R22 DOCOSAHEXAENOIC ACID (DHA) IN CYSTIC FIBROSIS

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An increased metabolic rate of Arachidonic Acid (C20:4n-6, AA) and its derivatives (prostanoids: prostaglandins, leukotrienes, thromboxanes) has been suggested as starting process of the biochemical and clinical derangements described in CF patients. This process might lead, on one hand, to reduced pools of linoleic acid (C18:2n-6, LA), the metabolic precursor of AA, and, on the other, to a continuous sub-inflammatory status affecting several organs and increasing the energy demand of patients. Within these processes, docosahexaenoic acid (C22:6n-3, DHA), the main derivative from the parental alpha-linolenic acid (C18:3n-3, ALA), maybe also reduced in CF as a secondary result of the sub-inflammatory status requiring high rates of AA conversions to prostanoids. This biomechanism could indirectly increase the consumption of DHA, whose role in hampering the metabolic conversions of AA derivatives has been shown in experimental models. The characteristics of the dietary treatment of CF patients could contribute to low DHA levels in blood and tissues, as result of both a low supply coupled with an increased use for energy production. Reduced levels of DHA in blood and tissue specimen have been observed in CF patients, with the lowest levels found in those with the most severe CF genotypes. As a consequence, the ratio of AA to DHA in blood and/or tissues has been proposed as marker of disease (a higher value of the ratio corresponding to worst conditions). Infants and children could be particularly exposed to the negative consequences of a high AA, low DHA status, since they physiologically need both AA and DHA for growth and development. Therefore, CF can be proposed as a model of increased AA availability for inflammatory processes where DHA is consumed, and where both these compounds are diverted from their biological and-points. The observed positive effects of early breastfeeding on later markers of disease progression are consistent with this hypothesis, since human milk represents a natural source of DHA within a balanced mixture of long-chain polyunsaturated fatty acids. To substantiate this hypothesis, well-controlled clinical trials are needed to check whether DHA supplementation, along with an adequate and balanced dietary regimen, could restore the fatty acid status and the AA/DHA ratio, with positive effects on functional markers of the disease, such as lung ventilatory measures and/or indices of infections and morbidity as well as nutritional status, growth and development.

R23 TOWARDS A EUROPEAN CLINICAL TRIAL NETWORK

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In the past decades, the prognosis of patients suffering from CF has been improved considerably by means of a very intensive supportive therapeutic management. However, in spite of all achievements, the life expectancy and especially the quality of life of CF patients remains very limited compared to those of their healthy peers. Thus, there is a considerable need to optimise the existing therapies and to develop new treatment options. With the better understanding of the basic pathophysiology of CF there is even hope that a curative treatment will be found.

For decades many committed CF centres have contributed greatly in clinical research aimed at improving patient care. The added value of neonatal screening for CF has also been documented. Thanks to meticulous follow-up and more intensive treatment early on, in recent decades, the rate of deterioration of lung function is slower and the current yearly drop in FEV1, the best surrogate outcome parameter, is only around 1–2%. Only appropriately powered trials will bring CF clinical research further. Increasingly large multi-centre trials will be the only option. For this reason, ECFS wants to intensify the clinical research in the field of CF in terms of quality and quantity. In order to be able to offer the structures required for a quick and professional implementation of clinical studies, the ECFS initiated the foundation of the Clinical Trial Network (ECFS-CTN). The ECFS-CTN aims to be a network in which interested CF trial sites, fulfilling criteria with regard to quality of care as well as performance of clinical research, will voluntarily form an alliance. The aim of forming the ECFS-CTN is the consistent performance and successful completion of clinical studies in accordance with good clinical practice guidelines. Innovative therapies could thus be available to CF patients more quickly. The successful model of the American cystic fibrosis foundation therapeutic development network (CFF-TDN) will work inspiringly to develop the road map for the emerging European clinical trial network.

R24 MONITORING OF ANTI-*PSEUDOMONAS AERUGINOSA* THERAPY IN EARLY PHASES OF THE INFECTION

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Chronic colonization by *Pseudomonas aeruginosa* (*Pa*) is an unfavorable event in the prognosis of cystic fibrosis (CF) patients. In the absence of a cure for CF, prevention of chronic colonization by eradicating the germ upon initial isolation appears to be a simple and efficacious way to improve long-term prognosis. The efficacy of eradication therapy, regardless of the type of drug used and duration of treatment, is on average 75%. Patients colonized by *Pa* and treated immediately should be monitored microbiologically, using molecular and immunological techniques. Classical monitoring using growth cultures does not distinguish between regrowth of suppressed but uneradicated strains and colonization by a new strain. Therefore, genotyping of strains isolated in the first and next colonization episodes should be performed. In our experience, re-colonization by *Pa* following eradication therapy is mainly caused by strains of different genotype, suggesting acquisition from an external source. A short *Pa*-free period is mainly due to transient suppression of *Pa* growth. True eradication followed by acquisition of a new *Pa* genotype occurs in most cases only after a *Pa*-free period >6 months. Patients with a *Pa*-free interval of <6 months had a 6-times higher chance that the *Pa* was not eradicated in comparison with those with a germ-free interval of >6 months.

To improve results of bacteriological cultures, amplification via PCR of a specific gene fragment of *Pa* directly on respiratory samples was performed. This approach identifies a higher number of patients with *Pa* than conventional growth culture and can supplement *Pa* identification.

The potential use of immunological markers for monitoring eradication therapy has been evaluated. There are several methods for investigating anti-*Pa* antibodies but none of these can be considered a gold standard. Our research indicates that 45% of patients have an increase of IgA anti-*Pa* antibodies before isolation of the germ. A rise in antibody titers indicates probable infection and eradication treatment may then be initiated, even in the absence of microbiological detection of *Pa*. In these cases, growth cultures should be carried out at briefer intervals.

Monitoring of eradication treatment cannot rely only on microbiological evidence but requires investigation of other potential markers as well.

R25 NEW DIAGNOSTIC AND THERAPEUTIC APPROACHES IN CYSTIC FIBROSIS RELATED DIABETES (CFRD)

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Diabetes is a frequent co-morbidity in cystic fibrosis (CF). Although CFRD has always been considered a mild form of diabetes mellitus (DM) with a low risk of severe complication, it is associated with a worse prognosis in affected patients, due to deficient anabolizing action of insulin and, probably, to increased risk of infections related to hyperglycemia. To date, the only accredited therapy of CFRD is insulin, which is performed according to the therapeutic protocols used in Type 1 and Type 2 diabetic patients.

For a better management of CFRD condition a continuous and close collaboration with the diabetologic consultant is mandatory, to import to CFRD patients the new diagnostic and therapeutic approches in DM management. Research in the diabetic field has been developed in these last years, ranging from inhaled insulin to islet transplantation, and from gene therapy to stem cells. However, these approches will need to be formally tested in rigorous clinical trials before they can become part of the daily lives of people with DM. Moreover, some of these new therapies, as inhaled insulin, do not suit with the underlyng disease in CFRD patients. In our opinion, up to date, CFRD caregivers, waiting for future better tools to manage the problem, have to work, above all, on reducing negative impact that abnormal glucose metabolism has on the CF patients' future. New therapeutic protocols, studying "when" and "what" to use in the condition of glucose abnormal metabolism in CF, have to be implemented. An Italian multicenter randomized clinical trial about the use of Glargine Insulin in CF patients, affected by glucose intolerance, is ending the patients' recruitment.

R26 CLINICAL IMPACT OF TOBI® ON CYSTIC FIBROSIS PATIENTS WITH CHRONIC *PSEUDOMONAS AERUGINOSA* COLONISATION: RESULT OF A POST-MARKETING SURVEILLANCE

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Aim: A number of studies have shown a beneficial effect of nebulized antibiotics in cystic fibrosis (CF) patients with chronic *Pseudomonas aeruginosa* (PA) infection. We performed a post-marketing surveillance with the aim of comparing the clinical outcome before and after starting TOBI in a CF population with chronic PA colonization.

Methods: Our study is part of a larger post-marketing surveillance study which involved 21 Italian Cystic Fibrosis Centres and included 603 patients using TOBI, of whom 86 with first PA infection and 517 with chronic PA colonisation. Spirometric, clinical and therapeutical data were collected at 12 months before starting TOBI, at TOBI start, and at 6 and 12 months after TOBI start.

Results: Out of 517 patients with chronic PA infection, 422 used TOBI for 12 months. Although the mean number of days of hospitalization and of intravenous

(IV) antibiotic treatment did not change significantly before and after 12 months of TOBI, the number of oral antibiotic treatment days was reduced significantly (27.2 vs 20.1; $p < 0.001$). Overall when comparing the mean predicted FEV1 before 12 months from TOBI start with that after 12 months, no significant change was found. Between the 12 months before and TOBI start, mean FEV1 showed a decrease of 3.5% of the predicted value (from 69.1% to 66.7%), while 6 months after TOBI start, it increased to 68.3%. This increase was most apparent in patients of 13 to 18 years of age (from 74.5% to 78.6%). After the second other 6 months of treatment, mean predicted FEV1 showed a slight decrease (–1.6%). During 12 months of follow-up, 12% of patients interrupted TOBI because of side effects (mainly tinnitus, bronchospasm, dysphonia).

Conclusions: Our study showed that TOBI seems to stabilise the mean value of predicted FEV1 after 12 months of treatment but does not increase it. TOBI treatment did not have a significant effect in reducing neither the days of IV antibiotics nor those of hospitalisation, while it was associated with a decrease in the length of oral antibiotic treatment. Post-marketing surveillance studies have an important role in describing the effectiveness of treatment in the real non-controlled clinical context.

R27 EARLY EVALUATION OF CF LUNG DISEASE

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CF patients have lung inflammation and infection beginning early in life. This leads to an irreversible structural damage that is heterogenously distributed throughout the lung. Adequate monitoring of CF lung disease is paramount to tailoring treatment to a patient's need. Pulmonary function tests are insensitive to detect localized or early damage. Computed Tomography (CT) is the most sensitive tool to detect structural abnormalities at an early stage of CF lung disease.

Important structural changes that can be detected at an early stage are airway wall thickening, air trapping, bronchiectasis, and mucus plugging.

Chronic inflammation leads to airway wall thickening which is present in young children with CF. This is more severe in peripheral than in central airways. On CT peripheral airways cannot be observed directly because they are too small relative to the CT resolution; they can be identified indirectly as hypodens areas on expiratory chest CT representing air trapping areas often combined with hypoperfusion. Mucus plugging and bronchiectasis are other important structural changes, the latter being an irreversible and progressive widening of an airway becoming evident in the majority of patients in childhood. The goldstandard to diagnose bronchiectasis is through a volumetric CT scan taken at end-inspiration.

Monitoring with CT should start in infancy at regular time interval. Unfortunately, there are some major hurdles to overcome in young children: (1) CT should only be done in radiology departments with experience in using the lowest possible radiation dose; (2) life long radiation exposure should be limited to acceptable levels. Radiation dose has been brought down substantially in the last decade and it is likely that it will be further reduced in the near future. A CT scan should be done at end inspiration and end expiration during a breath hold; in children younger than 4 yrs the procedure should be done under general anesthesia. This requires a dedicated team with experience in using the controlled volume technique. The risk of atelectasis can be avoided when the CT is made using a modern fast scanner during spontaneous breathing that allow to do a whole CT scan in less than 1 second. The sensitivity of this method to diagnose air trapping is probably less compared to the controlled volume technique. In children older than 4 yrs it is possible to do a whole lung in- and expiratory scan each during a breath hold.

Only few therapeutic studies in young children are available that included chest CT. Hence, how to deal with abnormalities observed on CT scans of young children is not straight forward. When the CT scan is normal the pulmonary condition is probably good; if major abnormalities are being observed more aggressive therapy should be considered.

R28 EUROPEAN CYSTIC FIBROSIS SOCIETY CONSENSUS ON GENETIC TESTING

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This paper describes the conclusions of a consensus conference to address the use and interpretation of CF mutation analysis in the clinical setting.

The limitations of genotyping technology, the choice of mutations to be tested, the clinical context in which the test is being taken can all influence how genetic information is interpreted. Although the diagnosis of cystic fibrosis is usually straightforward, care needs to be exercised in the use and interpretation of genetic tests; genotype information is not the final arbiter of a clinical diagnosis of cystic fibrosis or cystic fibrosis transmembrane regulator protein related disorders. The diagnosis of these conditions is primarily based on the clinical picture, and supported by measurements of CFTR function (sweat testing, nasal potential difference and so on) and genetic analysis. None of these is sufficient on its own to make a diagnosis of CF or CFTR related disorders.

The consensus addressed the prognostic value of particular genotypes and concluded that, although useful in group studies, individual genotype can not accurately predict the pulmonary outcomes in people with CF.

Genotype analysis is a very valuable tool in the diagnosis and assessment of people with cystic fibrosis and CFTR related disorders; the availability of relatively cheap DNA sequencing of the entire CFTR gene has thrown up numerous novel mutations and sequence variants but these must be interpreted with due acknowledgement of the limitations of primary DNA sequence analysis.

Finally, and of paramount importance, good communication between clinicians and medical genetic laboratories is vital. Information must be exchanged clearly and completely between the laboratory and the clinician, and subsequently communicated to the patient in an understandable way.