

## PROTOCOL BIOPSY AND SUBCLINICAL REJECTION IN PATIENTS AFTER KIDNEY TRANSPLANTATION TREATED BY TACROLIMUS (PROGRAF)

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The article deals with the contribution of tacrolimus (Prograf) to improvement in kidney transplant results. Tacrolimus, in comparison with cyclosporine significantly reduces the incidence of acute rejection and improves survival of grafts as well as patients. Based on the literature, the primary immunological differences between tacrolimus and cyclosporine effects are pointed out. These differences explain the better immunosuppressive effectiveness of tacrolimus. Based on analysis of the results, subclinical rejection problems and significance of protocol biopsy for present-day transplantology are discussed. There is also a critical analysis of the questions, which priority, in relationship to the expanding availability of immunosuppressive substances currently has high interest for nephrologists researching subclinical rejection.

Tacrolimus (Prograf, formerly FK 506) is a macrolid, which is a product of the *Streptomyces tsukubaensis* fungus. It was discovered in Japan in 1985. For clinical application in the field of kidney transplantation it was used for the first time in 1994 in the UK<sup>1</sup>. Tacrolimus, together with cyclosporine, are calcineurin inhibitors, which are fundamental for currently used immunosuppressive protocols. The mechanism of their effect is described in detail in a number of easy to understand publications<sup>2</sup> and is not a subject of this report. In spite of that, there are a number of immunological, metabolic and clinical differences which those related to subclinical rejection will be mentioned.

The results of the initial multi-central, randomized studies were published in 1997. These studies showed that graft recipients treated by tacrolimus had a lower incidence of acute rejection episodes than the individuals treated by cyclosporine. In European study were selected 448 patients. Two thirds were treated by tacrolimus and one third by cyclosporine. The incidence of the acute rejection verified by biopsy in patients treated by tacrolimus was 24.1 %, while in patients treated by cyclosporine 43.4 % (ref.<sup>3</sup>). In an American study, 412 patients were random selected, 205 were treated with tacrolimus and 207 received cyclosporine. In patients treated with tacrolimus, the incidence of histologically verified acute rejection was 30.7 %, whereas in patients treated by cyclosporine it was 46.7 % (ref.<sup>4</sup>). Statistical analyses confirmed, that the reported differences in incidence of the acute rejection were significant ( $p < 0.001$ ). When tacrolimus was used, signifi-

cantly less corticoreistant acute rejection were observed (11.3 % vs. 21.6 %). After the first year, neither graft nor patient survived in either group, meaning patients treated by tacrolimus or cyclosporine, varied.

Similar results were achieved in comparative studies, which compared tacrolimus and cyclosporine in the form of microemulsion. For example, in a six-month randomized study involving 560 individuals from 50 European transplant centers, incidence of acute rejection verified by biopsy in patients treated by tacrolimus was 19.6 %, whereas in patients treated by cyclosporine microemulsion, it was 37.3 %. There was also less rejections resistant to the corticosteroids in the patients with tacrolimus – 9.4 % vs. 21.0 % (ref.<sup>5</sup>). These results were statistically significant ( $p < 0.0001$ ).

One of the greatest problems in present transplant medicine is the fact, that kidney transplant function is limited. Therefore a very important aspect of selection of the prophylactic immunosuppressive therapy is not one-year survival of graft, but, most importantly, assessment of effect of the immunosuppressive substance on the medium and long-term transplant results.

A prospective analysis of a population of 1.007 transplanted individuals recently published by Japanese authors showed an excellent immunosuppressive potential of tacrolimus from the medium term point of view<sup>6</sup>. In a population of 1.007 patients treated by tacrolimus, one-year graft survival rate was 94.8 %, two-year rate 92.6 % and three-year survival rate 90.4 %. The whole population at the same time included also 100 individuals who

received ABO incompatible kidney from a living donor and 146 patients with transplanted kidney from non-heart beating donors, that is from sub-group, where transplant results are generally poor. Even though the Japanese group did not compare results of the tacrolimus and cyclosporine therapy, they confirmed the results of earlier comparative studies. Both the American<sup>7</sup>, and European<sup>8</sup> multi-center randomized studies showed a significantly better graft survival rate in patients treated by tacrolimus in comparison with the patients treated by cyclosporine three or four years after the transplantation.

Long term monitoring of the results of the tacrolimus and cyclosporine therapy also confirmed, that the immunosuppressive therapy, the basis of which is tacrolimus, significantly reduced risk of failure of the graft function without increasing, at the same time, incidence of undesirable effects of the long-term immunosuppression. According to the results of the American multicenter, comparative, randomized study<sup>9</sup>, the five-year graft survival rate in the tacrolimus group was 63.8 %, whereas in the cyclosporine group only 53.8 %. The difference in the patients survival rate in both groups, however, did not reach statistical significance.

Development of molecular-biological techniques helps clarify the cause of the better immunosuppressive effectiveness of tacrolimus in comparison with cyclosporine. As the most important facts resulting from the immunological research and related to this can be considered the finding, that tacrolimus, unlike cyclosporine, lowers mRNA expression for IL-10, increases apoptosis of T lymphocytes activated by anti-CD3, decreases production of antibody against HLA as well as HLA antigens and significantly decreases proliferation of vascular endothelial cells<sup>10-12</sup>. From the long-term point of view, particularly significant is the fact, that tacrolimus, unlike cyclosporine, has no effect on levels of transforming growth factor (TGF  $\beta$ ), which has a significant fibroproliferative potential and is considered the key factor in development of the chronic rejection nephropathy<sup>2</sup>.

In the last few years, the nephrology department of the 3<sup>rd</sup> Clinic of Internal Medicine has concentrated its efforts on, among other things, study of subclinical rejection. The subclinical rejection of transplanted kidney is defined by histological presence of rejection changes in the graft, but it is not reflected in the clinical image and the creatinine concentration is stabilized in normal range<sup>13</sup>. The subclinical rejection diagnostics has so far been possible only by means of the protocol biopsies, which means the biopsies that are performed regardless of the graft function according to a selected timetable. Introduction of the protocol biopsies was made possible by development of automatized biopsy equipment and wide availability of sonographic examinations. Today, the graft biopsy is considered a relatively safe invasive method with a minimal risk of clinically serious complications.

A number of observations indicate, that the subclinical rejection is not a benign unit but may have a significant negative effect on fate of the graft<sup>14</sup>. Presence of subclinical rejection is a sensitive indicator of effectiveness of the immunosuppressive treatment. It has been proved, that

the subclinical rejection treatment in the first six months after the transplantation improves long-term function of the graft. Particularly, a Winnipeg group's research confirmed, that two-year survival of the graft with subclinical rejection, which had been re-treated by methylprednisolone pulses was 14 % better than in the patients who were not treated (97 % vs. 83 %)<sup>15</sup>. Alternatively, cellular infiltrate, particularly in the area around peritubular capillaries and tubules, especially if accompanied by disruption of basement membranes, leads to progressive interstitial fibrosis and probably plays the decisive role in pathogenesis of chronic rejection nephropathy<sup>16</sup>.

There is no substitute of contribution of the protocol biopsies to the patients with delayed start of the graft function where the acute rejection cannot be clinically recognized<sup>17,18</sup>. Shapiro detected acute rejection by the seventh day after transplantation in 21 % and borderline changes in 36 % of patients with delayed start of graft function<sup>19</sup>. Protocol biopsies also contribute to diagnostics of early stages of chronic rejection nephropathy. Seron et al. detected signs of chronic rejection nephropathy in 42 % biopsies in a population of 98 patients 3 months after the transplantation<sup>20</sup>. By using the protocol biopsy, we can finally better diagnose cyclosporine nephrotoxicity<sup>21</sup>, or some very serious, especially viral infections, primarily nephropathy associated with the BK-virus<sup>22</sup>.

Information about subclinical rejection received up to now has been obtained from patients treated by cyclosporine. In our own population of 33 individuals after kidney transplantation, 18 females and 15 males, average age of 48.5 who were treated with a prophylactic combination of cyclosporine A + mycophenolate mofetil (azathioprin) + Prednisone, we observed high incidence of the subclinical acute rejection in the first three months after the kidney transplantation. Evaluation results of 66 protocol biopsies performed 3 weeks and 3 months after transplantation are shown in table No. 1. As can be seen from the table, the third week after transplantation, the acute subclinical rejection was diagnosed in 30.3 % of the patients and three months after the transplantation subclinical rejection was found in 27.2 % of monitored population. A similar incidence of the subclinical acute rejection is reported by the other authors working on the problem<sup>23</sup>. The mean values of creatinine in serum of individual diagnostic group are shown in table No. 2.

At present, multicenter studies are underway to evaluate the results of the protocol biopsies in patients treated with tacrolimus. The Rochester prospective study of a population of 114 patients treated by tacrolimus, mycophenolate mofetil and prednisone in the protocol biopsies performed three months after kidney transplantation showed subclinical rejection in only 2.6 % and border line changes in 11 % of patients<sup>24</sup>. This kind of incidence of subclinical acute rejection is extremely low, but it has to be taken into consideration that 56 % of the individuals in the evaluated population received induction therapy by thymoglobulin. Our patient population, who were treated primarily by tacrolimus and underwent the protocol biopsy, is as yet very small and from the statistical point

**Table 1.** Evaluation of protocol biopsies (Banff 1997).

First protocol biopsy 21 ± 2 days			Second protocol biopsy 90 ± 5 days		
Diagnostic group	Number	%	Diagnostic group	Number	%
N	12	36.4	N	12	36.4
SAR	10	30.3	SAR	9	27.2
AR	11	33.3	AR	12	36.4

N normal findings

SAR subclinical acute rejection

AR acute rejection

of view, for the time being, cannot be evaluated. No data related to the protocol biopsy findings in patients treated by sirolimus or everolimus have been published.

Discussion of significance of the protocol biopsies and opinions on the clinical significance of interstitial infiltrates in stable grafts continues<sup>23</sup>. For the time being, the protocol biopsies provide extraordinarily important information, which cannot be obtained in any other way. They can diagnose serious morphological defects of the graft which are not apparent during the clinical biopsy time and enable us to gain time during which an adequate therapy can prevent functional deterioration of the graft. Questions related particularly to timing and frequency of the protocol biopsies, better specification of pathogenic seriousness of infiltrating cells by means of molecular and immunological markers and finally also the type of optimal therapeutic strategy of subclinical rejection which could lead to improved function and survival of the graft still remain. With the expanding variety of available effective immunosuppressive substances, we cannot rule out the possibility that in future, the significance of protocol biopsies from the subclinical rejection diagnostics point of view will be limited or will be superseded by new, non – invasive examinations, development of which is intensively being worked on<sup>25</sup>.

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**Table 2.** Mean serum creatinine concentrations in diagnostic groups.

First protocol biopsy 21 ± 2 days		Second protocol biopsy 90 ± 5 days	
Diagnostic group	Serum creatinine (μmol/l)	Diagnostic group	Serum creatinine (μmol/l)
N	109 ± 13	N	102 ± 18
SAR	94 ± 18	SAR	108 ± 10
AR	274 ± 184	AR	224 ± 171

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