

## PLAN OF ACTIVITY

### Title of the research:

DIAGNOSIS AND THERAPY OF THE CORTICO-RESISTANT NEPHROTIC SYNDROME FROM FOCAL GLOMERULOSCLEROSIS

### Main seat of the research:

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### Cooperating Departments:

FACILITY	INSTITUTION	SUPERINTENDENT
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### Background of the project:

Focal glomerulosclerosis (GSF) is a sclerous lesion of part of the renal glomerulus, which is at first present in some glomeruli and then gradually spread to all the glomeruli of the kidneys.

In the clinical context of the cortico-resistant nephrotic syndrome, the discovery of a GSF lesion represents a negative prognostic element and characterizes children who very often and quickly develop an end-stage renal disease (ESRD).

As far as the causes and mechanisms that lead to GSF, some forms of cortico-resistant nephrotic syndrome have been recently identified as due to hereditary defects in the synthesis of the proteins of the basal glomerular membrane; therefore the spectrum of possible causes, previously confined to cellular immunological factors, has been extended and probably not concluded yet.

As per the genetic component, some mutations of structural components of the renal podocyte are reported. Therefore, the genes involved in this disease encode elements that constitute the basal membrane of the renal podocyte: proteins like podocin (NPHS2), nephrin (NPHS 1), CD2AP (CD2 related protein) and components of the cytoskeleton, like the  $\alpha$ -actin (ACTN4). Recent studies have reported mutations of the gene that encodes the podocin in some cases of sporadic GSF. Besides,

functional polymorphisms of the NPHS2 promoter have been identified too: these are considered not as the cause for the disease, but as a genetic susceptibility to develop it. There are then some cases of GSF that, under particular stressful circumstances like a renal transplant, develop the recurrence of the disease in the transplanted kidney.

As per the immunologic component, in the last 20 years many evidences have been found to show that GSF is associated to alterations of the immune system. GSF can in fact be associated to atopy, to lymphoproliferative disease, to an increased production of cytokines by the T helpers and to the production of factors of permeability of the basal glomerular membrane by the T lymphocytes.

These immunologic alterations could in their turn have a genetic base and be responsible for the recurrence of GSF in the transplanted kidney. Consequently, it is likely that different diseases hide behind the term GSF, and the determining factors are known only for some of them. A more precise diagnosis according to the categories known up to now represents then a first step to plan an individualized treatment. It is in fact known that patients with genetic defects don't respond to therapy with corticosteroids, nor to the immunosuppressive one: a diagnosis of a genetic kind would consequently allow to avoid long pharmacological treatments burdened by severe side effects. Transplant is instead the most rational therapy for these patients. For patients with GSF from immunological factors, the possible therapeutic solutions currently adopted imply on one side the immunosuppression of the cellular lines producing lymphokines, on the other side the elimination through plasmapheresis of the plasmatic factors responsible for the damage. The outcome of the different treatments proposed hasn't been conclusive though, at least for patients with severe manifestation of the disease.

Renal transplant represents a valid therapeutic option for patients affected by end-stage renal disease. There are patients who can hardly undergo a transplant, because affected by particular background disease like the Focal Glomerulosclerosis itself (GSF), associated to cortico-resistant Nephrotic Syndrome. This disease recurs in 25-50% of the cases after the first transplant and in 75-100% after the second one. The therapeutic strategies currently available to treat the recurrence of GSF in the transplanted kidney use immunosuppressive drugs and aphaeresis treatments. The immunosuppressive therapy centres on the use of corticosteroids associated to other drugs; corticosteroids are usually dispensed in intravenous way and then per os. The drugs used together with corticosteroids are calcineurin inhibitors, like high dosed of Cyclosporine A and also antilymphocytic agents, like mycophenolate mofetil, if not previously dispensed. The aphaeresis techniques [conventional plasmapheresis (PF) and immunoabsorption with A vitamin (IA)] are based on the assumption that there is a circulating proteinuric factor; these methodologies allow to make the activity of permeability in the plasma of these patients undetectable, with a consequent reduction of the proteinuria. The action of these therapeutic schemes doesn't always provide the expected results: in fact, the relapse of FSGS can happen even just a few hours after the transplant. It is then clear that there is still room for new therapeutic actions.

### **Main goals**

- To clarify the FSGS diagnosis with genetic studies aimed to identify hereditary and sporadic forms of the disease, through the analysis of the genes involved in it: NPHS1, NPHS2, ACTN4, CD2AP.
- To carry out immunologic studies and studies of proteomics on the affected patients' blood and urine, as markers of the disease and of its activity.
- To assess the therapeutic options in the different stages of the disease.

**Staff specifically addressed and competence required** (Department of Nephrology and Paediatric Haemodialysis, Foundation IRCCS, General Hospital, Mangiagalli and Regina Elena)

- Bursar of the Foundation "La nuova speranza – Lotta alla glomerulosclerosi Focale"

- Postgraduate in nephrology awarded a bursary abroad.

## **Main stages, work packages, protocols and methodologies**

1. **Epidemiologic study** on the cases of cortico-resistant nephrotic syndrome from GSF, treated at the Department of Nephrology and Paediatric Dialysis, according to the specially prepared questionnaire. Patients will be divided in 5 groups, corresponding to as many phases of the disease:  
phase A: patients with disease pre-ESRD  
phase B: patients with the disease, under dialysis pre first transplant  
phase C: patients with the disease, post first transplant  
phase D: patients with the disease, post second transplant  
phase E (control group): patients with working transplant

2. **Etiopathogenetic study:** being possible to propose a scheme that includes a multifactor pathogenesis of GSF, genetic and immunological, the following studies will be carried out on all the 5 groups of patients:

Genetic analysis: search for mutations in the genes encoding podocin (NPHS2), nephrin (NPHS 1), CD2 related protein (CD2AP) and  $\alpha$ -actin (ACTN4).

Immunological study:

Search for the plasmatic factor able to modify the permeability of the basal membrane of the podocyte. The existence of this factor is supported by many observations: a) the recurrence of nephrotic syndrome in the transplanted kidney, b) the injection in animal models of serum coming from patients with recurrence of the disease in the transplanted kidney provokes an alteration of the permeability of the basal membrane and nephrotic syndrome, c) the use of plasmapheresis or immunoabsorption with protein A causes a reduction or a remission of proteinuria in these patients. Immunological alterations: a series of surveys on the lymphocytic underpopulation and on the function of the lymphocytes in this type of patients have defined an anomaly of the T lymphocytes and therefore their role of causality in the GSF pathogenesis. Lymphocytes (in particular T-helpers) and cytokines produced by them (in particular IL2, INF- $\gamma$ , IL4, IL6, TNF- $\beta$ ) are therefore involved in it: the picture of anomaly in the production of cytokines could depend on an modified intracellular synthesis in the majority of the lymphocytic underpopulations. A possible new cause of nephrotic syndrome, related especially to the recurrence of GSF has been recently identified: the presence of costimulatory molecules CD80 (B7) expressed on podocytic level. CD80 are proteins of the membrane expressed in constitutive way on the dendritic cells and, after induction, on the surface of the lymphocytes B and of the APC. The ligands of these molecules are the CD28 and the CTLA4 present on the surface of the T lymphocytes: their bond fully activates the T lymphocytes in reaction to the antigen. Recent studies have demonstrated that these molecules are up regulated on podocytic level and contribute to the pathogenesis of the proteinuria, modifying the permeability of the podocytic membrane.

The presence of the plasmatic factor will be then searched in all the patients enrolled in our plan of activity and the immunological arrangement will be identified through the study of the various lymphocytic underpopulations involved in the disease according to the studies already carried out.

According to the results obtained, each group of patients will be divided into two subgroups: 1) patients with genetic mutations of the basal membrane of the renal podocyte and 2) patients with immunological alterations responsible for the damage of the basal membrane.

Proteomics: the term proteomics delineates a systematic analysis of the identity, quality and function of the proteins, and therefore denotes the study of all the proteins expressed in an organism, tissue or cell in a given moment; the same kind of cells expresses different proteins in relation to a physiologic or pathologic state (age, disease, environmental influences). This science uses specific methodologies, some of which still to be refined: bidimensional electrophoresis, mass spectrometry, microarray. The analysis of these proteins can be carried out both on blood and on urine. Recent studies have identified the existence of a set of urinary biomarkers in patients with Nephrotic Syndrome (albumin, transferrin,  $\alpha$ -antitrypsin, kininogen and kallikrein) and biomarkers distinctive of GSF (discovery of growth hormone-GH in the urine of patients affected by this disease). Other recent studies carried out on blood have identified circulating antibodies against podocytes and tubular cells (anti-actin antibodies/ATP synthase) in patients with Nephrotic Syndrome and GSF.

According to the studies reported above, we will investigate the presence of specific biomarkers as albumin, transferrin,  $\alpha$ -antitrypsin, kininogen and kallikrein in the urine and (anti-actin antibodies/ATP synthase in the blood, in all the patients affected by GSF in order to assess the activity of the disease and the effectiveness of the planned therapy.

**3. New therapeutic proposals:** for each single patient, they will be considered in relation to the pathogenesis of the disease. Given the limited number of patients, there won't be any randomization with different treatments.

Genetic forms won't be treated with immunosuppressive therapy in the pre-ESRD and pre first transplant stages (phases A and B), but only with conservative and then dialytic therapy. Because genetic forms recur only in 1-2% of the cases of transplant, in case of first transplant a conventional immunosuppressive therapy will be carried out. In case of a possible recurrence after the transplant (phases C and D), the therapy for the immunological forms will be carried out.

Non genetic forms, meaning the immunological ones, will require an aggressive immunosuppressive therapy both in phase A and in the following ones, because of the high rate of recurrence in the transplant:

- Use of new antibodies, like the Campath 1H (Alemtuzumab, anti CD-52) or LEA29Y (Belatacept, co-stimulating molecules CD80-CD86 inhibitor).
- Use of aphaeresis techniques, like the chemophotoaphaeresis, with or without plasmapheresis.

Specifically, for the immunological forms, after the failure of the conventional immunosuppressive schemes using Cyclosporine and then Tacrolimus + Mycophenolate, the following treatments will be carried out:

- Patients in phase A: chemophotoaphaeresis and plasmapheresis + immunosuppressive therapy (Campath 1H)
- Patients in phase B: chemophotoaphaeresis only after demonstration of effectiveness of the phase A treatment
- Patients in phase C: chemophotoaphaeresis and plasmapheresis + immunosuppressive therapy (Campath 1H)
- Patients in phase D: chemophotoaphaeresis only after demonstration of effectiveness of the phase A treatment

LEA29Y, characterized by less side effects, could replace Campath 1H, if available during the three years of the study.

**4. Follow-up assessment of the therapeutic response:** based on the assessment of proteinuria (Pru/Cru) and of the haematological parameters related to it, regularly monitored.

**Duration (36 months):**

The recruitment of the existing patients will take place in the first months of 2006 (epidemiological assessment). At the moment 35 patients are available, divided as below:

- Phase A: 15
- Phase B: 3
- Phase C: 5
- Phase D: 0
- Phase E: 12

During the whole duration of the study, it's possible to recruit new patients and the transit of patients within different groups.

In the following months, biological material will be taken for the basic etiopathogenetic study (genetic and immunological parameters, and also metabolic parameters). On 24h urine sample: proteins/creatinine; on blood sample: urea, creatinine, electrolytes, total protidemy + albumine, calcium, phosphorus, cholesterol and triglycerides. The time requirement needed to carry out these studies will vary from 6 to 12 months, according to the number of recruited patients, to the achievement of biological samples to examine and to the time to deliver the results, related to the methods used in external centres (Gaslini Hospital in Genova, Cattinara Hospital in Trieste) or in the Foundation labs (Medical Genetics).

In relation to the group one belongs to, different treatment and follow-up protocols will be defined, as describe above, and that will require an extended observation period of time. For example, for the patients under dialysis waiting for a transplant, the moment of the transplant will be time 0 to start the follow-up. Patients will be monitored in the following 24 months, in order to assess the influence of the genetic and immunological conditions on the outcome of the transplant.

During the period of the study, the patients enlisted in each group will be monitored with intermediate assessments, as described, to define the clinical evolution of the disease and the response to a specific treatment.