients with normal renal function but the incidence appears to rise to as high as 25% in patients with pre-existing renal impairment or in the presence of risk factors such as diabetes, advanced age, vascular disease and use of certain concurrent medications. The incidence of CIN/AKI after intravenous contrast administration is very low in general population. Radiologists and referring physicians should be familiar with the risk factors for renal disease, CIN and preventing measures. (www.actabiomedica.it)

Key words: contrast induced nephropathy, acute abdomen, Emergency Department, acute kidney injury, prevention strategy

Background

In clinical practice, physical examination of the patient, plasmatic dosage of C-Reactive Protein and White Blood Cells count alone are not always sufficient to discriminate the grade of urgency of abdominal diseases in patients admitted for acute abdominal pain in emergency surgery department (1).

Contrast enhanced Computed Tomography (CCT) is the most used imaging test to investigate acute abdomen (AA) because of its high sensitivity and specificity (1).

Contrast medium (CM) administration was linked to acute renal failure, above all in patients undergoing primary angioplasty or coronary procedures with higher dose of CM than in patients who received CCT with intravascular contrast medium in ED (2-4).

In literature, several studies showed that CM administration can lead to contrast-induced nephropathy (CIN) especially in "high risk" patients including the elderly and patients with chronic renal impairment, diabetes, congestive heart failure and anemia (2-8).

The American College of Radiology (ACR) stated that CIN is a real, albeit rare, entity and recommends more restricted use of intravenous contrast material among "high-risk" patients (9).

The "fear" of CIN makes radiologists reluctant to submit a patient to CCT with intravenous contrast administration if serum creatinine is minimally increased even in emergency situations, when making diagnosis is the priority to promptly manage the patient presenting with AA, and CCT is mandatory, especially when clinical findings and laboratory results are inconclusive.

Post-contrast induced Acute Kidney Injury (PC-AKI) is a general term used to describe a sudden deterioration in renal function that occurs within 48 hours after intra-venous administration of iodinated contrast agent (9).

PC-AKI is a correlative diagnosis and may occur regardless of whether CM was the cause of the deterioration (9).

CIN is a specific term used to describe a sudden deterioration in renal function that is caused by the intra-venous administration of iodinated CM; therefore, CIN is a subgroup PC-AKI (9).

CIN is considered the development of AKI after the administration of radiographic CM in the absence of other identifiable causes and is widely accepted as the third most common cause of hospital-acquired AKI; it occurs above all in patients undergoing primary angioplasty or coronary procedures which require intra-arterial high dose of CM than in patients who underwent to CCT with intravenous CM in emergency surgery department (2, 3, 9).

The incidence of CIN in the general population ranges from 0.6% to 2.3%, but, when focusing on specific high-risk patients, the incidence can increase to more than 40% (5-7, 9).

The precise pathophysiological mechanism of CIN is not entirely understood. The leading theories hypothesize that it results from hypoxic injury to the renal tubules induced by renal vasoconstriction or by direct cytotoxic effects of CM (9); alternatively, some experts have argued that PC-AKI is caused by coexisting risk factors and is only coincidentally related to CM, especially when administered intravenously (10, 11).

The osmolality and dose of CM are key factors determining its renal tolerability (12, 13).

Besides, CIN is reported to be a self-limited phenomenon: serum creatinine typically increases over 1 to 3 days, peaks at 4 to 5 days, and returns to baseline in 7 to 14 days. More severe CIN may be associated with oliguria and a delayed peak in serum creatinine and a slower return to steady state, which may remain above baseline values. In a small subset of patients, temporary or permanent dialysis may be required (14).

High risk patients are considered those with pre-existing renal dysfunction, acute or chronic renal failure, diabetes mellitus, congestive heart failure because of poor renal perfusion from atherosclerosis, chronic hypertension, or diminished cardiac output (7).

Risk factors for CIN (table 1) are classified in:

- Patient related risk factors, divided into major (pre-existing renal disease and diabetes), and minor (advanced age, female gender, hypertension and nephrotoxic drugs).
- Contrast related risk factors: related to concentration of CM, volume administered, repeated contrast administration within 24-48 hours (7-8).

Table 1. Risk factors for contrast induced nephropathy

Risk Factors for CIN non modifiable	Risk factors for CIN modifiable
• Pre-existing renal disease	• dehydratation
• Diabetes associated with renal disease	• Recent contrast administration (<72 hours)
• Acute tubular necrosis	
• hypotension	
• sepsis	
• Advanced age (>70 years)	
• cirrhosis	
• Nephrotic syndrome	
• Myeloma	
• Organ transplantation	
• Human Immunodeficiency Virus	
• Metabolic disorders	
• Hyperuricemia, hypercholesterolemy, hypercalcemia	

In this "potentially" at risk patients, usually radiologists prefer to perform a CT without contrast, often useless to make diagnosis or eliminate a suspected surgical abdominal disease.

Acute abdominal conditions may be particularly challenging in the elderly, as they have a diminished sensorium, which allows the pathology to advance to an emergency state before developing symptoms. The most frequent disorders that occurs in elderly patients are: mesenteric ischemia, intestinal perforation by colon rectal cancer, diverticulitis and cholecystitis (15).

CT with intravenous contrast provides anatomical details and has high diagnostic specificity (15).

A clear imaging is mandatory to guide emergency surgery in differential diagnosis with the aim to plan the definitive management of the patient, choosing the best surgical approach.

ACR established that CCT has superior diagnostic performance compared to un-enhanced CT and that failure to diagnose an important clinical entity carries its own risk (9).

Preventive measures such as pre-hydration can significantly decrease the risk of CIN in low and high-risk patients.

In emergency setting, the timing of diagnosis is fundamental to obtain the best outcomes, decreasing morbidity and mortality rates. In patients presenting AA, CCT is mandatory to differentiate between surgical and non-surgical conditions.

The fear of CI-AKI is one of the most frequent reason why CM is withheld from patients undergoing computed tomography and thus frequently compromises the diagnostic information gained from CT and delays treatment.

We decided to perform a systematic literature review about contrast-induced nephropathy aimed to quantify the "real" risk of developing CIN and/or AKI for patients presenting with non-traumatic AA after CCT with intravenous contrast administration and to understand if the risk of CIN can be considered a strong contraindication to perform CCT and to report available strategies, recommendations and guidelines to decrease this risk.

Materials and methods

The systematic review protocol was guided by the Preferred Reporting Items for Systematic Reviews and Meta-analyses Protocol (PRISMA-P) (20). The methodological approach included the development of selection criteria, definition of search strategies, assessment of study quality, and extraction of relevant data. Quality of the evidence will be evaluated using

the Grading of Recommendations Assessment, Development and Evaluation (GRADE) methodology (21).

Literature search strategy

A literature search was performed on the following online databases: MEDLINE (through PubMed), EMBASE and Cochrane Libraries using the combination of the following keywords and/or MeSH terms: "contrast induced nephropathy" OR "contrast enhanced tomography" OR "acute kidney injury" OR "contrast agent" AND "non traumatic abdominal pain" OR "acute abdomen" OR "emergency surgery" and ""emergency department".

In addition, the reference lists from the eligible studies and relevant systematic review articles were cross-checked to identify additional records. The literature search was performed on March 2018 and was restricted to articles published since 2000. Only studies were written in English and met the selection criteria were reviewed.

Study selection

The title and abstract from all references were screened independently and blindly by two reviewers using the pre-defined inclusion and exclusion criteria. Full-text copies of relevant reports were then obtained and reviewed independently by two reviewers for final inclusion decision. Two independent reviewers extracted data from included studies using a standardized data extraction form. Disagreements were resolved by consensus and by consultation with a third independent reviewer, when needed.

Study inclusion criteria

The study selection criteria were defined before initiating data collection for proper identification of studies eligible for the analysis. All studies in which the primary objective was to describe the role of contrast enhanced tomography in the management of AA, the incidence of CIN and related implication in emergency department in the diagnostic pathway of AA in patients aged ≥18 years, were retrieved and analyzed.

Types of study

Observational and prospective studies, meta-analyses, randomized controlled trial and epidemiological studies were considered eligible for inclusion in this systematic review. Conference abstracts, letters, retrospective studies, case reports and commentaries were not considered.

Exclusion criteria

The search was limited to studies published in English, analyzing data from a population of study aged ≥18 years.

Data extraction

Data extracted from the included studies were processed for qualitative and possibly quantitative analyses.

Quality of evidence

The Grading of Recommendations Assessment Development and Evaluation (GRADE) system (21-22) was used to enable consistent judgment of the "body of evidence" and was included in the systematic review. GRADE specifies four categories: high, moderate, low, and very low. In the context of a systematic review, the quality of evidence reflects the confidence that the estimates of the effect are correct.

Results

Out of the 2500 articles initially identified, 8 articles (table 2) met the inclusion criteria and were selected for the analysis.

The flow chart of study identification and inclusion/exclusion process is shown in Figure 1.

Limitations

This systematic review will address both CIN and PC-AKI because in literature the 2 terms we can't separate CIN from PC-AKI even if these terms are not interchangeable.

Table 2. Studies included in the systematic review

References	Type of study	Numb. of patients	Setting	Area of CCT	Incidence AKI in CCT group (%)	Incidence AKI in non-CCT group (%)
Mc Donald 2014 (12)	Retrospective matched	21346	multiple	any	4,8	5,1
Davenport 2013 (63)	Retrospective matched	20242	inpatient	any	8,3	8,6
Hinson 2017 (40)	Retrospective matched	12700	ED	any	10,6	10,2
Sonhaye 2015 (41)	Prospective observational	1292	ED	any	3,4	1,8
Haveman 2006 (38)	Retrospective	340	ICU	Abdo/pelvis	2,2	NR
Heller 2016	Retrospective	7863	ED	any	8,6	9,6
Kidoh 2013 (39)	Retrospective	470	multiple	abdo-pelvis	9,1	8,3
Tremblay 2005 (37)	Retrospective	95	ED/trauma	any	3,6	15,4

ED=emergency department; ICU=intensive care unit; NR=non-reported; AKI= acute kidney injury; CCT=contrast enhanced tomography

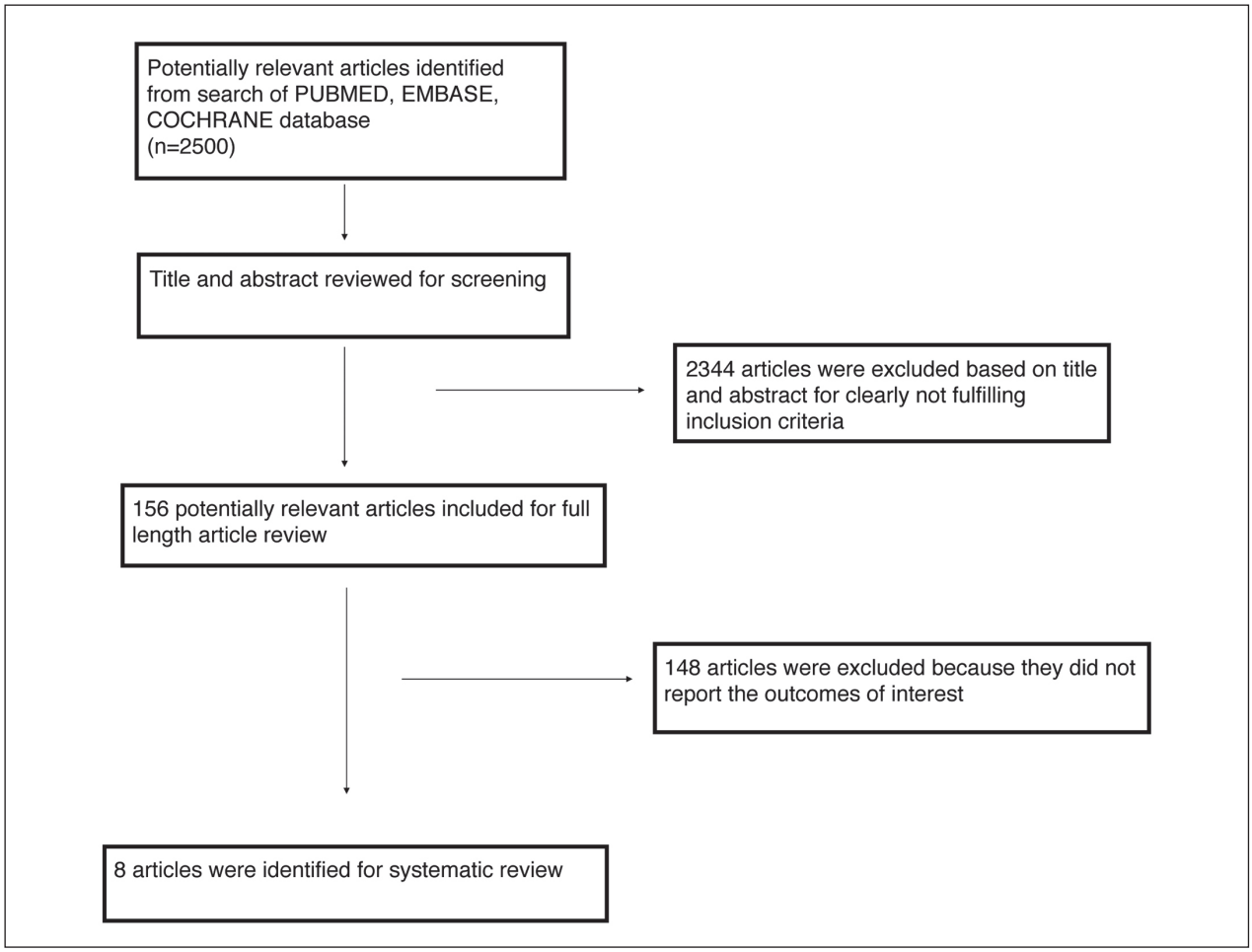

Figure 1. Search strategy according to PRISMA

• CIN/PC-AKI: definition, incidence, risk factors.

CIN is broadly defined as an absolute (≥0.5 mg/dl) or relative (≥25%) increase in serum creatinine compared with baseline after exposure to intravascular CM when alternative explanations for renal impairment have been excluded. (23).

The CIN Consensus Panel recommended using a relative increase in serum creatinine to define CIN given that this definition is independent of baseline renal function (24).

CIN typically develops within 24-72 hours post-exposure to contrast medium, with renal function returning to baseline level in two weeks. The overall incidence of CIN in the general population is <2%. In high-risk patients, including the elderly population and patients with chronic renal impairment, diabetes, congestive heart failure and anemia, the incidence of CIN is much higher (≥20%) (25).

The serum creatinine level returns within 1 to 3 weeks to baseline or a new baseline on serial follow up and contrast induced nephropathy is believed to resolve within 3 weeks. Sometimes it progresses to severe renal failure (serum creatinine >=3.0mg/dL) needing for dialysis and death (24-25).

The pathophysiological mechanisms of CIN is still unclear; iodine contrast in animal model has toxic effect for renal epithelial and endothelial cells and can increase renal tubular viscosity in vitro resulting in tubular obstruction and elevated interstitial pressure (24-25-26).

The term AKI was introduced to define abrupt damage to the kidneys whether permanent leading to acute then chronic renal failure, or temporary leading to short-term compromise in renal function. This damage is manifested by abnormal fluid balance, acid-base disturbances, and electrolyte imbalances (25). Acute renal failure is associated with high mortality rate (40-90%) (9-25).

PC-AKI is commonly defined as a rise in blood urea nitrogen, serum creatinine, or a decline in estimated glomerular filtration rate (eGFR) occurring in a narrow time window - typically 24-72 hours - after administration of iodinated CM (9).

The Acute Kidney Injury Network (AKIN) defines AKI if at least one out of of three conditions is met: (a) an absolute increase in serum creatinine levels by ≥0.3 mg/dL from baseline, (b) a relative increase in serum creatinine by ≥50% from baseline, or (c) a urine output reduced to ≤0.5 mL/kg/hour for at least 6 hours (24-26).

Urine output is not routinely measured in non-critically ill patients, consequently the first two of the 3 criteria listed above have been used to define AKI in recent studies on PC-AKI with intravenous CM (9, 10, 25).

The RIFLE (risk, injury, failure, loss, and end-stage renal disease) is a classification system which defines different stages of AKI based on changes in serum creatinine or eGFR and urine output.

It was developed by the Acute Dialysis Quality Initiative group in 2004 and introduced as measures for grading the level of kidney damage, the outcome of this damage, the interventions necessary for each class, and thus the mortality associated with this damage upon the administration of nephrotoxic drugs or contrast medium and classify patients (27, 28).

AKI reported incidence rates vary greatly depending on the patient population, the nature of the radiologic procedure, and definition of AKI. It can range from 0-11% (27).

PC-AKI is generally estimated to occur in approximately 5-15% of patients after contrast injection. A decreased baseline renal function has consistently been found to be a strong predictor of PC-AKI. Some comorbidities such as diabetes, proteinuria, hypertension, and dehydration, and nephrotoxic co-medications further increase the risk of AKI following angiographic procedures (3-5).

In clinical practice, there are different types of contrast agents with different severity of their side effects, used for varying diagnostic studies, the oldest of which is the ionic contrast agent that is also known as the first generation of CM. It is a hyper-osmolar agent that produces good images but causes more renal damage. Since the 1990s, low-osmolar CM (2 to 3 times plasma osmolality) have been the standard of care for intravascular injection. The second generation of the CM is a non-ionic agent that has lower osmolality in comparison to the first generation. The newest agent is a non-ionic iso-osmolar contrast agent; it is isotonic to plasma and it has an even lower osmolality than the

second generation, thus associated with less incidence of CIN. Iodixanol is the only iso-osmolar CM available for intravascular injection. in patients with intra-arterial administration and renal insufficiency, iodixanol is associated with a reduced risk of CIN compared with iohexol (low osmolar contrast agent), whereas no significant difference between iodixanol and other low osmolar CM could be found (30, 31).

The risk of post-PC-AKI has been shown to be significantly higher with high-osmolar CM compared to low-osmolar CM, but the iso-osmolar CM iodixanol has had conflicting results in further reducing risk even in vulnerable patients (12, 13, 31-33). In diabetic population, iodixanol is not associated with a significant reduction of CIN risk. Iodixanol is associated with a reduced risk of CIN compared with iohexol, whereas no significant difference between iodixanol and other low osmolar CM could be found (31).

Several studies have found evidence of a dose-dependent risk increasing with CM volume administered during the procedure (3, 4, 32).

In their meta-analysis of controlled studies of intravenous CM, McDonald et al. (32) report an overall AKI rate of 6.5% in the non-contrast CT group averaged over varying definitions of AKI. Davenport et al. found AKI incidence rates in the non-contrast group of 8.6% and 12.4% based on the AKIN and more traditional CIN criteria, respectively (33). Using an absolute increase of serum creatinine 0.5 mg/dL from baseline to define AKI, McDonald et al. (13) found AKI rates in the non-contrast control group of 4, 9, and 13 % for patients with a baseline creatinine of <1.5, 1.5-2, and ≥2 mg/dL, respectively. These background rates of AKI need to be considered when assessing whether CM exposure increases the frequency of AKI.

The incidence of AKI is substantially higher following coronary angiographic procedures than following contrast-enhanced CT because the patient population undergoing coronary procedures typically has more advanced vascular disease and a higher rate of hemodynamic compromise and is thus more predisposed for AKI than the average population undergoing contrast-enhanced CT (12, 32, 33). Furthermore, in CCT, the contrast agent dose administered is lower than in angiographic procedures (3, 4, 12, 13, 32, 33).

The site of CM injection (intra-arterial versus intravenous) may also have a direct influence possibly due to a higher initial concentration of CM in the renal vessels, since it has been demonstrated for aortography that the risk of AKI is greater if the CM is injected immediately proximal to the renal arteries (3, 4, 23).

Furthermore, coronary angiography leads a variety of iatrogenic risk factors for AKI, which may increase the risk of PC-AKI and are unrelated to the CM itself. It is well known that cholesterol emboli and microemboli from scraping of aortic plaque occur in a high percentage of patients during invasive angiographic procedures. Iatrogenic (micro-) embolization of renal parenchyma may contribute to AKI following angiographic procedures. Other potential complications of coronary angiography including arrhythmias, hemorrhage, myocardial infarction, or aortic dissection can all lead to hypotension or reduced cardiac output and thus contribute to post-procedural AKI which may be falsely attributed to the CM (23).

In the recent literature, CIN is reported to be a self-limited phenomenon but concern remains that intravenous iodinated contrast material exposure can lead to irreversible nephrotoxicity. Although self-limiting in most cases, PC-AKI carries a risk of more permanent renal insufficiency, dialysis, and death. Levy and colleagues retrospectively compared the outcomes of 174 patients who developed AKI after CM administration for various procedures with matched controls who received CM but did not develop AKI (34). This study found a significantly increased risk of in-hospital mortality (34% versus 7%) for those patients who developed post-AKI. However, it has been pointed out that AKI in most of these patients was probably not due to contrast material but to other comorbid and iatrogenic risk factors (23, 35).

Most available evidence on the outcome of PC-AKI relates to intra-arterial CM administration for cardiac catheterization or other angiographic procedures. In a retrospective study of patients with pre-existing renal insufficiency undergoing percutaneous coronary interventions, Gruberg and colleagues found a significantly increased risk of in-hospital mortality (15% versus 5%) for those patients who had a ≥25% increase in serum creatinine within 48 hours following coronary procedures compared to those who did not (35).

The incidence of AKI requiring dialysis after percutaneous coronary interventions is <1% in most published cohorts (13, 32, 33).

An adverse prognostic value of PC-AKI has also been demonstrated for longer-term mortality after percutaneous coronary interventions. In summary, the literature has consistently demonstrated that patients who develop postcontrast AKI after catheterization procedures have a significantly higher risk of death during the hospital stay (34, 35).

Unlike intra-arterial CM for cardiac catheterization or other angiographic procedures, the risk of adverse outcome from post-contrast administration is lower for intravenously administered CM. In an analysis of 6 prospective studies including >1,000 total patients undergoing contrast-enhanced CT with an overall PC-AKI rate of 5.1%, there was no case of dialysis or death resulting from PC-AKI (36).

• Is intravenous contrast administration for CCT safe in emergency?

Tremblay et al (37) carried out a retrospective analysis of data from 95 trauma patients to assess if the benefits outweigh the risks of intravenous contrast in trauma patients who present with an elevated serum creatinine. The incidence of AKI after CCT with intravenous contrast administration reported was 3.6% versus 15.4% in the control group. This result suggested that the benefits outweigh the risks for proceeding with iv contrast in trauma patients with an elevated creatinine.

Haveman et al (38) in a previous retrospective analysis investigated the incidence of AKI in ICU patients and concluded that CT with modern contrast was associated with a very low incidence of nephropathy in predominantly non-diabetic surgical ICU patients and that intravenous contrast should only rarely be withheld in these patients.

Furthermore, Kidoh et al (39) demonstrated that there were no significant differences in the incidence of AKI between the low-contrast dose and unenhanced CT protocols (9.1% vs 8.3%, P=0.77) in patients with renal insufficiency.

Sonhaye et al (40) confirmed these data with a prospective review of 620 patients admitted to the emergency room undergone CCT using intravenous contrast and 672 patients who received CT without intravenous contrast. Among the patients who received intravenous contrast, 3% developed CIN during their admission. At discharge, no patient had continued renal impairment. The multivariate analysis of all patients who had serial creatinine levels (including those who did not receive any contrast load) shows no increased risk for acute kidney injury associated intravenous contrast (odds ratio=0.619, p value=0.886); only diabetes remains an independent risk factor of acute kidney injury (odds ratio=6.26, p value=0.031).

The fear of causing or exacerbating renal damage should not be a reason for with-holding contrast studies.

• The importance of CT Scan in emergency surgery

Acute abdominal pain is a common condition of admission in ED. The term "acute abdomen" defines a clinical syndrome characterized by the sudden onset of severe abdominal pain requiring emergency medical or surgical treatment.

In an analysis of more than 10,000 patients presenting with acute abdominal pain the etiology could not be determined in one-third of these cases; of those patients in whom a diagnosis was made, 28% had appendicitis, 9.7% acute cholecystitis, 4.1% small bowel obstruction, 4% acute gynecological disease, 2.9% acute pancreatitis, 2.9% acute renal colic, 2.5% perforated peptic ulcer, and 1.5% acute diverticulitis (42).

Various potentially life-threatening diseases can cause acute abdominal pain; thus a rapid and accurate diagnosis is essential to reduce morbidity and mortality.

Physical and laboratory examinations are often non-specific, and the clinical presentation of many entities overlaps. Therefore, diagnostic and efficient imaging evaluations are indispensable.

Computed tomography (CT) has gained widespread acceptance as the first-line imaging modality in patients presenting with acute abdominal pain (1, 43).

Although clinical data, physical examination and laboratory tests guide the clinician in diagnosis, they are not sufficient to reach definitive diagnosis especially if pain spreads throughout the abdomen rather than involving a specific region or abdominal quad-

rant, particularly among the elderly, obese and immunocompromised patients.

The best diagnostic imaging test in these clinical scenarios is contrast enhanced multidetector CT (42-44).

In a cohort study comparing ultrasound and CT in 1021 consecutive patients, CT was significantly more sensitive than ultrasound (89% *vs* 70%, $p<0.001$), although the approach achieving the highest sensitivity was a diagnostic strategy combining an initial ultrasound scan, followed by CT, only when ultrasound examination yielded negative or inconclusive findings (1).

To compare the effect of an initial early CT examination versus standard practice on the length of hospital stay, diagnostic accuracy, and mortality of adults presenting with AA, Sala et al. conducted a randomized controlled trial involving 205 adults presenting with acute abdominal pain; the study showed that early abdominal CT in patients with acute abdominal pain improves diagnostic certainty, even if it does not reduce the length of hospital stay and 6 month mortality (45).

Tsushima et al. prospectively analyzed data about 125 adult patients presenting with acute abdominal pain to determine the value of CT on the diagnosis and treatment plan of these patients; authors concluded that CCT frequently changed the initial clinical diagnoses, increasing the diagnostic yield (46).

Catena et al focused on the diagnostic impact of CT scans in abdominal trauma and in non-traumatic acute abdomen. The aim was to guide emergency surgeons and physicians in the choice of the best radiological exam taking in account sensitivity and specificity of CCT for the different diseases underlying acute abdominal pain (47).

Moreover, in emergency setting time is the essence for survival and to obtain decreasing in morbidity and mortality rates related to the AA.

CCT with intravenous contrast administration in patients presenting with non-traumatic AA has the advantage to give information about the presence and feature of fluids or abscesses in the abdominal cavity, about the cause of small or large bowel obstruction, it allows to detect the site of a gastrointestinal perforation and to check vascular changes in the small or large bowel wall.

CCT is mandatory to make diagnosis in some life-threatening gastrointestinal emergencies as in case of acute mesenteric ischemia, gastrointestinal perforation, obstruction, diverticulitis and to early manage the patient with the best operative or non-operative strategy.

• How to prevent CIN

CIN is not as frequent as it was believed in the past few years in general population, but it occurs in high risk patients (23). This suggests using all precautions that may prevent contrast media-nephrotoxicity in all patients, especially in high-risk patients.

All the available studies about CIN prevention strategies suggest to identify patients at risk for developing CIN before administering CM. Methods to identify patients at risk include use of patient questionnaire, a review of the patient's complete medical history looking for comorbidities as hypertension, renal disease, dyslipidemia, hyperuricemia, diabetes, heart failure, myeloma, treatment with nephrotoxic drugs, and measurement of serum creatinine before CM administration (table 1).

The absence of risk factors for renal disease effectively eliminates the likelihood of a patient having renal impairment (48).

Renal function is usually assessed with a serum creatinine (sCR), which is used in either the Cockcroft-Gault or modification of diet on renal disease formula to estimate glomerular filtration rate (eGFR). The risk of CIN increases as the estimated glomerular filtration rate falls, particularly below 60 ml/min (48-50).

In all patients admitted with AA, suspected to have an acute surgical pathology, it is suggested to:

- discontinue all potentially nephrotoxic drugs (aminoglycosides, vancomycin, amphotericin B, dipyridamole, metformin, and nonsteroidal anti-inflammatory drugs). Special attention should be paid to the use of metformin, because of its prevalent renal excretion and its tendency to cause a severe lactic acidosis. Thus, this medication should be discontinued 12 hours before the administration of contrast agent and not be resumed until at least 36 hours after the procedure, or even longer if the serum creatinine has not returned to baseline (50).

- Provide as soon as possible an adequate hydration of the patient. Iso-tonic fluids were significantly less risky than half iso-tonic fluids for developing CIN (51).

In high-risk patients it may be useful to implement the i.v. infusion of 0.9% saline solution at a rate of about 3 mL/kg/hour, 1 hour before and for the 6 hours after the procedure, for procedure scheduled the same day. At least 300-500 mL of IV hydration should be administrated before contrast is given (10).

The European Renal Best Practice (55) recommends volume expansion with either isotonic sodium chloride or sodium bicarbonate solutions, rather than no volume expansion, in patients at increased risk for CIN.

All potentially surgical patients should be hydrated by IV fluids since admission.

Other agents are used to prevent CIN but they should not be considered as a substitute for hydration; the most used are N-acetylcysteine (NAC), beta blockers such as nebivolol, adenosine antagonists, simvastatin, furosemide, dopamine and dopamine agonists, recombinant human erythropoietin (55-59).

Most of these agents has been studied in patients undergone intra-arterial CM administration.

There is no evidence of outcomes regarding the application of short-term prophylaxis protocols for contrast-induced nephropathy (CIN), that may be most feasibly convenient, in emergency settings.

The European Renal Best Practice does "not recommend using prophylactic intermittent hemodialysis or hemofiltration for the purpose of prevention of CIN (55).

In preventing CIN, the radiologist has a major role, in choosing to administrate the least nephrotoxic iodinated agent as iodixanol (iso-osmolar CM) and iopamidol (low-osmolar CM) at the lowest dosage possible that would produce good imaging and enable diagnosis of the underlying cause of AA (58).

High doses of contrast agents are required in coronary angiography and percutaneous coronary interventions. For these procedures some formulas have been suggested to calculate the dosage that is least dangerous for renal function: (a) Cigarroa's formula: 5 mL of contrast per kg b.w./serum creatinine (mg/dL) with maximum dose acceptable of 300 mL for diagnostic coronary arteriography; (b) Laskey's formula: volume of contrast to eGFR ratio with a cut-off point of the ratio at 3.7 for percutaneous coronary intervention (58).

Discussion

CIN and CCT with intravenous contrast administration: dogma or reality?

The strongest currently available evidence on the incidence of PC-AKI following intravenous CM administration consists in a meta-analysis of observational studies.

Twenty-eight studies involving 107,335 participants were included in the final analysis.

Included articles specifically compared rates of renal insufficiency, need for renal replacement therapy, or mortality in patients who received intravenous contrast versus those who received no contrast.

Meta-analysis demonstrated that, compared with non-contrast CT, contrast-enhanced CT was not significantly associated with either AKI (odds ratio [OR] 0.94; 95% confidence interval [CI] 0.83 to 1.07), need for renal replacement therapy (OR 0.83; 95% CI 0.59 to 1.16), or all-cause mortality (OR 1.0; 95% CI 0.73 to 1.36).

Therefore, given similar frequencies of AKI in patients receiving non-contrast CT, other patient- and illness-level factors, rather than the use of contrast material, likely contribute to the development of AKI.

Before that study, another meta-analysis of controlled studies demonstrated a similar incidence of AKI, dialysis, and death between patients who received CM and control group.

This meta-analysis was performed by Mc Donald et al. (32) to examine the incidence of AKI and other outcomes in patients exposed to intravenous CM compared with patients who underwent an imaging examination without contrast medium or were otherwise unexposed (control group).

Thirteen non-randomized studies were included for meta-analysis with a total of 25,950. In the group that received contrast medium (contrast medium group), risk of AKI (RR=0.79; 95% confidence interval [CI]: 0.62, 1.02; P=.07), death (RR=0.95; 95% CI:

0.55, 1.67; P=.87), and dialysis (RR=0.88; 95% CI: 0.23, 3.43; P=.85) was similar, compared with the risk of AKI in the non-contrast medium group. This pattern was observed regardless of i.v. contrast medium type, diagnostic criteria for AKI, or whether patients had diabetes mellitus or renal insufficiency.

It is important to remind that all controlled studies included in the meta-analysis had a nonrandomized study design, which inevitably makes them vulnerable to selection bias, since patients perceived to be at risk for AKI are more likely to receive non-contrast CT examinations.

In the following studies, statistical methods of propensity score adjustment were used to neutralize differences in AKI risk factors between the contrast-enhanced and the non-contrast CT group and thus neutralize the effects of selection bias. McDonald et al. (13) found that after propensity matching there was no significant difference in AKI incidence between contrast-enhanced and non-contrast group. Subgroup analysis was performed for patients with a baseline serum creatinine of <1.5, 1.5-2, and ≥2 mg/dL, and no significant difference between exposed and nonexposed patients was found in either group.

Mc Donald and al. (64) examined the association of intravenous iodinated contrast material administration with the subsequent development of PC-AKI, emergent dialysis, and short-term mortality using a propensity score-adjusted analysis of a cohort of intensive care unit (ICU) patients who underwent CT examination and confirmed that intravenous contrast material administration was not associated with an increased risk of AKI, emergent dialysis, and short-term mortality in ICU patients with pre-CT eGFR >45. An increased risk of dialysis was observed in patients with pre-CT eGFR ≤45.

Hinson and al. performed a single-center retrospective cohort analysis with the aim to determine whether intravenous contrast administration for computed tomography was independently associated with increased risk for AKI and adverse clinical outcomes. They reported that contrast administration was not associated with increased incidence of AKI and was not associated with increased incidence of chronic kidney disease, dialysis, or renal transplant at 6 months. They demonstrated also that clinicians were less likely to prescribe contrast to patients with decreased renal function and more likely to prescribe intravenous fluids if contrast was administered (65).

Analyzing all data available about PC-AKI after intravenous administration of CM, evidence strongly suggests that the risk caused by CM is much smaller than previously thought based on noncontrolled studies. For patients with a baseline creatinine of <1.5 mg/dL and an eGFR of ≥45 mL/min/1.73 m2 the risk of PC-AKI is likely to be nonexistent.

In emergency setting the first aim is making diagnosis.

Acute abdominal pain is one of the most common conditions that calls for prompt diagnosis and early treatment. Having the correct diagnosis is the essential premise to set up the best management for the patient. In patients who are acutely ill, delays in imaging may adversely affect patient care.

The "golden hour" concept can be applied also to the evaluation of AA patients: rapid intervention improves the outcomes. The relationship between timing and mortality is well known in literature and as CCT in evaluation of trauma patients is mandatory both in hemodynamically stable patients and in unstable patients after adequate resuscitative maneuvers to assess and treat all the lesions.

CCT with intravenous administration of CM is the most used imaging technique to investigate life threatening causes underlying AA and it is mandatory when intestinal ischemia, small or large bowel obstruction, diverticulitis or peritonitis are suspected diseases.

The risk of CIN is negligible in patients with normal renal function but the incidence appears to rise to as high as 25% in patients with pre-existing renal impairment or in the presence of risk factors such as diabetes, advanced age, vascular disease and use of certain concurrent medications (66, 67).

Patients admitted for non-traumatic AA in emergency surgery department are often elderly, dehydrated and present with hypotension, acute or chronic nephropathy or acute pathologies that independently affect the risk for developing CIN; this are widespread conditions which cannot limit diagnostic CT in emergency.

At admission, before beginning clinical evaluation, laboratory and imaging exams, the patient with AA should:

- be hydrated as early as possible;
- stop medications as anticoagulants and nephrotoxic drugs.

Delaying inpatient CT scans worsens patient outcomes and increases morbidity and mortality rates.

Explorative laparoscopy or laparotomy are not the best diagnostic tools in case of high-risk patients.

The radiologist should not refuse to perform CCT if it is necessary for diagnosis, even in patients at high risk for CIN.

Preventing measures to decrease the risk of CIN and improve outcomes should be applied.

The incidence reported of AKI in patients undergoing contrast IV administration for CCT is not high as thought before.

Conclusions

The benefits of diagnostic information gained from contrast enhanced TC in assessing AA are fundamental in some clinical scenarios. Radiologists and referring physicians should be familiar with the risk factors for renal disease and CIN. The incidence of CIN/AKI after intravenous contrast administration is very low in general population.

Since volume expansion is the only proven preventive strategy, in emergency setting it is advised to start volume expansion as early as possible before contrast medium administration.

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