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FATTY LIVER AND GUT MICROBIOTA: A NEW MULTI-DIMENSIONAL APPROACH FOR PERSONALIZED PREVENTIVE MEDICINE

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Abstract. Cardiovascular, degenerative, hepato-digestive, metabolic and neoplastic diseases are major causes of death; all of them are beckoned years before by fatty liver that can quantify by non-invasive ultrasound methods. Such a measure is sensitive and reproducible and qualifies as mirror of general health to monitor the efficacy of preventive care in pre-symptomatic subjects. One major determinant of alimentary and general health is the gut microbiota that regulates hepatic gene expression, lipid metabolism and contributes to hepatic inflammation and obesity. The microbiota can be dynamically modified by probiotic/prebiotic supplementation, however a direct gut microbiota profiling by stool metagenomics is limited by sampling error. The study of blood and/or saliva metabolites (metabolomics) and circulating antimicrobial antibodies provide an indirect microbiota profiling. Studies need to be performed to test whether variation of metabolomics and antimicrobial antibody levels correlate with the in vivo bacteria dynamics. The non-invasive measure of fatty liver in combination with of the gut microbiota characterization by metagenomics, metabolomics and anti-microbial enzyme immune assays will provide an innovative technological approach to stratify individuals with fatty liver for both prevention, outcome prediction and personalized treatment and to identify new aetiologies, diagnostic and prognostic biomarkers and therapeutic targets.

Key words: Fatty Liver, Gut-Microbiota, Probiotics, Steatosis, Ultrasound

Metabolic syndrome (MetS) and its complications (diabetes and cardiovascular diseases), degenerative, hepato-digestive, and neoplastic diseases are major causes of death: familiarity or early symptoms are used for preventive care. Fatty liver (>5% of total liver weight, steatosis, fatty liver disease, FLD) is associated with higher mortality for all these pathologic conditions.[1-3] The prevalence of FLD is rapidly increasing worldwide, in 20-30% of the overall population and more than 60% in the elderly:[1-3] a large cohort study reported that fatty liver was associated with 26% higher 5-year overall health-care-costs, mostly by cardiovascular and metabolic diseases.[3] FLD is an indolent liver pathology unless it is complicated by inflammation, steato-hepatitis (SH) which may progress rapidly to cirrhosis and hepatocellular carcinoma.[1-3] A great deal of new knowledge in the physiopathology of fatty liver has accumulated in recent years revealing the complexity of the mechanisms involved.[4] All most recent guidelines and expert opinions for the management of FLD prompt a new systems medicine approach for the study of the interplays between major physiology systems which control our vital relations with the environment: brain and nervous, endocrine, digestive (gut, liver and microbiota) and immune systems.[1,4] The first mandatory step is a consistent and reliable measure of intrahepatic fat (IHF). Liver biopsy is invasive and hampered by sampling errors because it represents only a minimal part of the liver and in about one third of cases the intrahepatic fat distribution is not homogenous.[1-3] The gold standard is magnetic resonance (MR) spectrometry and nowadays, it is also possible to quantify IHF using new non-invasive methods based on software using algorithms of multiple standardized imaging parameters provided by common ultrasound instruments.[5,6] They provide reproducible and precise measures particularly in mild and intermediate forms of steatosis, which are the most important to be monitored for an accurate and timely preventive care before the development of irreversible complications of FLD and MetS. Using these new techniques, it is possible to evaluate in clinical trials as well as in clinical practice whether drugs and/or changes of life style or alimentary habit determine an

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effective reduction of IHF in the single subject.

The liver is target of many signals from distant tissues, particularly from visceral adipose tissue and gut, both of them inextricably linked with the gut microbiota.[4,7] The impact of different diet components on liver and plasma lipid composition is mediated by the gut microbiota that regulates hepatic gene expression and cholesterol metabolism,[7] plays a pivotal role in many signaling pathways regulating intra-hepatic inflammation[8] and contributes to development of fatty liver and obesity.[9] The complexity of the interplay between liver and gut microbiota accounts also for the association between fatty liver and irritable bowel syndromes (IBS).[10] The gut microbiota modulates both local and systemic immune responses and controls the trans-membrane translocation of bacteria.[11,12] A specific action of Bifidobacterium strains producing exopolysaccharides (EPS) conditions their adhesion to the gut mucosa.[12] For instance EPS of Bifidobacterium longum W11 resistant to rifaximin was shown to trigger the production of cytokines from in vitro Con-A stimulated mononuclear cells.[13,14] Bifidobacterium longum W11 is resistant against rifaximin and might contribute to its efficacy in both IBS and hepatic encephalopathy.[15,16] Moreover, it has been reported a marked reduction of Bifidobacterium species (B. bifidum, B. longum and B. adolescentis) in obese, NAFL and NASH children when compared with healthy control.[17] These data suggest that Bifidobacterium may have a protective role in the development of NASH, NAFL and obesity due to their marked reduction in patients with these disorders. All these evidences suggest that the measure of IHF qualifies for a simple reliable mirror of general health to be used to monitor the efficacy of alimentary and preventive care particularly in the pre-symptomatic subject.[18] A major determinant/partner of both alimentary/general health and liver function is the gut microbiota that can be dynamically modified by probiotic and prebiotic supplementation. However, in spite of the consistent evidence of the importance of the gut microbiota there is an unmet need of appropriate methods to study the heterogeneity and dynamics of the different microbial species.[9,11] In fact, the gut microbiota profiling by the metagenomics of the stools is inevitably limited by the sampling error caused by the differential compartmentalization of the microbiota species along the long gastrointestinal tract. An indirect evidence of the presence and growth of some microbial species can be achieved by the differential profiling of blood and/or saliva metabolites (metabolomics).[19-20] On the other hand is well known that the immune system produces antibodies against microbes colonizing the gastro-intestinal mucosa and their serum levels correlate with the microbial load.[21] Furthermore, it is well known that when a given microbe triggers inflammation the immune system beckons its pathogenesis by producing specific IgM type antibodies quantitatively related with the inflammatory burden. Exploiting this host-microbe relation the quantification of anti-microbial antibodies (IgG, IgA e IgM) in serum and/or saliva might provide a new opportunity for profiling the heterogeneity of the gut microbiota and to study its dynamics in different physic-pathologic conditions. This approach will be fostered by new regulatory standards for probiotic production requiring that bacterial viability is assessed not only by Plate Count methods but also by methods like flow cytometry capable to detect also viable bacteria that have adapted to the environmental stress by becoming dormant and do not form colonies.[22] The production of antimicrobial antibodies necessary to detect microbes by flow cytometry for probiotic characterization and enumeration will prompt also the setting up of enzyme immune assays for quantification of human antimicrobial antibodies in serum and saliva. Studies will be performed to test whether the logarithmic variation of the circulating antibody levels correlate with the *in vivo* bacteria growth. The combination of metagenomics with metabolomics and multiple anti-microbial enzyme immune assays (Figure 1) will provide an innovative approach for a more precise three-dimensional (3D) characterization of the dynamics of the gut microbial species. Hopefully the results of the new studies will help to identify new aetiologies, diagnostic and prognostic biomarkers and therapy targets for a better stratification of the patients with fatty liver for both prevention, outcome prediction and personalized treatment.

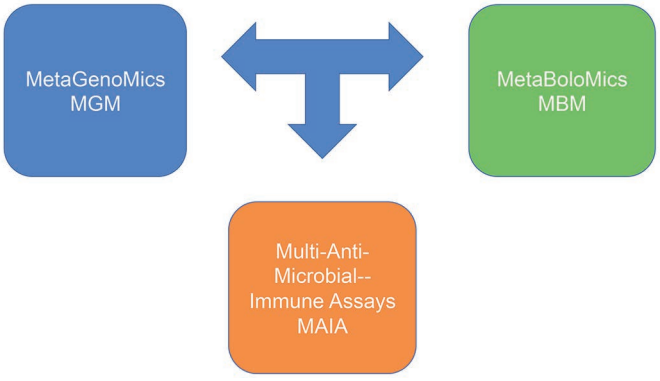

Figure 1. Multi-system microbiomics for personalized medicine.

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