

APHERESIS IN PRIMARY FOCAL SEGMENTAL GLOMERULOSCLEROSIS OF NATIVE AND TRANSPLANTED KIDNEYS: A THERAPEUTIC PROTOCOL.

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ABSTRACT : *Background.* Patients with focal segmental glomerulosclerosis (FSGS) develop nephrotic syndrome and terminal renal failure in most cases. FSGS reappears in 15-50% of transplanted kidneys and frequently causes the graft loss. Sera from patients with FSGS of native or transplanted kidneys contain some proteinuric or permeability factors (PF) which can be removed by means of plasma exchange (PE) or protein A Immunoabsorption (IA).

Methods. We suggest a therapeutic protocol, for patients with biopsy proven FSGS of native or transplanted kidneys, resistant to steroid and immunosuppressive therapy, based on the association of PE or IA to conventional drug therapy. Daily proteinuria, renal function, serum albumin and circulating levels of PF (permeability test) will be monitored at regular time intervals during the apheresis cycle, which will be intensive at the beginning (8-10 sessions in 4 weeks) and very gradually discontinued.

Results. We will consider *satisfactory* remission the reduction of proteinuria below 1 g/day, improvement of renal function, normalization of serum albumin level (> 3.5 g/dl). *Partial* remission will be considered: proteinuria < 3 g/day, serum albumin level between 3 and 3.5 g/dl. Permeability test, if positive at baseline examination, should be negative after apheresis.

Conclusions. The primary endpoint of our protocol is: lasting remission (satisfactory or partial) after the apheresis suspension. Secondary endpoints are: maintained remission with continuing apheresis sessions, correlation between permeability activity and disease activity, identification of responders and non responders patients on the basis of positive permeability test.

Key words: *FSGS, transplanted kidney, proteinuric factor, plasma exchange, protein A*

immunoabsorption.

INTRODUCTION

Approximately 76% of children and 55% of adults affected by primary or idiopathic focal-segmental glomerulosclerosis (FSGS) present nephrotic syndrome (1). 50% of patients with proteinuria in nephrotic range ($>3,5$ g/24h) will develop terminal renal failure after 6-8 years; this period will extend to 10 years in 20% of patients with non nephrotic proteinuria (2). FSGS re-appears in 15-50% of transplanted kidneys and in half of these cases it develops into renal function loss (3,4). In case of a second transplant, relapse is much more frequent (4). Studies carried out since the Seventies revealed the presence in FSGS patients of blood factors (5), called "proteinuric" or "permeability" factors, which have partially been isolated only in the last 10 years (6-8). The exact chemical-physical features and biological behaviour of the factors are still unknown as well as their prevalence rate in patients with native or transplanted kidney FSGS, and their prognostic and therapeutic significance. Recently published data seem to indicate that the presence of circulating permeability factors has a significant predictive value for the recurrence of proteinuria in children with FSGS who have received a renal allograft (9). The possibility of a proteinuric circulating factor has fostered the use in these patients of therapeutic apheresis techniques: published studies have demonstrated that conventional plasmapheresis (PE) and prot. A immunoabsorption (IA) reduce the permeability activity in patients' plasma and decrease proteinuria (9-14).

It is, however, well known that pharmacological therapy can achieve total or partial remission of proteinuria of FSGS both in children and adults, particularly if corticosteroid treatment is prolonged and cyclosporin is added to the therapy of corticosteroid resistant patients (15-20). The examination of many case histories leads to the following conclusions:

1. The percentage of total or partial remission in primary FSGS in native kidneys (including both children and adults) varies from 39 to 60%: this is the case of steroid-sensitive patients or responders. The key to remission is the length of steroid treatment: 1-1,5 mg/kg/die (not more than 80 mg) for 3-4 months, to be gradually decreased during a period of not less than 6-8 months (average remission time: 3 months).
2. If relapse occurs more than six months after suspension of steroid treatment (steroid-dependent or relapsing patients), the steroid treatment can be repeated. In the case, instead, of early relapse, the number of remissions can be increased by adding 2-2,5 mg/kg/die of Cyclophosphamide (Cy) for 2 months or 5 mg/kg/die of Cyclosporin (CsA) for 6 months (to be gradually decreased by 25% per month until suspension). There are no major differences between the two drugs even if the use of Cy leads to a more stable remission, while when using CsA, remission seems to be dependent on continued use of the drug.
3. When there is no remission after the first 4 months of therapy, about 25% of steroid-resistant patients would achieve remission, if the above mentioned amounts of Cy were added. This percentage would increase to 45% if the above mentioned quantities of CsA are added. The use of other cytotoxic agents, such as Chlorambucil, Methotrexate,

Vincristine, as well as ACE-Inhibitors has not yet been sufficiently documented to consider further.

4. In relapsing FSGS in transplanted kidneys, almost half of the cases present partial or total remission as a result of intensification of the treatment: increased steroid and CsA dosage, addition of Azathioprine (Aza) or Mycophenolate Mofetil (MMF) if not previously used. If, after two months of intensified treatment the patient does not achieve remission, he/she can be considered as a “non responder” and, therefore, as a case evolving into terminal renal failure and return to dialysis.

OBJECTIVES

This therapy protocol aims to:

- Establish whether PE and IA induce remission if added to the pharmacological treatment.
- Verify whether remission is related to the decrease or disappearance of protein permeability activity
- Contribute to the identification of protein permeability factors.
- Establish the appropriate “dose” of apheresis.

PATIENTS

Criteria for inclusion in the study:

In case of biopsy proven primary FSGS in native kidneys, all patients (children and adults) presenting proteinuria in steroid-resistant or steroid-dependent nephrotic range, and frequent relapses regardless of Cy and CsA treatment;

In case of relapsing FSGS in transplants, all patients (children and adults) with proteinuria in nephrotic range not responding to the intensification of a steroid or immunosuppressive regimen; they must have a biopsy proven diagnosis in their native as well as in transplanted kidney. The recurrence of FSGS is considered related to the original disease if proteinuria occurs within 12 months after transplantation.

Regarding the examination of new cases, waiting at least one year from the approval of this protocol is suggested.

Criteria for exclusion:

in native kidney FSGS: secondary forms (oligomeganephronia, unilateral renal agenesis, single kidney as a result of surgery, vesicourethral reflux, residual cortical necrosis, focal and segmental sclerosis in different GNs, systemic vasculitis, pyelonephritis, diabetes, vascular nephropaties, existing cancers or cancers which have been surgically treated within the last 3-5 years);

in transplant FSGS: histologic evidence of acute or chronic rejection or CsA toxicity associated with glomerulosclerosis; surgical complications during transplants (obstructive or vascular);

in native kidney and transplant FSGS: age below 2 and over 65, intercurrent major infectious diseases (hepatitis B, C, CMV, tuberculosis), hepatic and heart failure, medullar depression (Hb < 7 gr/dl, leucocytes < 3000/ μ l, platelets < 80.000/ μ l) cancers, insulin-dependent diabetes, stable advanced renal failure (creat.> 3,00 mg/dl) revealed by ultrasonography (volume reduction) or renal biopsy (preponderance of sclerohyaline injuries). Renal failure, although severe, presenting good echographic renal conditions and biopsy showing recent or partially evolved lesions do not constitute reasons for exclusion from this study.

METHODS

Patients enrolled in the apheresis protocol must present the above mentioned clinical features, i.e. steroid-resistant or steroid-dependent, non responders to immunosuppressive treatment. We can therefore define three groups:

1. Patients with native kidney FSGS, who do not achieve remission after 4 months of steroid treatment (1 mg/kg/die) and 2 months of combined steroid (0.5 mg/Kg/die) + Cy (2-2.5 mg/Kg/die) or CsA (5 mg/KG/die) treatment.
2. Patients who had a remission after the combined steroid + Cy or CsA treatment, and present a first relapse after treatment suspension, and do not achieve remission after two months of new combined treatment (steroid 1 mg/Kg/die + Cy or CsA as above mentioned).
3. Patients who present a second relapse following a first remission reached through combined treatment, after the treatment suspension.

As regards patients with relapsing FSGS in transplants, please refer to the previous section. FSGS diagnosis must be confirmed through a histologic assessment.

During the first phase of the apheretic cycle, the steroid and immunosuppressive treatment already in course must be continued, despite having no results in reducing proteinuria: this behaviour is necessary in transplanted patients to avoid the risk of rejection; moreover, the drug treatment suspension or reduction in patients with native kidney FSGS could introduce an unpredictable variant in the response to the therapy and make this group not comparable to the transplant group.

Apheresis modalities: depending on technologies available in the centres taking part in this study, PE or IA will be used accordingly. In any event, patients characteristics should be comparable between the two techniques.

PE sessions: 3 per week during the first 2 weeks and 2 per week during the following 2 weeks.

IA sessions: 2 per week for 4 weeks.

The first 4 weeks including from 8 to 10 sessions are the most intensive period of the treatment. During every session, 2 blood volumes will be treated with IA (1.5 in children) and 1-2 volumes will be changed in PE (1 in children). The removed plasma is substituted with 4% albumin solution during PE whereas no reinfusion is needed during IA. PE can be performed either by plasmafiltration or blood centrifugation. IA is performed exploiting the properties of protein A which is able to adsorb immunoglobulins and PF (11,14). We use the Excorim IA System (Lund, Sweden) in which 2 protein A columns are alternatively perfused by plasma and regenerated (11)

Laboratory tests: before starting the apheresis cycle, the following tests will be carried out:

proteinuria, clearances, serum proteins, routine blood chemistries, presence of glomerular permeability serum factor by means of isolated glomerular assay (9, 21).

Assessments will be run as follows:

24h proteinuria: every session - clearances: once per week - permeability test (if positive): serum will be tested before/after the first two sessions (in the case of IA, the test will be run also on column eluate). After the first 2 sessions, permeability test will be run before the apheretic session once per week.

In order to assess the resynthesis rate of the permeability factor, some patients will undergo serial sampling, regardless of apheretic sessions, to be agreed with the laboratory running the test.

Sample size determination: with a 15% proportion of remission (due to misclassification and/or spontaneous improvement), a sample of at least 15 patients is required to detect a 35% of remission due to apheresis treatment (total remission 50%), for a significance level $\alpha=0.05$ (two tailed tests using normal approximation to the binomial distribution) and a power $(1 - \beta)=0.8$.

ASSESSMENT OF RESULTS

As the subjects are, by definition, non responders, to achieve what the literature usually considers as total remission will be difficult, i.e. proteinuria < 4 mg/h/m² for 3 consecutive days or, in adults, proteinuria < 0.2 or 0.5 g/die. We will therefore consider two possible positive results:

The following results may be considered to constitute a "satisfactory" remission:

proteinuria reduction below 1g/day
improved renal function (if previously reduced)
albuminemia $> 3,5$ g/dl

The following may be considered "partial" remission:

proteinuria below nephrotic range (< 3 g/day)
stable renal function
albuminemia > 3 g/dl, $< 3,5$ g/dl.

In all cases studied in the literature, the average remission time is 2 or 3 weeks. If no remission is achieved after the intensive treatment period, the patient will therefore be considered a "non responder" also to the apheresis treatment which will then be suspended. Should the patient respond, the apheresis treatment will be continued and run once a week for the first month and every other week for the following two months. Steroid and immunosuppressive treatment will be continued according to transplant requirement in case of recurrent FSGS, or gradually decreased, after the intensive apheresis period, in case of native kidney FSGS: we suggest to decrease the steroid daily dosage by 5 mg every week and to not exceed 2-2.5 mg/Kg/die of Cy for 12 weeks. CsA administration can be tapered to 4-3 mgr/Kg/die and discontinued within 18-24 weeks. Serologic, urinary and eluate tests will

be run every 2 weeks.

After 4 months period and 16-18 apheretic sessions, treatment will be suspended and proteinuria, renal function and permeability test (when positive at the beginning) will be monitored once a week.

According to data available in the literature, we can consider three kinds of follow-up:

1. Persistent remission even after apheresis has been suspended.
2. Reappearance of proteinuria after suspension. Some cases demonstrated an increased proteinuria 3-4 weeks after the last session. Some of the patients become apheresis dependent, undergoing sessions every 2 or 3 weeks for years, thus maintaining remission.
3. Reappearance of the disease with proteinuria in nephrotic range and renal failure which could either respond or not to a new intensive period of treatment.

With regard to this study protocol, monitoring of patient's proteinuria, serum proteins, renal function and permeability test every month for a year of follow up is suggested.

Primary endpoint: persistent remission (however defined) of the disease even after apheresis has been suspended.

Secondary endpoints:

- Maintained remission with continuing apheresis sessions
- Evidence of relation between permeability factor and FSGS activity
- Identifying "responder" and "non responder" patients by means of positive results of the permeability test.

For permeability tests, please send serum and eluate samples stored at -20° C and packed in dry ice to:

Dott. Michele Carraro, Istituto Di Medicina Clinica Università di Trieste, Ospedale Cattinara, strada

di Fiume 447, Trieste, 31149. Tel 040/3994962, Fax 040/912881.

For any communication, please refer to: Dott. Luigi Moriconi, U.O. Nefrologia e Dialisi, Ospedale degli

Infermi, Piazza 20 Settembre, 56027 S. Miniato, A.S.L. 11. Tel 0571/248526-530 Fax 0571/248526-588 e-mail: lmrc43@infinito.it or nefrosm@tin.it

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